UNIT-IV
SYLLABUS

Developmental Stage II: Cell division in cleavage – Chemical changes–Patterns of embryonic cleavage – Morula and Blastula – Role of egg cortex – Morphogenetic gradients – Fate map – Gastrulation – Primary organ, Rudimental organs, Organizer – Morphogenetic movements-invagination, extension, ingression movements and locomotion.

Cell division in cleavage

Cleavage is the repeated mitotic division of zygote to form a solid ball of cells called morula which later changes into a hollow ball of cells called blastula.

Cleavage of human zygote occurs within the fallopian tube. It is holoblastic, i.e., it divides the zygote completely into daughter cells or blastomeres.

The first cleavage takes place about 30 hours after fertilization. It divides zygote longitudinally into two blastomeres (one slightly larger than the other). The second cleavage occurs within forty hours after fertilization. It is at right angles to the plane of the first resulting in four blastomeres. The third cleavage takes place about 72 hours after fertilization. During these early cleavages, the young embryo moves slowly down the fallopian tube towards the uterus (Fig. 3(B).10).

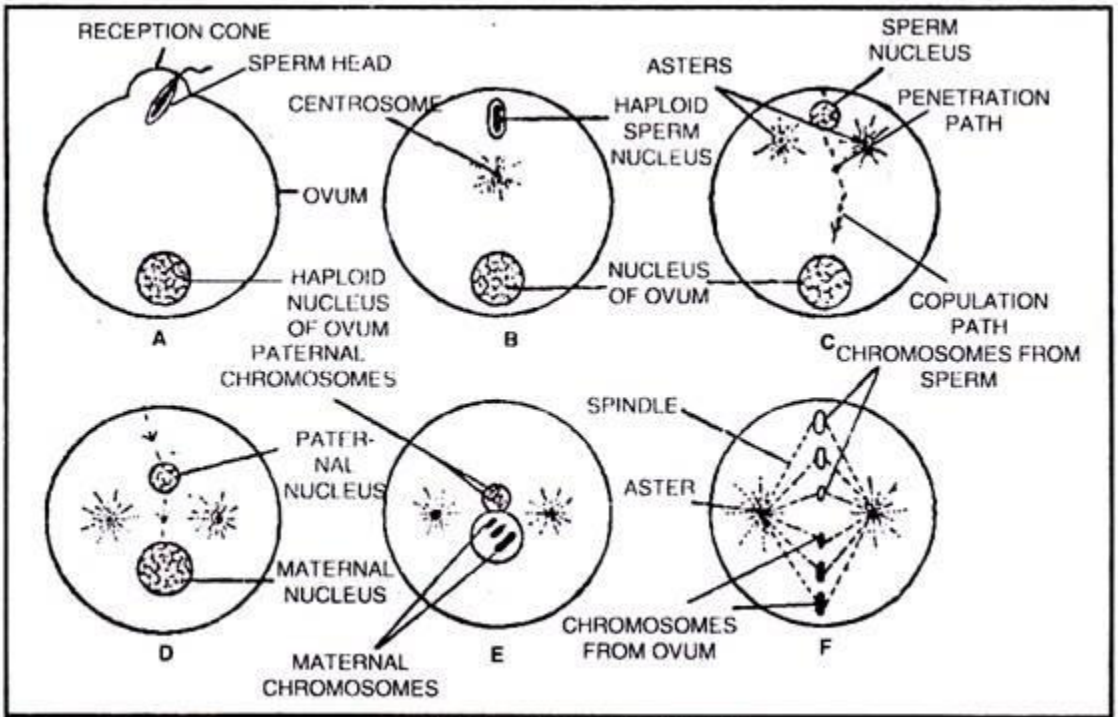
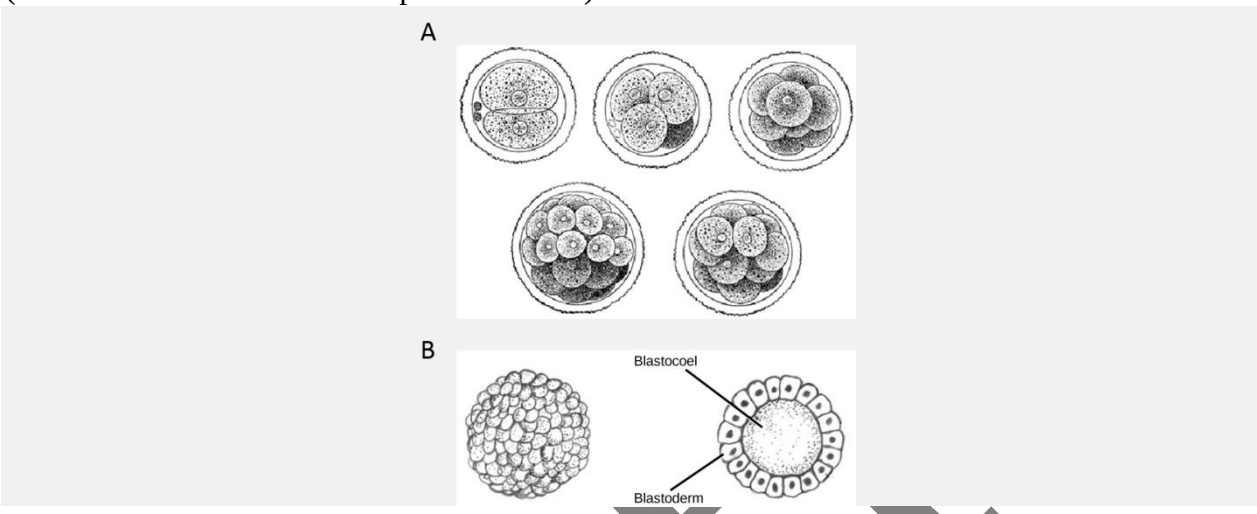
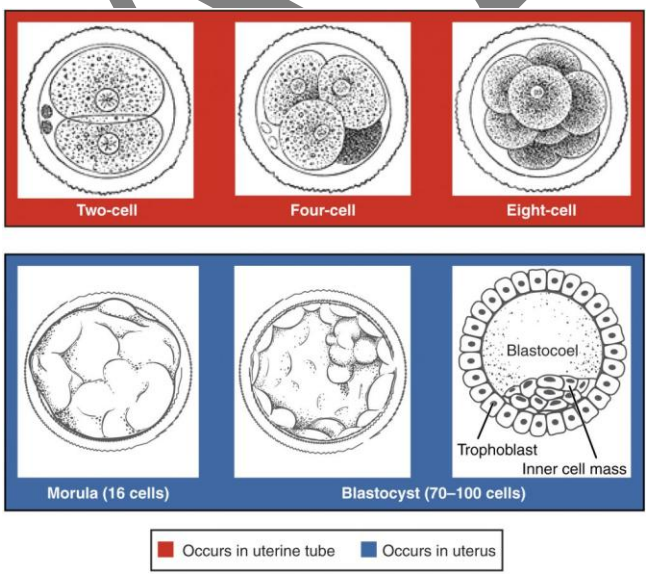


Fig. 3(B).9 Process of fertilization

At the end of the fourth day, the embryo reaches the uterus. It looks like a mulberry and is known as morula. This solid ball like morula has thirty two cells. In human zygote the cleavage is radial (blastomeres are arranged in radial plane around the polar axis) and indeterminate type (fate of each blastomere is not predetermined).



(a) During cleavage, the zygote rapidly divides into multiple cells without increasing in size. (b) The cells rearrange themselves to form a hollow ball with a fluid-filled or yolk-filled cavity called the blastula



Pre-embryonic cleavages make use of the abundant cytoplasm of the fertilized egg as the cells rapidly divide without changing the total volume.Â The rearrangement of the cells in the mammalian blastula to two layers the inner cell mass and the trophoblastâ€"results in the formation of the blastocyst.

Capacitation:

During the early cleavage in mammals capacitation occurs. It occurs at 8-cell stage when the loosely attached blastomeres are held tightly due to production of proteins called cohesions on their surface.

Significance of Cleavage:

(i) It converts a unicellular zygote into a multicellular embryo.
(ii) It maintains the cell size and nucleo-cytoplasmic ratio of the species.
(iii) Cleavage produces large member of cells or blastomeres required for the building of offspring's body.
(iv) During cleavage quick mitotic division of blastomeres occurs following which there is no growth of blastomeres.
(v) Cleavage brings about the distribution of cytoplasm among the blastomeres.

Planes of Cleavage:

During early cleavage, distinct geometrical relationships exist between the blastomeres, i.e., each plane of cell-division bears a definite relationship with each other.

The planes of division are:

a. Meridional plane of cleavage:

When a furrow bisect both the poles of the egg passing through the median axis or centre of egg it is called meridional plane of cleavage. The median axis runs between the centre of animal pole and vegetal pole.

b. Vertical plane of cleavage:

When a furrow passes in any direction (does not pass through the median axis) from the animal pole towards the opposite pole.

c. Equatorial plane of cleavage:

This type of cleavage plane divides the egg halfway between the animal and vegetal poles and the line of division runs at right angle to the median axis.

d. Latitudinal plane of cleavage:

This is almost similar to the equatorial plane of cleavage, but the furrow runs through the cytoplasm on either side of the equatorial plane.

Types of Cleavage:

Considerable amount of reorganisation occurs during the period of cleavage and the types of cleavage depend largely upon the cytoplasmic contents.

Different types of cleavage encountered in different eggs are catalogued below:

a. Holoblastic op total cleavage:

When the cleavage furrows divide the entire egg.

It may be:

Equal:

When the cleavage furrow cuts the egg into two equal cells. It may be radially symmetrical, bilaterally, symmetrical, spirally symmetrical or irregular.

Unequal:

When the resultant blastomeres become unequal ir size.

b. Meroblastic cleavage:

When segmentation takes place only in a small portion of the egg resulting in the formation of blastoderm, it is called meroblastic cleavage. Usually the blastoderm is present in the animal pole and the vegetal pole becomes laden with yolk which remains in an tihcleaved state, i.e., the plane of division does not reach the periphery of blastoderm or blastodisc.

c. Transitional cleavage:

In many eggs, the cleavage is atypical which is neither typically holoblastic nor meroblastic, but assumes a transitional stage between the two.

Effects of Yolk in Cleavage:

The fertilized egg in most cases contains yolk, which are inert bodies. During division these bodies exert mechanical influences. In the egg of Amphioxus, the yolk is thin and remains uniformly distributed. Therefore the division is complete and early divisions occur at a very quicker rate.

The amphibian egg contains yolk which is localised at the vegetal pole. Here division initiates from the animal pole and extends towards the vegetal pole, where the progress of cleavage slows down considerably.

Consequently, the animal pole divides faster than the vegetal pole. The eggs of reptiles and birds are fully laden with large masses of yolk, thus restricting the cytoplasm and nucleus on the periphery as a circular disc on the animal pole. Here the lines of cleavage divide only the small animal pole region. Such effects of yolk on cleavage pattern influence the pattern of further development.

Mechanism of Cleavage:

The incidence of cleavage provides unique opportunity to study the mechanism of cell division and specially the role of different cell organelles during division.

Opinions differ regarding the accumulation of force for the initiation of cleavage and following factors are believed to be responsible for controlling the cleavages:

(a) Localised expansion of cortex.
(b) Increased stiffness of the cortical cytoplasm.
(c) Increase of tangential force activity in the cortex.
(d) Contractile nature of the regions near the cortex and
(e) Formation of new cell membrane from the subcortical cytoplasm.

Though the abovementioned factors are not clearly understood, it is evident that three structures present within the cell: Cortical layer, Spindle structures and Chromosomes play the important part.

The energy which is required during the process is supplied by the metabolic activity of the developing egg. Besides the factors involved in segmentation, there are cleavage laws which govern the behaviour of the cells during cleavage.

Sach's rules:
The blastomeres tend to divide into identical daughter cells and a cleavage furrow tends to cut the previous cell at right angles.

Hart wig's laws:
The position of nucleus is vital and it tends to lie at the centre of the protoplasmic content of the cell. The nucleus exerts influence on cleavage. The long axis of mitotic spindle usually coincides with the long axis of the protoplasmic content. During cleavage the long axis of the protoplasm has the tendency to cut transversely.

Balfour's law:
The rate of cleavage is inversely proportional to the amount of yolk material present in the egg.

Chemical Changes during Cleavage:
Significant chemical changes go on in the fertilized egg during cleavage.

They are:

Increase of nuclear material:
During cleavage a steady increase in nuclear material (predominantly DNA) is observed. Cytoplasm of the egg is the source of such nuclear material. Cytoplasmic DNA contained in mitochondria and yolk platelets are available.

RNA synthesis:
During cleavage messenger RNA (mRNA) and transfer RNA (tRNA) are synthesised during cleavage, especially in late stages.

Synthesis of proteins:
Throughout the period of cleavage there is steady and spectacular increase in protein synthesis.

Patterns of embryonic cleavage

Cleavage in Different Chordates:
The pattern of cleavage differs in different animals. The following account will give an idea of the process of cleavage in different chordates.

a. Amphioxus:
The cleavage in Amphioxus is typically holoblastic (Fig. 5.10). The first cleavage is meridional. The second cleavage is also meridional but at right angle to the first one. Four equal blastomeres are produced. The third cleavage is latitudinal and occurs slightly above the equatorial plane resulting in the production of eight blastomeres—four are smaller called the micromeres and four are larger known as the macromeres.

The micromeres are situated towards the animal pole and the macromeres towards the vegetal pole. The fourth cleavage is meridional which involves all the eight cells resulting in the formation of eight micromeres and eight macromeres. The fifth cleavage planes are latitudinal.

Each micromere is divided into an upper and lower micromere and each macromere likewise divides to form an upper and lower macromere. The fifth cleavage planes produce thirty-two blastomeres. The sixth cleavage planes are nearly meridional involving all the thirty-two cells resulting in sixty-four cells.

At the 64-cell stage a conspicuous space is produced at the centre and this space becomes filled with a fluid. When the eighth cleavage planes take place, the blastula becomes pear- shaped and the blastocoel becomes large.

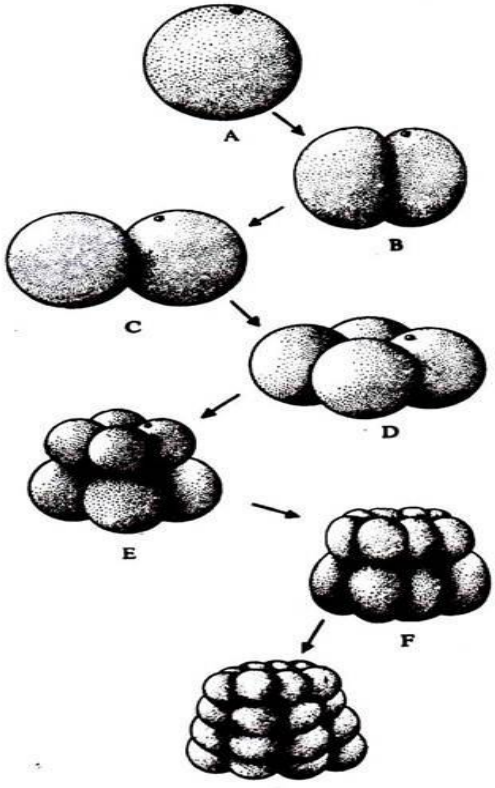


Fig. 5.10. Early cleavage pattern in the egg of Amphioxus. A. Fertilized egg. B–C. First cleavage. D. Second cleavage. E. Third cleavage. F. Fourth cleavage. G. Morula stage.

b. Frog:

The egg of frog is telolecithal with a considerable amount of yolk localized towards the vegetal pole. The cleavage is holoblastic in nature, but differs considerably from that of Amphioxus because of larger quantity of yolk.

The first cleavage plane is meridional which occurs at about 3-3½ hours after fertilization. But the time depends largely on extrinsic factors. The first cleavage starts at the animal pole and gradually travels towards the vegetal pole. Thus the egg is bisected along the poles. Two blastomeres of equal size are produced. The second cleavage is almost meridional but oriented at right angles to the first cleavage plane (Fig. 5.11).

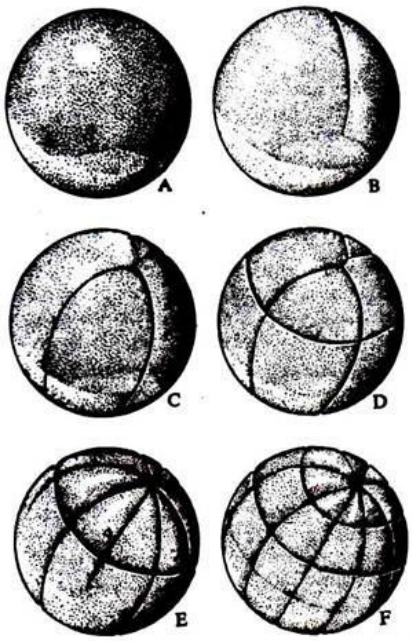


Fig. 5.11. Semidiagrammatic representation of the cleavage pattern in the egg of frog. A. Fertilized egg. B. First cleavage. C. Second cleavage. D. Third cleavage. E. Fourth cleavage. F. Morula stage.

The four blastomeres thus produced are not qualitatively identical, because the grey crescent material is present in two of the four blastomeres. Each blastomere contains dark pigment at the animal pole and yellowish yolk towards the vegetal pole. The third cleavage is latitudinal and occurs at right angles to previous cleavage planes but passes slightly above the equator.

The furrow produces eight unequal blastomeres, four micromeres in the animal hemisphere and four macromeres in the vegetal part. The fourth cleavage planes are meridional which involve the micromeres first and pass on slowly towards the yolk-laden macromeres of the vegetal pole.

In Amphioxus, the cleavages occur in a synchronous fashion, while in frog considerable degree of irregularities (asynchronism) appear in later stages. But it is certain that the micromeres always continue to divide at a faster rate than do the macromeres.

At the eight-celled stage, a small space makes its appearance between the four micromeres. As development goes on, this space becomes conspicuous and forms the blastocoel. The floor of the blastocoel is formed of macromeres. The blastocoel (or segmentation cavity) is eccentrically located and becomes displaced towards the animal pole as development proceeds.

c. Chick:

Typical meroblastic cleavage occurs in chick, where the segmentation activity is restricted only at the blastodisc or germinal disc (Fig. 5.12). Thus the cleavage is incomplete.

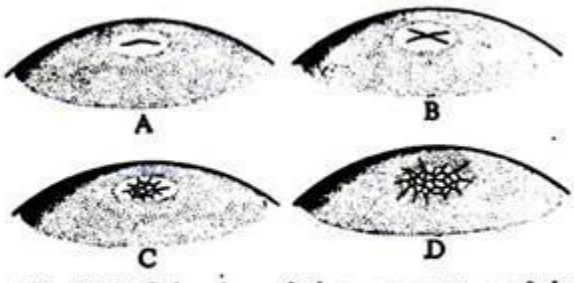


Fig. 5.12. Side view of cleavage pattern of the egg of chick. A. First cleavage. B. Second cleavage. C–D. Subsequent cleavages.

The first cleavage starts as a meridional furrow near the centre of the blastodisc at about 4½ hours after fertilization when the egg reaches the isthmus of oviduct. This furrow cuts across the blastodisc and passes towards the vegetal pole but does not reach the pole. The second cleavage is also meridional, but approximately at right angles to the first one. The third cleavage is vertical.

The fourth cleavage is also vertical but the division is not synchronous. As a consequence eight central cells encircled by twelve marginal cells are produced. From this point onward the cleavage becomes irregular and a disc containing smaller cells appears.

This disc remains firmly connected with the underlying yolk. Soon a cleft appears which separates the disc in the middle from the underlying yolk. The new cavity in between is known as sub-germinal space (Fig. 5.13).

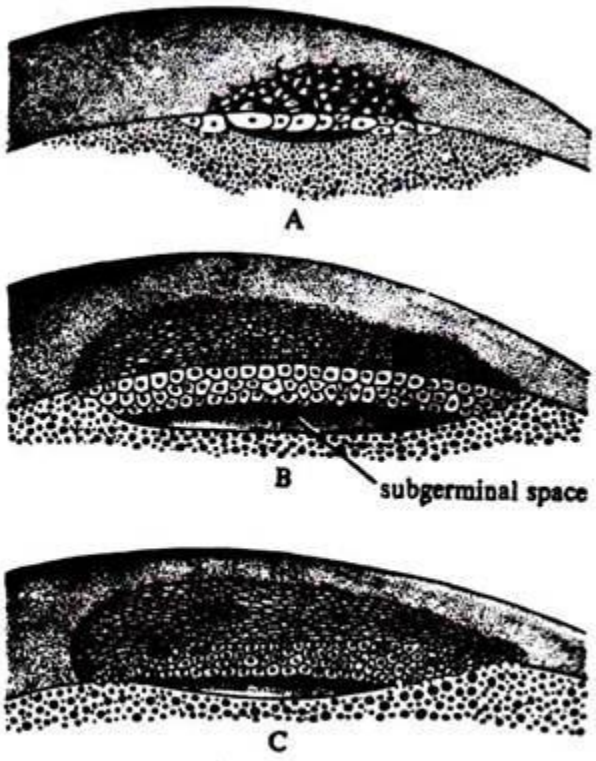


Fig. 5.13. Sectional view of chick blastula showing the formation (A–C) of subgerminal space (after Huettner).

Thus at the end of segmentation, the disc contains many-layered small cells which are connected with the yolk only at the periphery. This disc is then termed as blastoderm, the cells of which still continue to divide.

The peripheral part which lies in contact with yolk possesses granular cells called area apaca and the inner layer having clear portion is called area pellucida. At one end of area opaca, aggregation of cells takes place. This denotes the formation of future posterior side.

d. Rabbit:

The egg of rabbit is small and does not contain any yolk (i.e. alecithal type of egg). The cleavage is holoblastic and nearly equal. Irregularities and a synchronism become the rule in the cleavage of rabbit like all other eutherian mammals.

The first cleavage is vertical resulting in the formation of two unequal blastomeres. The second cleavage is also vertical but runs at right angle to the first. The third cleavage is horizontal but slightly above the equator.

Subsequent divisions are rapid and irregular. The blastomeres thus produced become clustered together to form a solid cellular ball called morula. Two types of cells (small and large) are recognised in the morula.

The large cells lie at the centre. Soon a cavity appears inside the cell mass on one side. The cavity gradually increases which shifts the central cell mass to one side. The stage is called blastocyst stage. The inner cell mass in the centre is attached with the outer cell layer (trophoblast) of the blastocyst.

The cavity is called the blastocoel or sub-germinal cavity (Fig. 5.13A) which is filled with a fluid. The inner cell mass remains attached at the embryonic knob towards the animal pole. From this embryonic knob, the embryo arises. The trophoblast which encloses the blastocoel and the embryonic knob participates in the formation of placenta. The trophoblastic cells overlying the embryonic knob is called cells of Rauber.

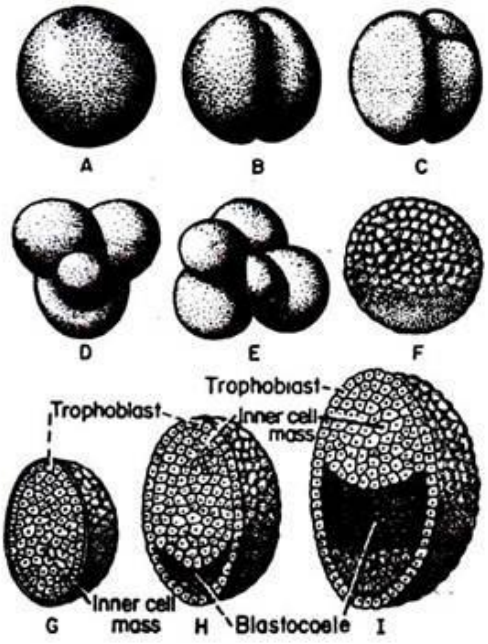


Fig. 5.13A. Showing the process of cleavage in Rabbit.
A=Zygote, B–E=Cleavage stages, F=Morula, G=Section of morula, H & I=Differentiation of inner cell mass and Trophoblast.

Importance of Cleavage in Embryonic Pattern

The cleavage phase of development and blastulation are extremely significant, because the blastoderm is morphologically elaborated in such a way that the important presumptive organ forming areas of the future embryo are segregated into definite districts of the blastoderm.

Such orientation of the organ forming areas in the blastoderm permits an ordered movement of these areas during gastrulation to take up their fateful position. So the period of cleavage and blastulation is regarded as the phase of preparation for future differentiation.

The cells which are produced at the end of segmentation resemble the zygote—but do they possess the same potentiality as the zygote itself. Driesch (1891), in order to get an answer,

separated the two blastomeres at the two-celled stage and found that both the blastomeres developed into complete embryos.

His conclusion was that each blastomere has the full potentiality to be an entire embryo. But in 1900, Roux showed that if one of the blastomeres of the two-celled stage is killed, the remaining one produces 'half embryo'.

He claimed that each cleavage results into the segregation of specialization in the blastomeres and this is irreversible. This experiment demonstrates that an organising or controlling centre is elaborated to control the development process.

The experiment of Spemann and others have shown that it is the grey crescent region which plays the vital role in the process of determination and the blastomeres which are formed due to segmentation are neither completely regulative nor irreversibly determined.

Fig. 5.14 shows the importance of grey crescent in the development of amphibian embryo. It has been experimentally established that the grey crescent in the amphibian blastula transforms into the dorsal lip of the blastopore which acts as an instigator and controller of the gastrulation process.

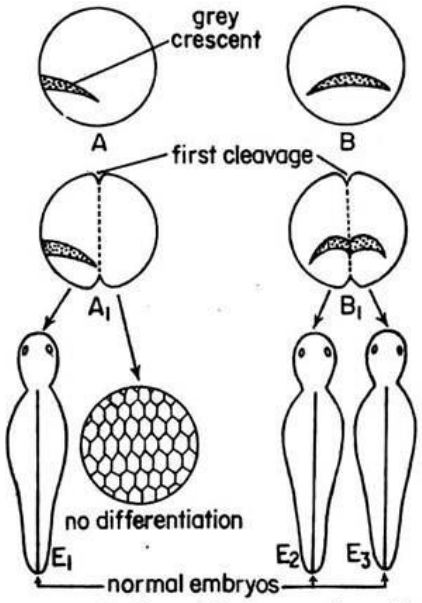


Fig. 5.14. Significance of grey crescent in amphibian development. A. Side view. B. Top view. A_1. Separation of two blastomeres, one with grey crescent and the other without grey crescent. The blastomere lacking the grey crescent fails to differentiate. B_1. Both the blastomeres contain grey crescent and develop into normal embryos. E_1–E_3 Normal embryos.

TABLE EMBRYOLOGY–2
Comparative account of cleavage in Amphioxus, Frog and Chick

Points	Amphioxus	Frog	Chick
1. Type of cleavage	1. Holoblastic	1. Holoblastic	1. Meroblastic
2. First cleavage plane	2. Meridional (Equal)	2. Meridional (Equal)	2. Meridional (Equal)
3. Second cleavage plane	3. Meridional, but at right angle to first one (Equal)	3. Same as Amphioxus	3. Same as Amphioxus
4. Third cleavage plane	4. Latitudinal, slightly above the equator of egg (unequal)	4. Same as Amphioxus	4. Vertical
5. Fourth cleavage plane	5. Meridional (Equal)	5. Meridional (Equal)	5. Vertical
6. Fifth cleavage plane	6. Latitudinal (unequal)	6. Irregular	6. Irregular
7. Sixth cleavage plane	7. Nearly meridional	7. Irregular	7. Irregular
8. Regularity in cleavage	8. Synchronism prevails upto sixth cleavage.	8. Asynchronism starts from fifth cleavage.	8. Asynchronism becomes the rule from the fifth cleavage
9. Blastula	9. Becomes pear-shaped with a large and spacious blastocoelic cavity. The wall is composed of one layer of cells.	9. Rounded and the blastocoelic cavity is located towards the animal pole. The epiblast has micromeres while the hypoblast has macromeres.	9. Cleavage is restricted to a round blastodisc, which is demarcated from the underlying yolk by subgerminal space. The blastodisc is divided into two zones—(*i*) area pellucida and (*ii*) area opaca.

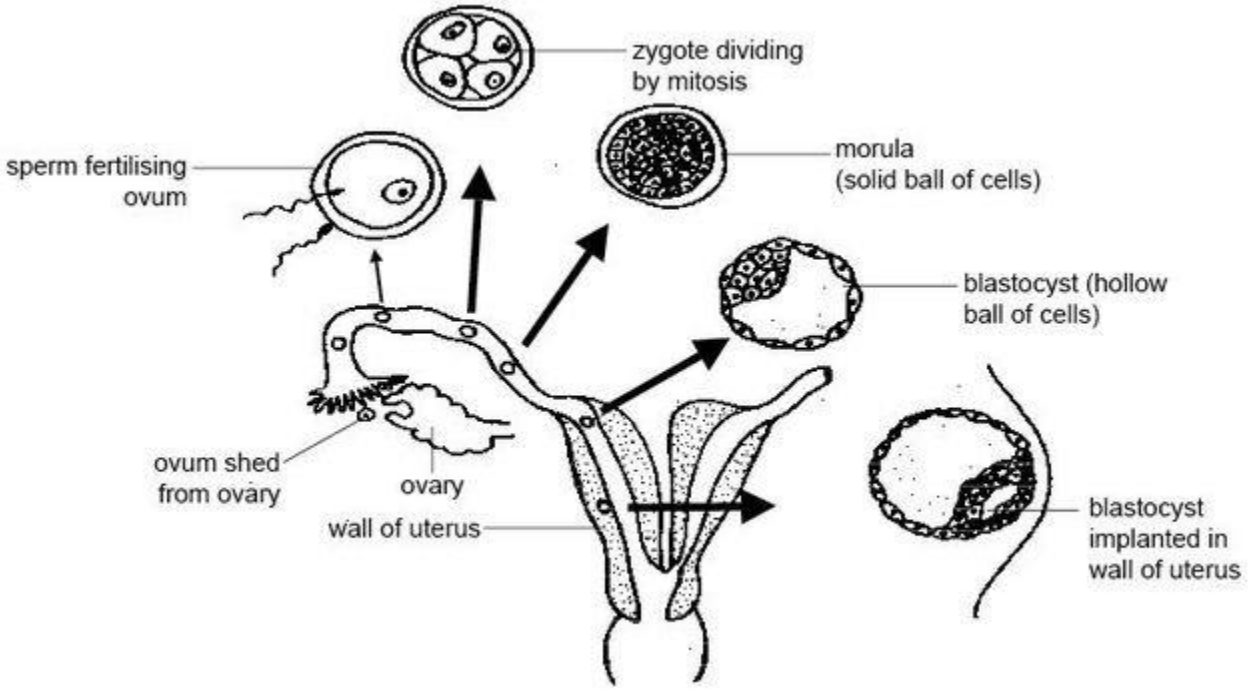
Morula and Blastula

The difference between morula and blastula is to do with the different developmental stages of the egg. Major developmental stages of egg after the fertilization are zygote, morula, blastula, and embryo. Fertilization is an important biological process resulting in the first embryonic stage, the zygote. Formation of morula and blastula are considered as the early embryonic developmental stages of animals. After the formation of the zygote, it transforms to the next stage, called the blastula. This transformation process is governed by a unique embryological biological process called **cleavage**. During the cleavage, series of mitotic divisions take place in zygote to produce daughter cells, which are genetically similar to their parent cell. These new daughter cells are now called **blastomeres**. With the time, morula differentiates into blastula,

which has a higher cell number and different structure. In this article, differences between morula and blastula will be outlined.

Morula:

Morula is a **ball-like mass of cells formed by the cleavage of the zygote**. Morula usually consists of 16 – 32 cells. The first cleavage in human zygote occurs in the fallopian tube, about 30 hours after fertilization. Second and third cleavage take place about 60 hours and 72 hours after fertilization respectively. Cleavage increases the number of cells, but not result in growth. Thus, morula has the same size as the zygote. As a result of subsequent cleavage division, morula forms into a centrally located inner cell mass and a surrounding layer, the outer cell mass. During the embryonic development, the inner cell mass forms the tissues of the embryo while the outer cell mass gives rise the **trophoblast**, which lately develops into the placenta. Morula reaches the uterus within 4-6 days after fertilization.



Blastula:

Once the morula is formed, the trophoblast cells in the center of the morula start to secrete fluid into the center of the morula forming a fluid-filled space, called **blastocoel**. Now the embryo resembles **a hollow ball-like structure** known as the blastula. Blastocoel is surrounded by single cell layer known as trophoblast or **trophectoderm**. Blastula is present in all vertebrates during their embryonic development. However, in mammalian species, blastula consists of an inner cell mass on the inner surface on one side of the blastula, whereas no such inner cell mass is found in non-mammalian species. The face of blastocyst, where inner cell mass is attached is known as **embryonic pole** or **animal pole**, whereas the opposite side is called **abembryonic pole**.

During the development of blastula, zona pellucida starts to disintegrate, which enhances the growth of the embryo.

Difference between Morula and Blastula

• During the embryonic development, zygote transforms to morula and then morula transforms to blastula.
• The cells inside the morula are larger than the cells that form blastula.
• The number of cells in blastula is larger than that in morula.
• Morula is a solid structure with no fluid-filled cavity inside. But blastula is a hollow structure, due to the presence of fluid-filled space called blastocoel.
• Trophoblast cells are present in blastula unlike in morula.
• Unlike in blastula, morula consists of an inner and outer cell masses.
• Duration of the formation of morula is lower than the formation of the blastula .

Morphogenetic gradients

Morphogenesis refers to all those processes by which parts of a developing system come to have a definite shape or to occupy particular relative positions in space. It may be regarded as the architecture of development. Morphogenetic processes involve the movement of parts of the developing system from one place to another in space, and therefore involve the action of physical forces, in contrast to processes of differentiation (see below), which require only chemical operations. Although in practice the physical and chemical processes of development normally proceed in close connection, for purposes of discussion it is often convenient to make an artificial separation between them.

There is an enormous variety of different kinds of structures within living organisms. They occur at all levels of size, from an elephant's trunk to organelles within a cell, visible only with the electron microscope. There is still no satisfactory classification of the great range of processes by which these structures are brought into being. The following paragraphs constitute a tentative categorization that seems appropriate for the present state of biological thought on this topic.

Morphogenesis by differential growth

After their initiation, the various organs and regions of an organism may increase in size at different rates. Such processes of differential growth will change the overall shape of the body in which they occur. Processes of this kind take place very commonly in animals, particularly in the later stages of development. They are of major importance in the morphogenesis of plants, where the overall shape of the plant, the shape of individual leaves, and so on, depends primarily on the rates of growth of such component elements as the stems, the lateral shoots, and the vein and intervein material in leaves. In both animals and plants, such growth processes are greatly influenced by a variety of hormones. It is probable that factors internal to individual cells also always play a role.

Although differential growth may produce striking alterations in the general shape of organisms, these effects should probably be considered as somewhat superficial, since they only modify a basic pattern laid down by other processes. In a plant, for instance, the fundamental pattern is determined by the arrangement of the lateral buds around the central growing stem; whether

these buds then grow fast or slowly relative to the stem is a secondary matter, however striking its results may be.

Morphogenetic fields

Many fundamental processes of pattern formation (*e.g.*, the arrangement of lateral buds in growing plants) occur within areas or three-dimensional masses of tissue that show no obvious indications of where the various elements in the pattern will arise until they actually appear. Such masses of tissue, in which a pattern appears, have been spoken of as "fields." This word was originally used in the early years of the 20th century by German authors who suggested an analogy between biological morphogenetic fields and such physical entities as magnetic or electromagnetic fields. The biological field is a description, but not an explanation, of the way in which the developing system behaves. The system develops as though each cell or subunit within it possessed "positional information" that specifies its location within the field and a set of instructions that lays down the developmental behaviour appropriate to each position.

There have been several attempts to account for the nature of the positional information and of the corresponding instructions. The oldest and best known of these is the gradient hypothesis. In many fields there is some region that is in some way "dominant," so that the field appears as though organized around it. It is suggested that this region has a high concentration of some substance or activity, which falls off in a graded way throughout the rest of the field. The main deficiency of the hypothesis is that no one has yet succeeded in identifying satisfactorily the variables distributed in the gradients. Attempts to suppose that they are gradients of metabolic activity have, on investigation, always run into difficulties that can only be solved by defining metabolic activity in terms that reduce the hypothesis to a circular one in which metabolic activity is defined as that which is distributed in the gradient.

Recently, a new suggestion has been advanced concerning position information. Most processes within cells normally involve negative feedback control systems. These systems have a tendency to oscillate, or fluctuate regularly. In fact, any aspect of cell metabolism may be basically oscillatory in character; the cycle of cell growth and division may be only one example of a much more widespread phenomenon. The substances involved in these oscillations are likely to include diffusible molecules capable of influencing the behaviour of nearby cells. It is easy to envisage the possibility that there might be localized regions with oscillations of higher frequency or greater amplitude that act as centres from which trains of waves are radiated in all directions. It has been suggested that positional information is specified in terms of differences in phase between two or more such trains of transmitted oscillations.

Certain types of field phenomenon may involve an amplification of stochastic(random) variations. In systems containing a number of substances, with certain suitable rates of reaction and diffusion, chance variation on either side of an initial condition of equilibrium may become amplified both in amplitude and in the area involved. In this way, the processes may give rise to a pattern of differentiated areas, distributed in arrangements that depend on the boundary conditions.

Morphogenesis by the self-assembly of units

Complex structures may arise from the interaction between units that have characteristics such that they can fit together in a certain way. This is particularly appropriate for morphogenesis at the simple level of molecules or cells. Units such as the atoms of carbon, hydrogen, oxygen, nitrogen, and so on, can assemble themselves into orderly molecular structures, and larger molecules, such as those of tropocollagen, or protein subunits in general, can assemble themselves into complexes whose structure is dependent on localized and directional intermolecular forces. It seems that such comparatively large entities as the units that come together to form the head structures of bacteriophages or bacterial flagella are capable of orderly self-assembly, but the chemical forces that give rise to the interunit bonds are still little understood.

Processes that fall into the same general category as self-assembly may occur within aggregates of cells. The units that self-assemble are the cells themselves. Interaction and aggregation may be allowed to occur in assemblages of cells of one or more different kinds. In such cases it is commonly found that the originally isolated cells tend to adhere to one another, at first more or less at random and independently of their character, but later they become rearranged into a number of regions consisting of cells of a single kind. When the cells in the initial collection differ in two different characteristics, for instance in species and organ of origin, the assortment in some cases brings together cells from the same organ, in other cases cells from the same species. Mixtures of chick and mouse cells, for instance, reassort themselves into groups derived from the same organ, whereas cells from two different species of amphibia sort out into groups from the same species more or less independently of organ type.

This morphogenetic process probably has only a restricted application to the formation of structures in normal development, in which only in a few tissues (*e.g.*, the connective system) do cells ever pass through a free stage in which they are not in intimate contact with other cells, and cells of different origin do not normally become intermingled so as to call for processes of reassortment. To explain normal morphogenetic processes of plants and animals one must look to the results that can be produced by the differential behaviour of cells that remain in constant close contact with one another. Several authors have shown how striking morphogenetic changes could be produced within a mass of cells that remain in contact, but that undergo changes in the intensity of adhesion between neighbouring cells, in the area of surface in the proportion to cell volume, and so on.

Fate map

A fate map is a diagram of an egg or blastula, indicating the fate of each cell or region, at a later stage of development. Fate maps are essential tool in most embryological experiments. They provide researchers with information on which portions of the embryo will normally become which larval or adult structure. The analysis of the fate of each blastomere after first and second cleavage is called cytogeny or cell lineage study.

Construction of Fate Map:

Fate map of different types of animals have been constructed by the following methods:

i. Observing Living Embryos:

In some invertebrates, the embryos being transparent and having relatively few daughter cells that remain close to one another, it has been possible to look through the microscope and trace the descendants of a particular cell to the organ they subsequently formed.

This type of study was performed by Edwin G. Conklin (1905) in the tunicate, Styela partita, where the different cells contain different pigments. As for example, the muscle-forming cells always have a yellow colour.

ii. Vital Dye Marking:

Most embryos, however, do not have the facilities (transparent, few cells, different colours etc.) as described above in Styela partita. It was in 1929 that Vogt was able to trace the fate of different areas of amphibian eggs by applying vital dyes. These vital dyes stain the cells without killing them.

iii. Radioactive Labelling and Fluorescent Dyes:

A variation of the dye marking technique is to make one area of the embryo radioactive. A donor embryo is taken and grown in a solution containing radioactive thymidine. This thymidine base is subsequently incorporated into the DNA of the dividing embryo.

A second embryo, acting as the host embryo, is grown under normal conditions. The region of interest is cut off from the host embryo and is replaced by a radioactive graft from the donor embryo. The cells that are radioactive will be the descendants of the cells of the graft, and are distinguished by autoradiography.

iv. Genetic Marking:

Radioactive and vital dye marking have their own drawbacks such as dilution over many cell divisions and the laborious preparation of slides. One permanent way of cell marking is to create mosaic embryos having different genetic constitutions. The best example of such a marking is to graft quail cells inside a chick embryo. By doing so, fine-structure maps of the chick brain and skeletal system can be made.

Fate Map of Vertebrates:

i. Fate Map of Amphioxus:

The fate map of Amphioxus can be traced at an early stage prior to the onset of cleavage. The presumptive organ forming areas in the un-cleaved egg is given in Fig. 5.32. The future endodermal cells lie at the vegetal pole and would subsequently form the floor or hypoblast of the blastula. The area at the animal pole would form the presumptive ectodermal cells.

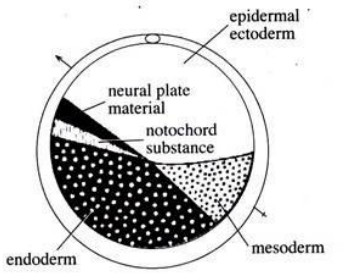


Fig. 5.32 : Presumptive organ forming areas in the uncleaved egg of *Amphioxus*.

The ventral grey crescent area at the future posterior end of the blastula, in between the future ectoderm and endoderm, forms the future mesoderm. Another area, the dorsal crescent, in between the ectoderm and endoderm on the anterior side, gives rise to the notochord and neural cells. The presumptive ectodermal, mesodermal, notochordal and neural cells would subsequently form the epiblast of the blastula.

ii. Fate Map of Frog:

The blastula of Xenopus at the 32 cell stage gives no indication as to how the different regions will develop. However, by following the fate of individual cell, or group of cells, the fate map of the blastula can be made. One way of making the fate map is by staining the various parts of the early embryo with a lipophilic dye such as dil and observe where the labelled regions end up.

Another sophisticated way of labelling the blastomeres is by injection of high molecular weight molecules such as rhodamine-labelled dextran, which cannot pass through cell membrane and are, therefore, restricted to the injected cell and its progeny. These cells can be easily detected later, under a UV microscope.

The fate map of the Xenopus blastula (Fig. 5.33) shows the presence of yolky macromeres at the vegetal pole which gives rise to the endoderm. Depending upon the position of the blastopore, the endodermal area can be divided into the sub-blastoporal and supra-blastoporal endoderm.

The cells toward the animal pole gives rise to the ectoderm, which becomes further subdivided into epidermis and the future nervous tissue. The epidermal ectoderm forms at the ventral side of the animal hemisphere, while the neural ectoderm forms at the dorsal side. The mesoderm forms a belt-like region, known as the marginal zone, around the equator of the blastula.

The mesoderm becomes subdivided along the dorsoventral axis of the blastula. The most dorsal mesoderm gives rise to the notochord. From this ventrally, the- mesoderm is differentiated by the somites (which gives rise to muscle tissue), lateral plate (which contains heart and kidney mesoderm) and blood islands.

In Xenopus, a thin outer layer of presumptive endoderm overlies the presumptive mesoderm in the marginal zone (Fig. 5.33C).

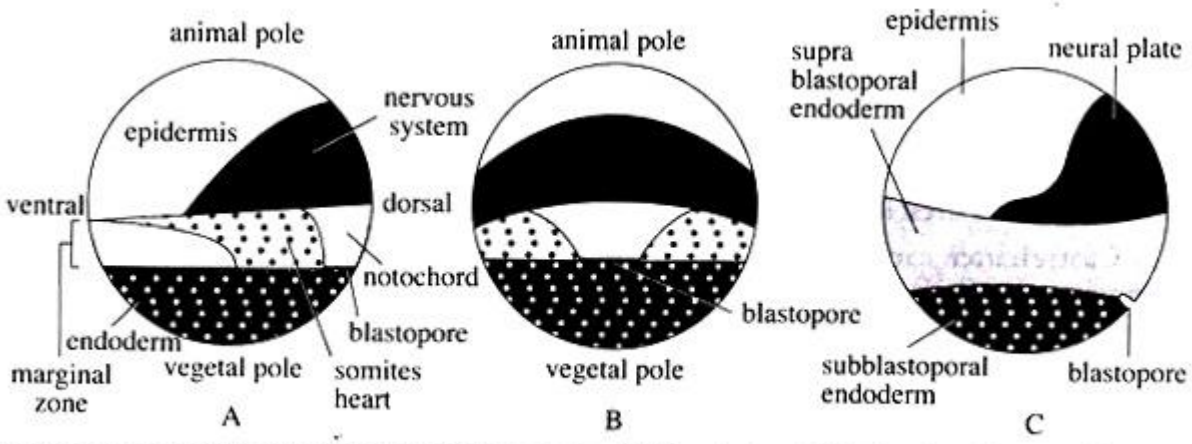


Fig. 5.33 : Fate map of *Xenopus* late blastula (A) Lateral view. (B) Dorsal view. (C) Exterior view showing a thin outer layer of presumptive endoderm overlies the presumptive mesoderm in the marginal zone.

iii. Fate Map of Chick:

Before going through the fate map of chick one should go through the formation of area pellucida and area opaca, and also through the format iod of hypoblast and epiblast. From the study of the above formations, it becomes clear that the hypoblast does not contribute any cells to the formation of the embryo proper, rather they contribute to the formation of a portion of the external membranes.

Recent studies with cell adhesion molecules (CAMs), it has become possible to construct the fate map of chick epiblast (Fig. 5.34). All the three germ layers of the embryo proper is formed by the epiblastic cells. The epiblast also forms a considerable amount of extra-embryonic (mesoderm) membrane.

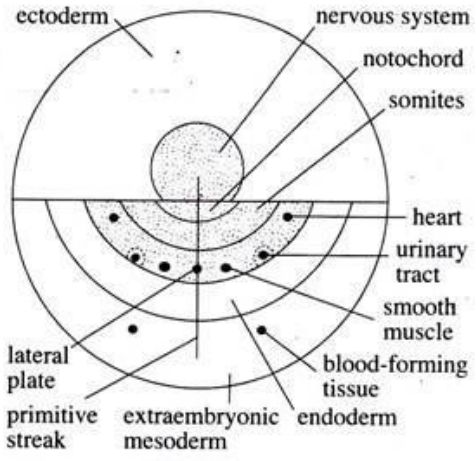


Fig. 5.34 : Fate map of chick embryo.

The fate map of chick (Fig. 5.34) reveals that the cells of the epiblast are organised around the notochord and nervous system. The neural ectoderm is present as a knob-like structure facing

towards the anterior side. The cells at the anterior part of the epiblast form the ectoderm, while the cells at the posterior side gives rise to mesoderm (body proper), endoderm and extra-embryonic mesoderm.

Usefulness of Fate Map:

The fate map of organisms is helpful in tracing the morphogenetic movements of the cells and the ultimate positions they take up. However, they tell us nothing about the tissue developmental potentialities during morphogenesis.

Gastrulation

The blastula passes into the stage called gastrula by the process—Gastrulation. This process is extremely important in the ontogenetic process of an animal, because the blue-print of the future organisation is laid down during this phase.

During this crucial and dynamic process major presumptive organ-forming areas of the blastula become reorganised in a fashion that allows their ready transformation into the fundamental body plan of a species. Gastrulation is essentially a process of migration of cells from one place to the other in the embryo. Besides movement of cells, considerable nuclear differentiation also takes place.

In almost all animals it results in:

(i) The establishment and differentiation of three primary germinal layers—ectoderm, mesoderm and endoderm,

(ii) The establishment of nuclear differentiation and

(iii) The beginning of the control of genetic factors over development.

2. Basic Mechanism in Gastrulation:

The process of gastrulation involves following three cellular activities, cell-movement, cell-contact and cell-division. All these mechanisms are carried in a nicely co-ordinated and integrated way.

Number of factors are believed to be responsible for this coordination, but it has not been possible to pin point the final answer. It is undeniable that this process is controlled largely by intrinsic factors which are correlated with the external as well as internal conditions.

3. Methods used to Study Gastrulation:

The correct observation of incidences during gastrulation was started from the findings of W. Vogt in 1923. Vogt used vital dyes (Janus green and Neutral red) to mark the cells in an early gastrula and noted that cells during gastrulation actually migrate from one place to the other.

The vital dye technique of Vogt resulted into the application of several other methods:

(i) Visible differences in the cytoplasmic particles were used as natural marker,

(ii) Taging of the cells with carbon particles and

(iii) Taging of the cells with radioactive substances.

4. Morphogenetic Movement of Cells in Gastrulation:

During gastrulation, cells from one region of embryo move to another to take up their future fateful position. Two terms, emboly and epiboly which are quite opposite in their meanings, are generally applied to explain the process of movement.

Emboly means the throwing in or insertion of cells and epiboly signifies the extending upon. The movement of cells establishes a particular form and involved in organ formation in embryo—so this movement is designated as the morphogenetic movement. Fig. 5.15 shows the movement of cells in gastrulation.

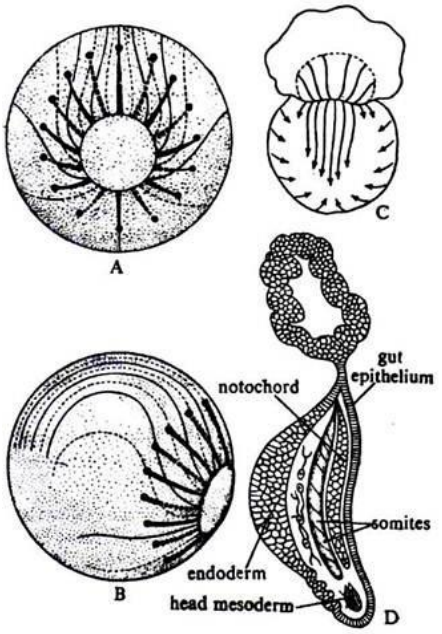


Fig. 5.15. Showing morphogenetic movement in amphibian gastrulation. A–B. Normal gastrulation. C–D. Exogastrulation. Note the failure of formation of nervous structures in the sectional view (D) of the embryo. Heavy lines indicate the movement of cells on the surface of the embryo while the thin lines show the movement inside the gastrula in A and B.

Fundamentally, the morphogenetic movement is similar but the details of the process vary greatly.

Following types of cells movement occur:

Epiboly:

It involves the extension along the anteroposterior axis and peripheral divergence.

Emboly:

The inward movement of cells is classified into different types depending on the behaviour of migrating cells.

These are:

(i) Invagination:

It denotes the infolding of a layer of cells to form a cavity encircled by infolded cells. Generally in the gastrulation of Amphioxus and frog, the wall of the blastoderm is pushed inside the blastocoel. This creates a new cavity called the archentecon which communicates with the exterior by a blastopore.

This process of inpushing goes on and the inpushed layer forms the walls of the cavity. The archenteron (or primitive gut) completely obliterates the blastocoel.

(ii) Involution:

It implies the inward lotation of cells as seen in the gastrulation of amphibian and avian eggs. From one end near the edge of the blastoderm, the cells begin to move inwards to form the inner lining of the blastoderm.

(iii) Convergence:

It means the movement of cells to a particular region of the gastrula. In amphibian egg, the migration of cells to the external edge of the blastoporal lip is designated as convergence. The same phenomenon of convergence of cells is seen in the formation of primitive streak in chick embryo.

(iv) Divergence:

This phenomenon is opposite to convergence, when involuted cells diverge to take up their future positions inside the gastrula.

(v) Infiltration:

During this process, cells of the blastoderm infiltrate near the bottom of the blastocoel to form a second layer as seen in the gastrulation of chick.

(vi) Delamination:

This is a process of separation of a group of cells from others to form discrete cellular masses.

(vii) Extension:

The elongation of presumptive areas after they have moved inside the embryo is called the extension.

(viii) Cell proliferation:

It means the increase in the number of cells during gastrulation.

(ix) Concrescence:

It is similar to convergence. The cells from two sides migrate anteriorly along one axis, but in convergence the cells from two sides unite together and then move anteriorly.

The above terms are coined for the convenience of analysing the events in gastrulation. Recent observations have established that it is essentially a phenomenon of integration. It was, therefore, felt necessary to understand the whole process for a meaningful comprehension of individual event.

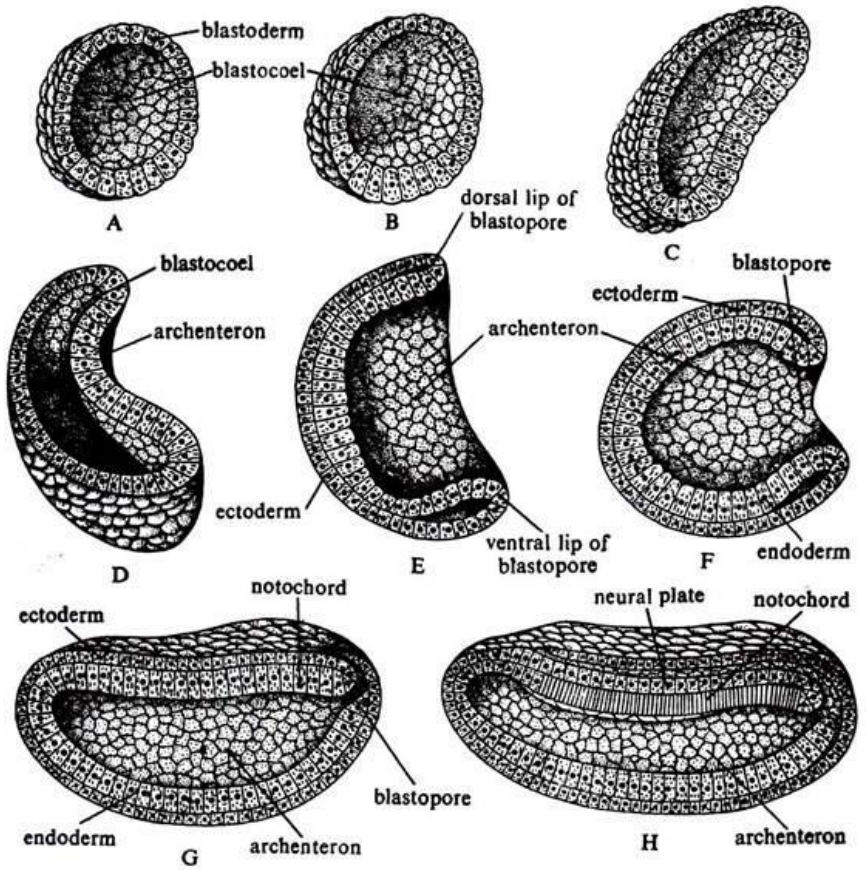


Fig. 5.16. Gastrulation in *Amphioxus*. A. Blastula. B–D. Beginning of invagination. E. The embryo becomes a double-walled cup with broad blastoporal opening. F. Constriction of blastopore. G–H. Completion of gastrulation. A–H. Sectional views.

5. Gastrulation in Different Chordates:

i. Amphioxus:

The blastula of Amphioxus contains the potential endodermal cells at the vegetal pole, i.e., hypoblast which forms the floor of the blastula. The presumptive organ forming cells (i.e., notochordal, mesodermal, epidermal, etc.), form the epiblast.

The epiblast constitutes the roof of blastula. The blastocoel is large. The dorsal crescent (presumptive neural and notochordal cells) lies in the future dorsal lip region of the blastopore while the ventral crescent (mesodermal area) occupies the ventral lip.

With the onset of gastrulation, an increase of mitotic activity is observed in the dorsal and ventral crescent regions. With the activity of the different cells, the endodermal plate invaginates into the blastocoel. During this process of invagination, the dorsal portion moves at a faster rate to touch a point which marks the anterior end of the developing embryo.

The notochordal cells, occupying the mid-dorsal region of the blastopore, involute and occupy a mid-dorsal position in the developing archenteron. Then the ventral crescents gradually converge

on either side of the notochordal cells. Thus the roof of the archenteron is composed of mesodermal and notochordal cells.

This process of eraboly is accompanied by epiboly when the ectodermal and neural cells extend along the antero-posterior direction. The extension of ectodermal cells and the proliferation, involution and infolding of presumptive endodermal, notochordal and mesodermal cells result in the formation of a double-layered embryo (Fig. 5.17). The external layer forms the ectoderm.

The internal layer has a dorsomedian area of notochordal cells with two bands of mesodermal cells. The rest of the inner layer is formed of endodermal cells. Rapid cell proliferation, accompanied by emboly and epiboly causes an anteroposterior elongation of the gastrula.

As the developing gastrula elongates in the anteroposterior direction, the ventral crescent is gradually shifted dorsalward along the inner lateral side of the blastoporal lip. As a result of convergence, the mesoderm comes to lie on the two sides of the notochordal material at the dorsal blastoporal lip.

At the end of gastrulation the blastopore becomes smaller and is closed by ectodermal overgrowth. A neuroenteric canal is formed between the archenteron and developing neural tube.

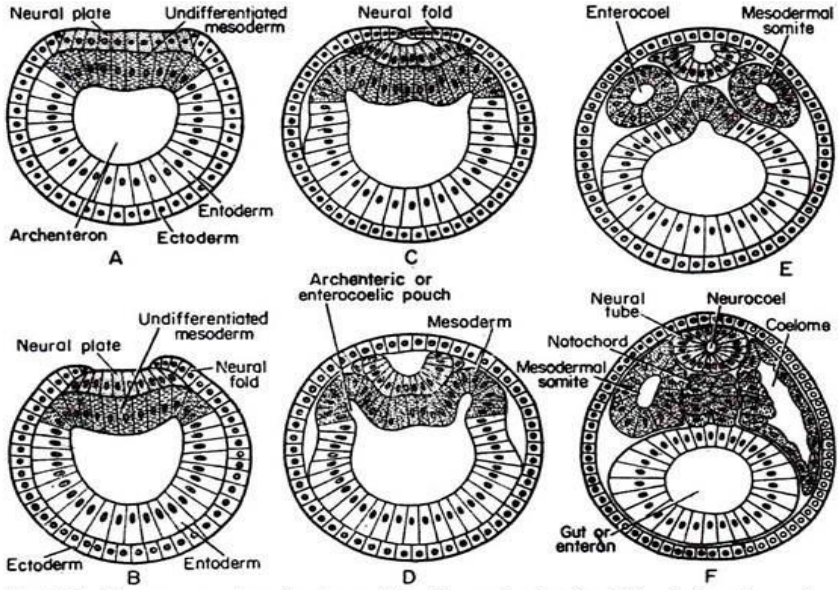


Fig. 5.17. Transverse sections of embryo of Amphioxus showing the differentiation of mesoderm. A–B. Gastrula stage. C–F. Post-gastrula stages. Note the early phase of formation of mesodermal somite on the left and coelome on the right side (after Huettner).

Mesoderm differentiation:

The transformation of the neural plate to form the neural tube is associated with the formative of a shallow groove on either dorsolateral walls of archenteron. The cells forming these two grooves are smaller than other cells. The grooves become deeper and their edges come together.

Such fusion results in the separation of a solid notochordal rod along the mid-dorsal line. These two lateral grooves become divided by transverse partitions into enterocoelic pouches which grow between the endoderm and ectoderm (Fig. 5.17).

The cavities of these pouches retain their connection with the archenteron at the beginning which become subsequently lost. As a consequence paired hollow blocks of mesodmal cells are formed. Formation of hollow mesodermal blocks is observed only in the first two pairs of somites.

The posterior entcrococlic pouches are pinched off as solid blocks of mesodermal cells within which coelomic cavities are formed anew. This process is observed upto fourteenth pairs of somites. In the rest of the posterior segments, the two halves of the original folds meet to form a solid band of cells extending up to the blastopore. The mesodermal somites differentiate from the lateral bands.

On the basis of origion the mesoderm is divided into:

(a) Gstral mesoderm and

(b) Pristomial mesoderm.

The gastral mesoderm develops from the enterocoelic pouches, while the peristomial mesoderm differentiates from the lateral bands.

The somites or segmental mesoderms gradually grow ventrally on either side until they meet in the midventral line below the alimentary canal. The mesodermal sheet becomes double-walled enclosing coelome within themselves. The lateral plate mesoderm becomes thus splitted into (i) somatic mesoderm in association with ectoderm and (ii) splanchnic mesoderm in association with the endoderm.

ii. Frog:

In late amphibian blastula, the presumptive organ forming areas are oriented around the blastocoelic cavity.

The hypoblast is situated at the vegetal pole, while the epiblast is located at the animal pole. In the epiblast the notochordal cells, neural plate and epidermal areas are situated along the anteroposterior axis of the blastula with the notochordal cells located at the most posterior position.

At the end of the cleavage all the blastomeres remain stationary and none of them have shiftecf from its original position. But at the onset of gastrulation a great mass migration started to occupy their definite position in the developing embryo. Gastrulation begins with the appearance of a small cleft-like invagination at one side and just above the grey crescent (Fig. 5.18).

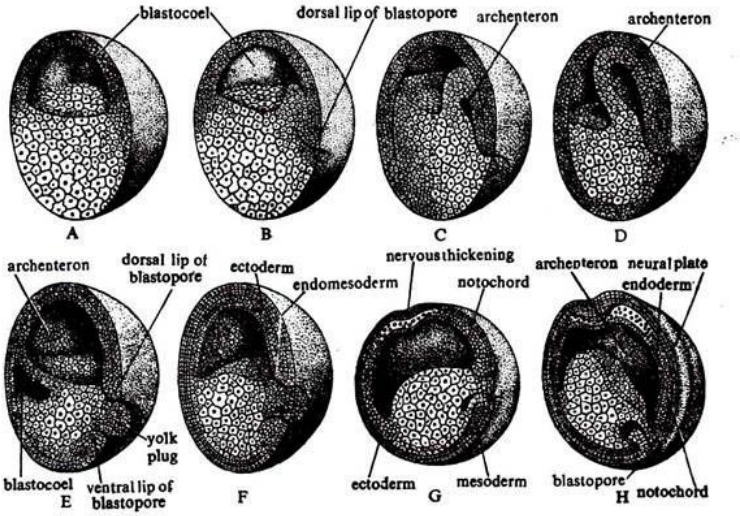


Fig. 5.18. Semidiagrammatic sectional views of the gastrulating embryo of Frog. (after Huettner). A. Blastula. B. Formation of blastopore. C–E. Enlargement of archenteron and gradual obliteration of blastocoel. F. Total obliteration of blastocoel and formation of endomesoderm. G–H. Completion of gastrulation showing the initiation of neural plate, notochord and differentiation of mesoderm as a middle layer.

This cleft-like invagination is crescent- shaped and represents the dorsal lip of the blastopore. As gastrulation progresses the crescent-shaped cleft continues to expand to assume a semicircular appearance, then becomes horse-shoe-shaped and finally forms a ring. This ring represents the blastopore. The blastopore becomes the focal point for gastrulation activities.

Migration of cells inside the gastrula starts along the newly-formed dorsal lip of blastopore and this inward pushing is caused by the endodermal cells which are folded inward (Fig. 5.19) and forward towards the future anterior end of the embryo. The upper margin of the blastopore is called the dorsal lip of the blastopore and the lower edge is designated as the ventral lip of the blastopore.

As invagination expands within the blastocoel, the prechordal plate cells from the upper part of the dorsal side move inward. The new cavity thus produced is called the archenteron which communicates to the exterior by the blastopore. With the further advancement of invagination, the archenteron continues to expand by obliterating the blastocoel.

The inward moving cells form a new border beneath the outer cells. The roof of the archenteron consists of the involuted layer which includes the endoderm and mesoderm. Beyond this layer lies the ectodermal layer. The floor of the archenteron is made up of a layer of endodermal cells, the derivatives of the large yolk cells which were located in vegetal hemisphere of blastula.

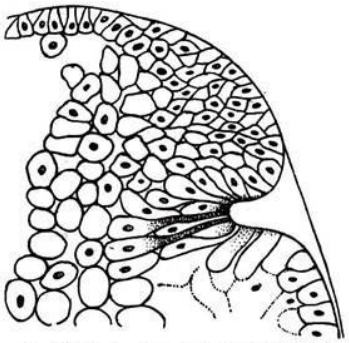


Fig. 5.19. Section through the blastoporal region of an early amphibian gastrula showing the streaming of blastoporal cells into the interior. As invagination begins, certain cells retain their attachment with the surface of the egg but become bottle-like in appearance.

When the inward movement of the cells is in progress through the dorsal lip, another type of movement occurs on the outer side. The pigmented cells of the animal hemisphere started to enclose the macromeres of vegetal hemisphere. After completing the enclosure, the outer cells reach up to the ventral lip of it.

A small mass of macromeres remains uncovered for a while and acts as a plug of the blastopore. It is called yolk plug. At this stage, embryo is made up of two distinct strata, each of which is composed of many layers of cells.

Differentiation of three primary germ-layers:

The blastula of frog is mono-layered which in course of gastrulation becomes converted into a triploblastic stage, i.e., three cell-layered. These three layers are designated as the primary germ-layers (embryonic ectoderm, embryonic mesoderm and embryonic endoderm). All the organs of the developing embryo develop from these three primary germ-layers.

(a) Ectoderm:

The pigmented cells of the animal pole, which spread to enclose the macromeres of the vegetal hemisphere become differentiated into ectoderm.

(b) Endoderm:

The dorsal and lateral sheets of cells which form the roof of the archenteron represent the endoderm as well as mesodermal material. Upon completion of gastrulation, the roof and sides of the archenteron become lined by a single layer of endodermal cells which have differentiated from the involuted several celled thick archenteron roof.

(c) Mesoderm:

As soon as the endodermal sheet becomes separated dorsally and laterally from the involuted cells, mesodermal sheet is being formed between the endoderm and ectoderm. The mesodermal sheet starts its differentiation anteriorly and then proceeds gradually backwards.

The mesodermal sheet is divided into two halves by a narrow band of median cells which develop into notochord. Laterally the mesodermal sheets grow downward and finally the right and left mesodermal sheets unite in the mid-ventral line to become a continuous mesodermal sheet.

The three layers thus formed are ectoderm, mesoderm and endoderm. It is the special feature in amphibian development that gastrulation results into the formation of mesoderm first and then the endoderm.

iii. Chick:

The blastoderm has a central area free from yolk which is called the area pellucida, while the germ-wall with the adhering yolk constitutes the area opaca. In course of development, the blastoderm becomes converted into a double-layered structure—the upper one is the epiblast and the lower layer is called the hypoblast.

The space between these two layers is called the blastocoel while the space below the hypoblast is the primordial archenteron.

The epiblast contains presumptive ectodermal and neural areas at the anterior portion while the posterior half comprises of presumptive notochordal and mesodermal cells. The hypoblast transforms into the endoderm and the epiblast is converted into ectoderm and mesoderm (Fig. 5.20).

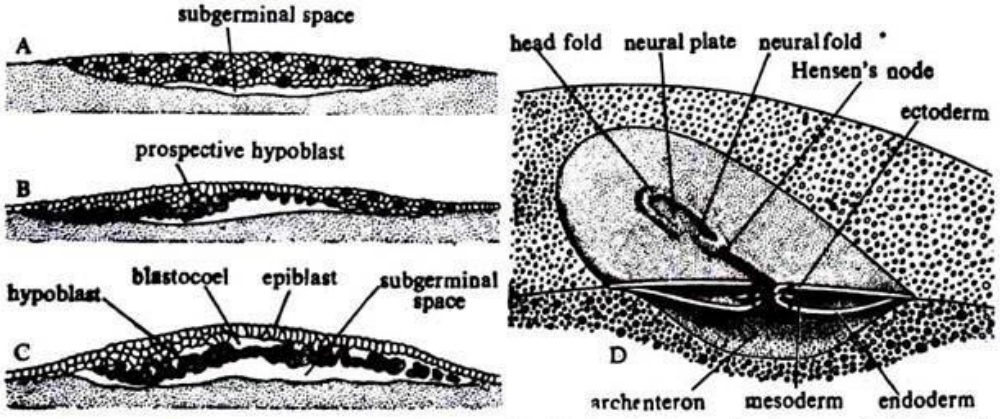


Fig. 5.20. Gastrulation in chick blastoderm. A–D. Sectional views. A. Large yolk-laden cells remain intermingled with smaller cells. B. The larger cells become concentrated in the subsurface position to form the prospective hypoblast. C. Separation of the hypoblast with the formation of blastocoel. D. Transverse section of primitive streak stage. Note the development of mesoderm.

At the initiation of gastrulation, the hypoblast cells from the posterior end start migrating towards the anterior end of the embryo along the median line. Immediately after the inauguration of the movement in the hypoblast, the cells of the epiblast overlying the migrating hypoblast move downward towards the hypoblast.

These involuted cells occupy a position between the epiblast and hypoblast and migrate to the lateral and anterior ends between the epiblast and hypoblast. Movement of cells in the blastoderm of chick during gastrulation has been studied by Spratt (1946) by carbon particle technique.

With the activities of the epiblast and hypoblast, the presumptive mesoderm cells from the posterior half of the epiblast move posteriorly and converge from the lateral sides towards the median line. These converging cells begin to accumulate at the posteromedian border of the area pellucida as a raphe-like thickened structure. This marks the appearance of the primitive streak (Fig. 5.21).

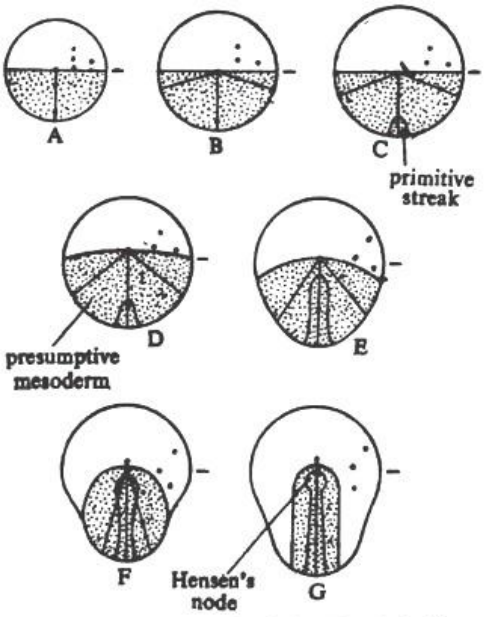


Fig. 5.21. Migration of cells (A–G) of the blastoderm to form the primitive streak in chick (Bodemer).

The migratory cells after coming to this region move inwards and migrate anteriorly and laterally. The migratory cells of the epiblast move downward and occupy the position between the epiblast and hypoblast. These cells then diverge anteriorly and laterally as a broad middle layer of mesodermal cells along the primitive streak.

Daring its forward movement, it approaches the presumptive notochordal areas. With the anterior movement the streak also starts to move backward. The primitive streak is fully formed at about 18-19 hours after incubation. Gradually the area pellucida changes from a round to pear-shaped appearance.

The primitive streak represents the posterior region of the developing embryo and the embryo proper develops anterior to it. It is also an area of cell proliferation arid rapid growth. The primitive streak becomes very conspicuous in early embryonic life. It consists of a groove (Primitive groove) which is flanked on both the sides by two ridges (Primitive ridges).

It terminates anteriorly in a primitive pit, and posteriorly in a primitive plate. Immediately anterior to the primitive pit (which represents the defunct neuroenteric canal) lies an elevation, Hensen's node or Head process.

In this area the mesoderm becomes thickened and projects from the primitive streak. With the formation of the Hensen's node, the primitive streak regresses posteriorly arid the major organ forming areas become well established! A groove appears on the outer surface of the head process and the two folds unite to form a tube.

The formation and closure of the groove continue posteriorly. The entire process may be compared with the action of a zipper. As die closure of the groove comes to the posterior end, the backward movement ceases leaving an opening at the posterior end.

In the gastrulation of chick, the mesoderm differentiates lastly from the epiblast by the process of involution, elongation, expansion and extension. The hypoblast gives rise to endoderm and the epiblast differentiates into ectoderm and mesoderm.

iv. Mammal:

In the mammalian blastula (Blastocyst) the formative area (germ disc) is restricted at one end. The germ disc is composed of epiblast and hypoblast. In the embryo of pig, the gastrulation activities are observed at two centres—the posterior end forms the primitive streak while the anterior end forms the Hensen's node.

The behaviour of these portions is almost similar to that observed in the gastrulation of chick. The mesodermal cells from the primitive streak move between the epiblast and hypoblast and form two wing-like areas.

The mesodermal cells are divided into:

(a) Embryonic mesoderm confined to the germ disc and
(b) Extra embryonic mesoderm.

6. Analysis of Gastrulation Mechanism:

Lillie (1913) established that the surface layer of the egg at first remains plastic but in course of development it loses its plasticity and becomes rigid. Spemann (1918) found that up to gastrulation, when the eggs are cut into two halves, each will form a complete embryo.

But after gastrulation, each half gives rise to half embryo. He also noticed that the half containing blastopole forms a complete embryo. He came to the conclusion that blastopore plays an important role in gastrulation.

Later he and Mangold (1924) grafted blastopore of one to the gastrula of another and demonstrated that the grafted blastopore influences the host tissue to form embryonic axis. They termed the blastopore as **"organiser"** and the influence of organiser as **"induction".**Spemann's lead was soon followed by different workers and considerable information became available regarding the nature of organiser.

It may be summarised that induction involves three distinct events:

(i) Evocation,
(ii) Individuation and
(iii) Competence.

The first two, evocation and individuation are the properties of organiser and the competence is the feature of the tissues on which the organiser acts.

In 1943, Holtfreter, working on the gastrulation mechanism of amphibian eggs, demonstrated that superficial cells are united by an extracellular surface coat and the beginning of invagination is due to the expansion of certain cells. This expansion according to him is caused by the change of surface tension due to the high pH of blastocoelic fluid.

Though many workers have questioned the findings of Holtfreter, it remained true that initiation of invagination is the property of localized cells. It may be due to local difference of pH or due to differential adhesiveness of the cells.

The work done to explore the nature of involution and epiboly also explained that the entire process is due to the nature of participating cells. It was demonstrated that the cells which are more adhesive are less mobile and on the contrary more mobile cells are less adhesive. Once this was understood attempts were made to explain the mechanism of gastrulation in terms of cellular adhesibility and cellular mobility.

In 1955, Townes and Holtfreter examined the interaction of different cell layers in amphibian gastrulae and demonstrated:

(a) That endoderm cells are less adhesive than mesoderm,
(b) That outer ectoderm is less adhesive than inner ectoderm and
(c) Mesoderm is less adhesive than inner ectoderm but more adhesive than endoderm.

Basing on this contention Stainberg (1964) proposed that disposition of different layers in a gastrula depends upon the adhesive nature of the cells. Outer ectoderm being less adhesive stays outermost. Mesoderm being less adhesive than inner ectoderm but more adhesive than endoderm remains in between the two.

Primary and rudimentary organ

An organ that has lost its basicsignificance in the process of the historical developmentof an org anism. Rudimentary organs form duringembryonic development but do not develop completely. Unlike larval organs, which are present only in embryos,rudimentary organs are retained for the entire lifetime ofan organism. Rudimentary organs were normallydeveloped organs in the ancest ors of any givenorganism, although in future generations their functionsare severely weakened or lost. In the latter case, theorgans can apparently perform some other function.

Examples of rudimentary organs in animals include thefibula in birds, the eyes in some cave and fossorialanimals, including Proteidae, mole rats, and moles, andthe remains of the hairy covering and the pelvic bonesin some whales. Rudimentary organs in man include thecoccygeal vertebrae , the hairy covering of the torso, theear muscles, the vermiform process of the cecum(appendix), and Morgagni's ventricles.

Sometimes rudimentary organs develop completely, forexample, the posterior pair of fins in wha les. There havealso been cases of humans being born with haircompletely covering their bodies a nd faces.Rudimentary organs assist in establishing the course ofphylogeny. Darwin believed that the existence ofrudimentary organs was one of the proofs that mandescended from animals.

Organizer

The effect of embryonic interaction or organizer is a morphogenetic effect by which one organic tissue transmits a chemical substance that influences other embryonic part to produce a structure that otherwise could not come into existence. The embryonic tissue which exerts such an influence is called an inductor and the chemical substance secreted by an inductor is known as evocators. The tissue on which evocator works and the tissue responses is known as responsive tissue. The action of the indicator through evocator is known as induction action or organizer

action. This process of induction influences greatly the protein synthesis mechanism of responsive tissues as a result of which definite structure forming cells become very active.

Origin of the concept of the organizer

Spemann's experiment (1924): A German embryologist Hans Spemann and his student Hilde Mangold (1924) performed transplantation experiment on a newt *Triturus cristatus,* an Urodela of class Amphibia. Spemann grafted a piece taken from the dorsal lip of early gastrula of *Rana* sp. to the lateral lip region of the early gastrula of *Triturus cristatus*. The embryo of *Rana* sp. is donor and the embryo of *Triturus* is the host. They observed that the cells of the grafted piece enter into the gastrula and form notochord and somites. In this embryo its own dorsal lip of blastopore forms neural groove, notochord, mesoderm etc. Similarly the grafted tissue influences to form notochord, neural groove and mesoderm. That is in the same embryo double set of notochord, nerve cord and mesoderm are produced. In this case donor tissue has secreted some chemical substances which has induced to form neural groove, notochord etc. in the host embryo. The donor tissue had pigments and the induced neural groove has also coloured pigments. After the completion of the gastrulation they observed that a larva has developed with two heads. One head is due to normal development and the other head production has been induced by donor tissue.

They examined the larva under the microscope and found that notochord, renal tubules, gut etc. have been formed by the tissue of the host embryo as a secondary set. If the donor tissue would not have been grafted such secondary structures wound not develop. From this experiment they concluded that dorsal lip of the donor had influenced greatly the tissue and thus has brought about change in the host tissue development. If it is not the fact then how a head had developed in the abdomen of the host. This secondary head formation is due to induction effect of donor tissue. This process of influencing other tissue was termed as induction by Spemann and the tissue that induced the tissue was known as the inductor or organizer.

Primary organizer:

Spemann continued his grafting experiments taking tissues from different zones of the gastrula and observed that except dorsal lip of the early gastrula other zone of tissue can not create any induction effect but when dorsal lip is grafted a complete embryo is formed. He named the dorsal lip as organizer as this dorsal lip organizes the developmental process of the embryo. According to him this dorsal lip induces to form neural tube and the neural tube then induces to form the eyes. The dorsal lip is composed of chorda-mesoderm and as it primarily acts as inducer so he named the dorsal lip or chordamesoderm as primary organizers.

Secondary, tertirary and quaternary organizers:

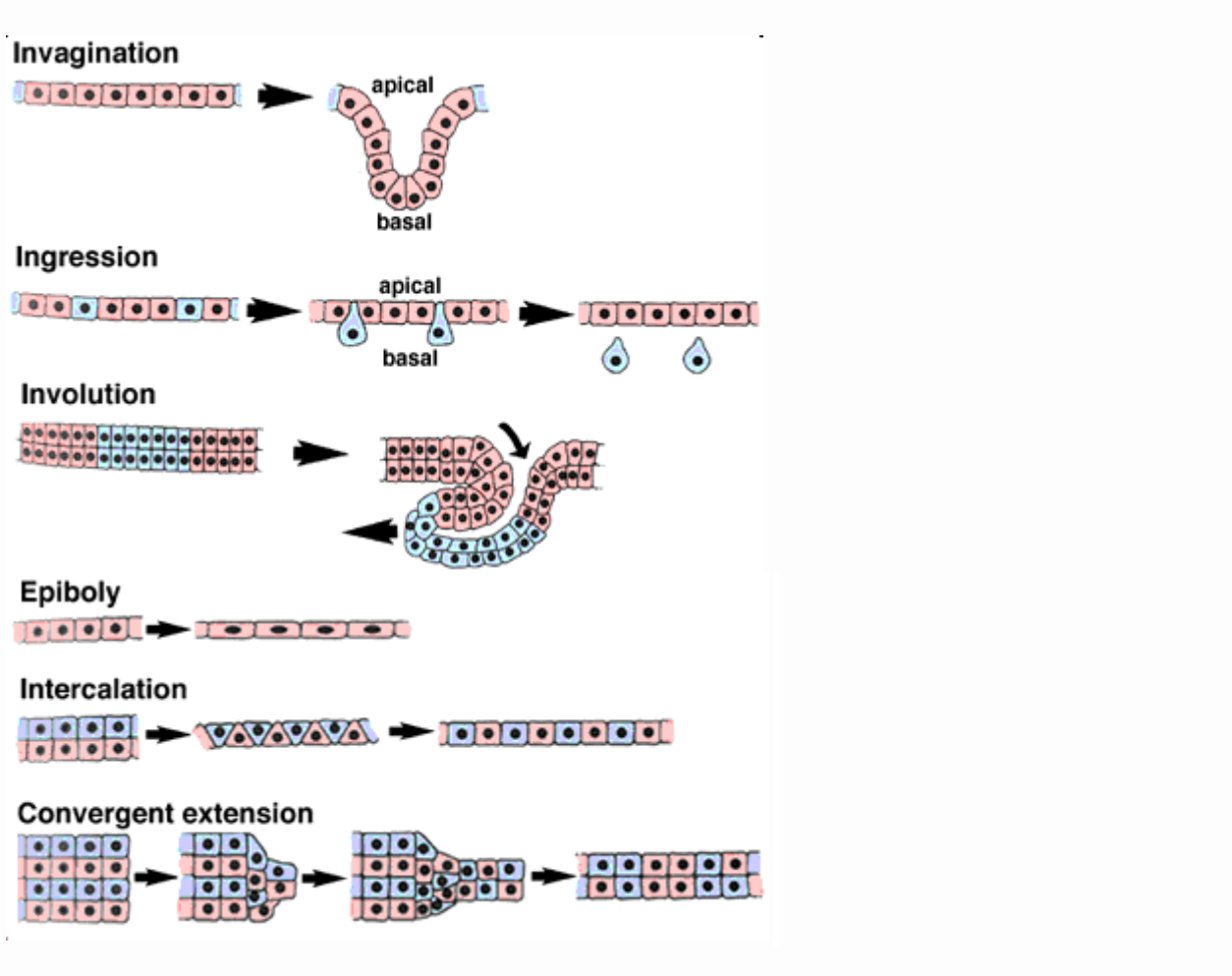
As the gastrulation proceeds due to primary organizer's induction primary organs begin to form and the early stages of organ development are known as organ rudiments. These organ rudiments themselves may act as organizer and then they are known as secondary organizer. Tissues formed by the action of secondary organizer may in turn induce further development. Then they are known as tertiary organizer. These successive stages of organizer activities start from the primary organizer.

How these organizers act in succession can clearly be understood from the examples of the development of eye in amphibian, chick etc. First of all due to induction effect of the primary organizer forebrain and within the forebrain eye forming cells are produced. These cells push out as a vesicle outside the forebrain. These vesicles are known as optic vesicle. This vesicle grows through the lateral mesenchyme and reaches the epidermis.

As soon as the vesicle comes in contact with the epidermis the outer layer of the vesicle invaginates to form a double layered optic cup. The inner layer of the optic cup is formed of sensory cells and the outer layer is formed of pigmented cells. They two together form the retina. The chemical substances secreted by the optic cup induce to form the lens between the optic cup and the epidermis. The peculiar thing is that if the optic vesicle is prevented from coming in contact with the epidermis there will be no lens formation. So the optic cup acts as secondary organizer. Similarly lens and retina together induce to form cornea so lens and retina together act as tertiary organizer and so on.

Morphogenetic movements

It is helpful to break the movements of gastrulation down into their component events wherever possible. In general, sheets of cells can engage in only a limited number of morphogenetic movements. This "morphogenetic repertoire" is helpful to keep in mind when we are presented with what seems to be an incomprehensible change in the shape of the embryo. Through careful observation and experimental manipulation, gastrulation can be analyzed in convenient organisms such as sea urchins. On this page, the various major morphogenetic movements that occur during gastrulation in diverse organisms are schematically represented. Some of these movements are only performed by epithelial cells, while others can be performed by both bona fide epithelial cells and by deeper, non-epithelial cells that nevertheless behave as integrated sheets of cells. The latter are poorly understood, but are common in amphibians as well as in higher vertebrates.



Invagination

During invagination, an epithelial sheet bends inward to form an inpocketing. One way to think of this in three dimensions is to imagine that you are poking a partially deflated beach ball inward with your finger. The resulting bulge or tube is an invagination. If the apical side of the epithelium forms the lumen (central empty space) of the tube, then the movement is termed invagination. If the lumen is formed by basal surfaces, then the movement is termed an evagination.

Ingression

During ingression, cells leave an epithellial sheet by transforming from well-behaved epithellial cells into freely migrating mesenchyme cells. To do so, they must presumably alter their cellular architecture, alter their program of motility, and alter their adhesive relationship(s) to the surrounding cells. Primary mesenchyme cells are an example of a mesenchymal cell type that emigrates out of an epithelium.

Involution

During involution, a tissue sheet rolls inward to form an underlying layer via bulk movement of tissue. One helpful image here is of a tank tread or conveyor belt. As material moves in from the edges of the sheet, material originally at the sites of inward rolling (shown in blue here) is free to move further up underneath the exterior tissue.

Epiboly

During epiboly, a sheet of cells spreads by thinning. i.e., the sheet thins, while its overall surface area increases in the other two directions. Epiboly can involve a monolayer (i.e. a sheet of cells one cell layer thick), in which case the individual cells must undergo a change in shape. In other cases, however, a sheet that has several cell layer can thin by changes in position of its cells. In this case, epiboly occurs via intercalation.

Intercalation

During intercalation, two or more rows of cells move between one another, creating an array of cells that is longer (in one or more dimensions) but thinner. The overall change in shape of the tissue results from cell rearrangement. Intercalation can be a powerful means of expanding a tissue sheet. A specialized form of intercalation is convergent extension.

Convergent Extension

During convergent extension, two or more rows of cells intercalate, but the intercalation is highly directional. Cells converge by intercalating perpendicular to the axis of extension, resulting in the overall extension of the tissue in a preferred direction. If we had a way to label cells from rows on either side of the axis of extension, they would be found to mix with one another as a result of these oriented intercalation events.

Possible questions

1. Give a brief note on patterns of embroyonic cleavage.
2. Explain the morphogenetic movements in detail.
3. Write a short note on morphogenetic gradients.
4. Explain in brief about gastrulation.
5. Give a note on role of egg cortex.
6. Explain in detail about primary organs and rudimental organs.
7. Write a short note on morula and blastula.
8. Explain in brief about fate map.

UNIT-V
SYLLABUS

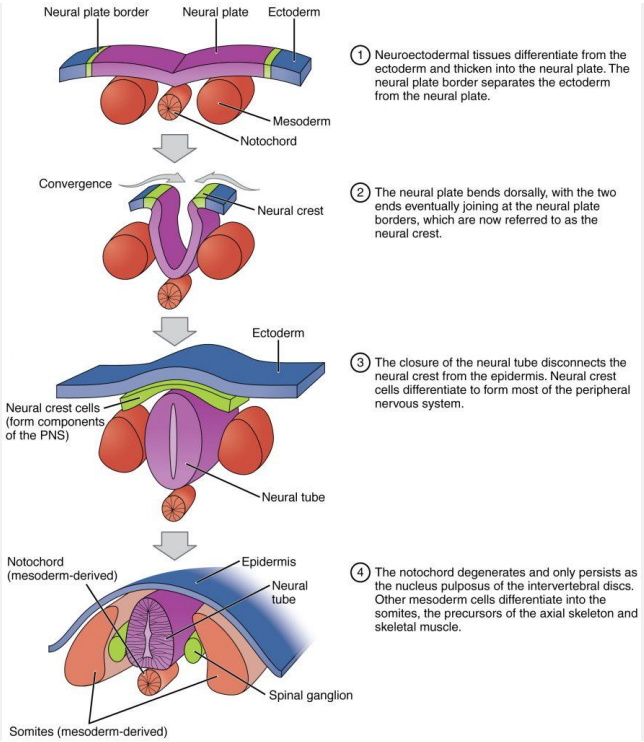
Developmental Stage III: Organogenesis: Induction and differentiation of Brain, eye, ear, limb, Heart, kidney, Development of Immune system, Genetic basis of differentiation – selective action of genes–gene action in development – Nuclear transplantation–apoptosis during development– aging–Teratogens and Teratogenesis.

Organogenesis:

Gastrulation leads to the formation of the three germ layers that give rise, during further development, to the different organs in the animal body. This process is called **organogenesis**.

In vertebrates, one of the primary steps during organogenesis is the formation of the nervous system. Interestingly, the nervous system originates from ectodermal, not mesodermal tissue.Â During the formation of the neural system, induction causes some cells at the edge of the ectoderm to become epidermis cells. The remaining cells in the center form the neural plate, which will go on to form the nervous system.

Immediately beneath the neural plate is a rod-shaped mesodermal structure called the **notochord**. The notochord signals the neural plate cells to fold overÂ to form a tube called the neural tube, as illustrated below. During later development, the notochord will disappear (it goes on to help form the spongy discs between the vertebrae), and the neural tube will give rise to the brain and the spinal cord.



The central region of the ectoderm forms the neural tube, which gives rise to the brain and the spinal cord. Illustration shows a flat sheet. The middle of the sheet is the neural plate, and the epidermis is at either end. The neural plate border separates the neural tube from the epidermis. During convergence the plate folds, bringing the neural folds together. The neural folds fuse, joining the neural plate into a neural tube.

Induction and differentiation of Brain, eye, ear, limb, Heart, kidney

Classification of induction:

Lovtrup (1974) classified induction into two principal classes.

Endogenous induction: Shapes and sizes of some of the embryonic cells changes after secreting inducting substances and this induction brings about differentiation of cells. As for example small cells of the dorsal lip carrying yolk granules act as endogenous induction.

Exogenous induction: When either by external influence or by contact any cell or tissue induces nearby tissue to differentiate, then it is known as exogenous induction. Exogenousinduction may again be of two types. As-

Homotypic: When the contact induction induces to form same types of cells, it is known as homotypic.

1. Heterotypic: When the contact induction induces different types of cell differentiation, it is known as heterotypic induction.

Embryonic induction in vertebrates:

Spemann observing the induction effect of dorsal lip named it as primary organizer but Ebert and Sussex (1974) said the formation of secondary embryo is due to cell differentiation of both the donor as well as of the host. They preferred to call the primary organizer of Spemann as embryonic inductor. As the primary organizer induces the epidermis for the formation of neural tube so now a days the primary organizer has been renamed as primary inductor or neural inductor.

Morphology of Neural inductor:

Vogt (1924) has shown by vital staining technique that cells of the dorsal lip of blastopore of a newt's gastrula, move interior and form the roof of the archenteron. If a block of tissue from archenteron roof is transplanted to the abdomen of another gastrula then from the abdomen created by the host gastrula tissue, a secondary larva is formed. All parts of the dorsal lip can not induce such induction. If only endodermal cells are grafted it will give rise to a partial embryo. If the anterior part is grafted it will induce to form the mouth, sensory organs head with the brain of the partial embryo. If the middle part is grafted it will give rise to eye and nasal cavities, lateral side induces to form posterior part of the head and if the posterior part is grafted then it will induce to form spinal cord, trunk and tail mesenchyme. From these experiments it can be concluded that the dorsal lip possess the regionality of its induction activity

Types of inductors:

On the basis of various experimental evidences Lehmon (1945) said that specific regionality of induction effects present in the dorsal lip of the blastopore. He further said that the roof of the archenteron definitely possess specific induction activities for the differentiation of head and

trunk regions. On the basis of the regional specificity he classified the inductors into three groups. They are:

Archenocephalic inductor: Due to induction effect of this inductor partial head, fore-brain, eye, nasal cavities are formed.

1. **Deuterencephalic inductor:** By its induction effect posterior portion of the head, ear cavities etc. are formed.

As arechenocephalic and deuterencephalic inductors induce the formation of different parts in the head region so they together are known as cephalic or head inductors.

1. **c) Spino-caudal inductor:** Their inductive influence leads to the formation of spinal cord and different structures of the tail region.

1. Brain:

The first important morphogenetic change following gastrulation is the development of the central nervous system. The central nervous system starts as a simple tubular nerve tube which, in course of development, transforms into brain, spinal cord and their associated structures.

The morphogenetic processes involved in this process are designated as neuralisation. It includes the separation of neural materials from the embryonic ectoderm, their migration inward to form a hollow nerve tube together with the segregation of neural crest cells. The nerve tube diff-erentiates into the brain and spinal cord, while neural crest cells develop into neuroblasts and many other structures.

Methods of Neuralisation:

Neuralisation occurs by two ways in different vertebrates.

These are:

(a) Thickened Keel Method:

In teleost, ganoid fishes and cyclostomes the neural materials become aggregated to form a thickened keel or ridge extending along the mid-dorsal axis of the body. This ridge separates itself from the overlying ectoderm and develops a lumen within to form a tube.

(b) Neural Fold Method:

It occurs in most of the vertebrates where neural cells become aggregated to form a neural plate. This plate folds inward to form neural groove. The neural groove transforms into a neural tube which sinks from the overlying ectoderm.

Events in Neural Morphogenesis:

After the completion of gastrulation, the ectoderm of the future dorsal side of the developing embryo tends to condense to form a thick and compact neural plate with elevated margins. This thickened part is called the neural (or medullary) plate or neural placode.

The plate is formed by two simultaneous processes:

(a) Elongation of prospective neural cells in the direction perpendicular to the surface of the developing embryo, and

(b) Shrinking the exposed surfaces both dorsally and ventrally.

The neural plate is pear-shaped, i.e. it is broader at the anterior part but gradually narrows towards the posterior end. This particular shape of the neural plate is crucial for shaping the future structures. The shaping of the neural plate is resulted as the consequence of regional differences in the cell contration. Fig. 5.25 relates the stages of neural morphogenesis in frog.

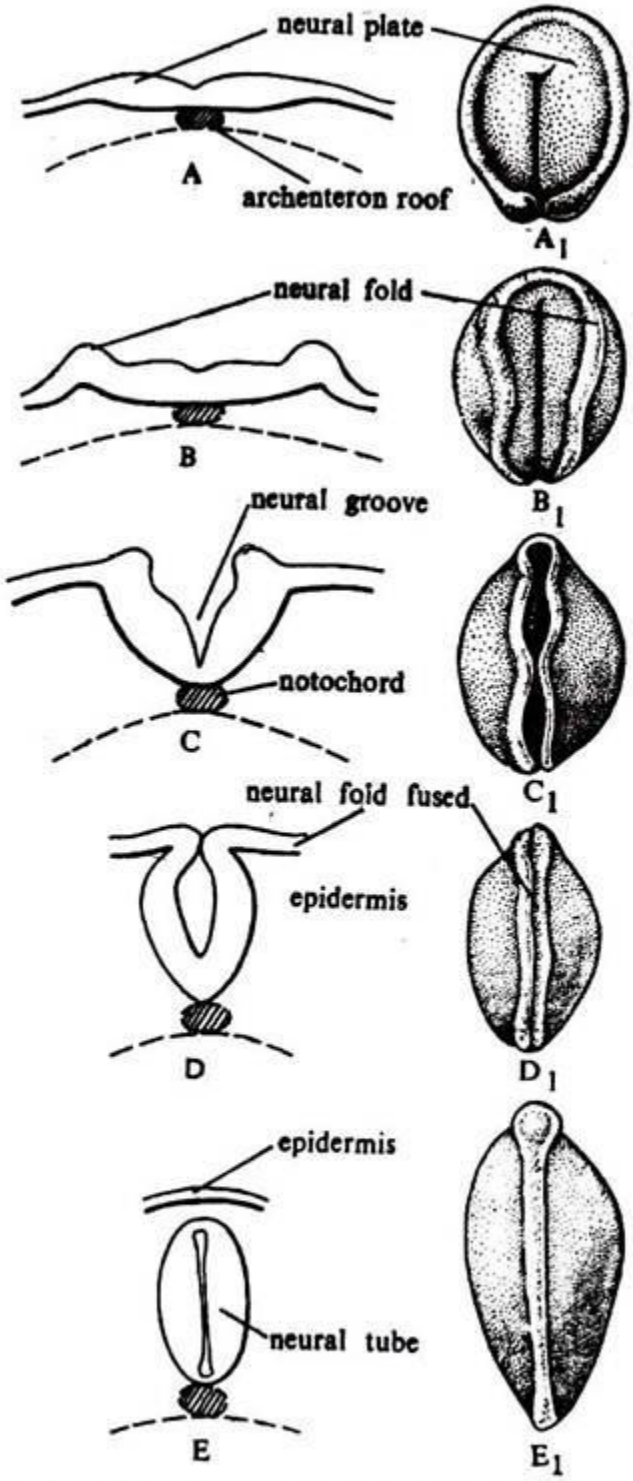


Fig. 5.25. Early stages of morphogenesis of brain. A–E. Sectional (Transverse) views. A_1–E_1. Dorsal views of entire embryos.

A depression appears along the entire length of the neural plate which folds downward to form a neufal or medullary groove. The formation of the neural groove is associated with the median and dorsal movement of the ectodermal layer attached to the lateral edges of the neural plate.

Thus the raised or folded margin of the neural groove is called the neural fold. The downward movement of the neural plate to form the neural groove depends largely on the lateral shifting of somatic mesoderm from the notochordal area to accommodate the invaginating neural groove. The lateral neural folds rise and meet along the middle line. This union begins from the anterior end and runs posteriorly.

With the union-of the folds, the outer ectodermal layers become continuous and the inner nervous layer, after fusion with the corresponding part, forms a tube and separates itself from the upper ectodermal layer. This tube-like structure is called the neural tube.

The cavity of the neural tube is called the neurocoel which is broader at the anterior end and opens to the exterior through an opening called the neuropore. The neuropore ultimately closes at the later stage of development.

Associated with the formation of neural tube neural crest cells become segregated on the two sides of the neural tube. These neural crest cells lie as two longitudinal strips of cells, one on each dorsal side of the neural tube.

Neural Crest and its Fate:

At the corners of the fusing neural fold during brain formation, groups of neural crest cells become detached to occupy a position over the neural tube. In course of development these cells leave their position and migrate to other parts of the embryo.

These cells are versatile in their developmental fate and develop neuroblasts of the spinal and sympathetic ganglia, Schwann sheath cells producing the myelin sheath and neurilemma of the nerve fibres, melanoblasts, chromaffin tissue of adrenal medulla, meninges, cartilages of the jaw, etc. Weston (1963) has shown the migration of neural crest cells.

The neural tube and neural crest cells labelled with radioactive isotopes are excised from the trunk of a developing chick embryo and transplanted to a normal (non-labelled) host in place of its counterparts.

It has been shown that the neural crest cells migrate along two ways:

(i) Dorsolaterally along the skin and

(ii) Ventrolaterally in relation to the neural tube.

Structural Differentiation of the Neural Tube:

The differentiation of the neural tube into the brain and spinal cord depends upon many intrinsic and extrinsic factors. The anterior part of the neural tube transforms into the brain while the posterior narrow part becomes elongated to form the spinal cord.

The broad anterior part is demarcated from the narrow posterior part by isthmus. Remarkable changes occur in the anterior part during its conversion into the brain.

This is caused by:

(a) Unequal thickening of the neural tube wall,

(b) Invaginations or evaginations of the wall and

(c) Various types of bending or folding (flexure formation).

Immediately after the formation of the neural tube, the anterior part swells up and two constrictions develop to divide the anterior part into three general regions: Prosencephalon, Mesencephalon and Rhombencephalon.

In course of development, prosencephalon and rhombencephalon become further subdivided thus giving rise to five parts: Telencephalon, Diencephalon, Mesencephalon, Metencephalon and Myelen- cephalon (Fig. 5.26).

Many factors are responsible in brain morphogenesis. Differential growth and intraventricular pressure are regarded to be the important morphogenetic factors in brain development, especially in flexure formation. Fig. 5.26 relates the development of flexures and different regions of the brain.

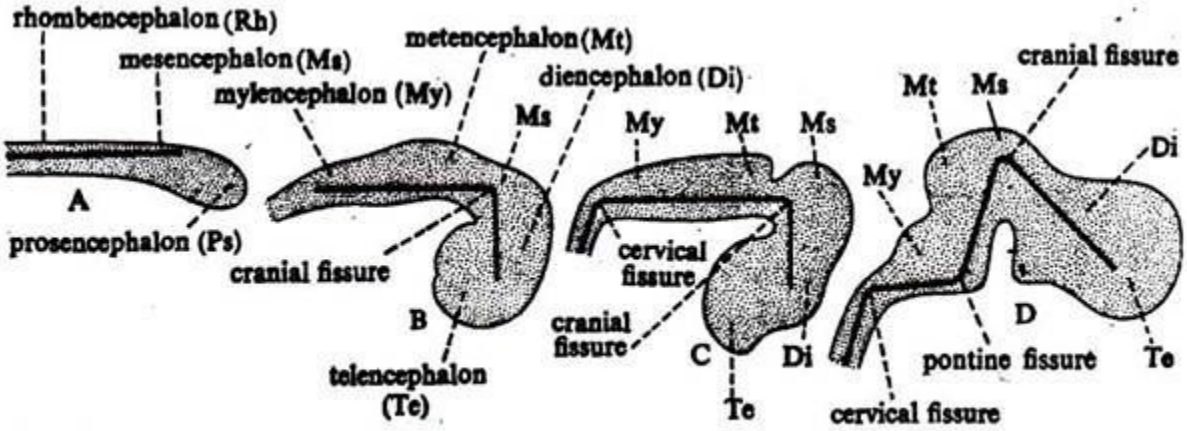


Fig. 5.26. Showing the regional differentiation of brain and the formation of flexures in chick. A. 48 hours. B. 72 hours. C. 4 days. D. 8 days.

Histogenesis in Brain Development:

The early neural tube is fairly uniform in structure. The walls are composed of neural epithelial cells which eventually differentiate into: (1) neuroblasts and (2) spongioblasts. The neuroblasts develop into nerve cells and fibres while the spongioblasts give origin to ependymal and neuroglial cells.

The neural epithelium is composed of pseudostratified columnar epithelial cells which form the primitive ependymal layer or matrix layer. Gradually the cells of the matrix layer migrate to cach lateral side to form a cellular layer called the mantle layer.

And lateral to the matle layer lies a cell-free marginal layer. The cells of primitive ependymal layer are usually called the germinal cells, some of which after a day or two following the closure of neural groove, develop neuroblasts and migrate first to the mantle layer (Fig. 5.27).

In the mantle layer, the cells differentiate into

(a) Neuroblasts and neurons and

(b) Spongioblasts and neuroglial cells.

The neuroglial cells give rise to astrocytes and oligodendrocytes. The neuroblasts do not remain evenly distributed but are aggregated into clusters. From mature neuroblasts, nerve cells and fibres grow out in a distinct pattern and turn the brain into a ‘working unit’.

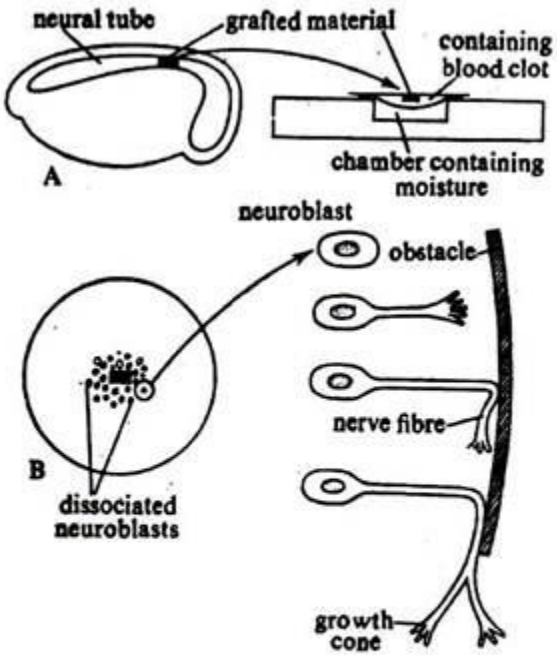


Fig. 5.28. Development of nerve fibre from a single neuroblast. A. A small portion of nerve tube is cultured within a blood clot in a specially designed culture chamber. B. Shows migration of neuroblasts and each transforms into a full-fledged nerve cell with fibres.

Development of Nerve Cells and Fibres:

Nerve cells originate from neuroblasts which develop from the neural tube, neural crests and cranial placodes. The actual stages of conversion of a small (neuroblast to a large cell-body of a nerve cell can be seen in tissue culture method reported first by Harrison in 1907. As in Fig. 5.28, a small fragment of neural tube is transplanted in a blood clot and kept sealed in a moist chamber.

Dissociation and dispersion of cells are the first observable events in tissue culture. The origin of nerve fibres is the most notable event in this process of conversion. Three theories are extant on this particular issue.

They are:

(a) Cell-chain theory. This theory relates that the fibre is laid down by chains of cells which surround the nerve fibre.

(b) Plasmodism theory. According to this theory the nerve fibre is laid down on preformed protoplasmic bridge.

(c) Outgrowth theory. The theory advocates that the fibre is formed as an outgrowth of a single neuroblast.

The tissue culture experiment gives support to the last concept and setties the long standing controversy regarding the issue. At the beginning, a thin strand of protoplasm emerges as outgrowth from one side of the neuroblast.

This outgrowth becomes amoeboid and creeps along the solid object. The outgrowth has developed a growth cone at the terminal end which may branch to form two or more growth cones. The growth of nerve fibre exhibits streotropism, i.e. it moves along solid object.

Causal Analyses in Brain Morphogenesis:

In the entire process of nervous system formation, a number of inductive events occur. In the amphibian eggs, the dorsal lip of blastopore acts as primary organizer to induce the inward moving cells to form chordamesoderm which in turn induces the dorsal ectoderm to be neuralised. The formation of the neural tube is also guided by the influence of regionally specific inductions.

The neural plate at the beginning is an oval, flattened plate and is formed by the ectodermal cells which have come from lateral regions to the dorsal side. The neural plate elongates rapidly, which is caused by the movement of cells. The cells first move towards the middle and then run in two directions: anteriorly and posteriorly.

The transformation of neural plate to neural tube which is called neurulation is also known to occur in vitro. It begins with a depression in the centre and curving of the edges which fuse together to form the tube. To search the motive force behind the formation of tube, the behaviour of cells in the centre and periphery is intimately studied.

Certain suggestions, like differential water uptake, differential cell divisions have been negativated. It is now claimed that elongation of the plate is due to migration of cells but curvature is caused by changes in the cell adhesion.

In further development, the anterior part of the tube swells up considerably to form brain vesicles. Considerable amounts of cell division and cell movement occur during the process. The different parts of the brain in course of its development induce the formation of structures like optic, auditory and nasal placodes on the outer ectodermal covering.

It must be remembered that mesodermal cells which immediately remain around neural tube are believed to play most important role in the epigenetic profess.

The formation of brain establishes:

(a) many histological features remain determined at neural plate stage and

(b) all the cells do not transform into neural element at the same time. On the contrary a gradient exists in the anterior-posterior plane.

2. Eye:

The early stages of eye development follow a generalised pattern in all vertebrates and the details of eye morphogenesis in chick will give an idea of the process in general. The eye is a very complicated structural unit. The development of eye reveals the incorporation of different tissues which follow an orderly fashion to give the geometry of pattern.

Because of the fact, the incidences involved in eye morphogenesis are regarded as a perfect model to explore the general problems of embryology.

The development of eye is discussed under three steps:

(a) Development of sensory areas.

(b) Development of lens.

(c) Development of associated structures.

Development of Sensory Areas:

Formation of Optic Placode and Optic Vesicle:

The primordial eye rudiment lies at the very anterior end of neural plate in the form of two closely placed oval areas, one on either side of the middle line. They are lined below by mesoderm. In course of the formation of brain, these two lateral sides of the forebrain, which are destined to be the future diencephalon, become thickened.

These parts are known as optic placodes. These two placodes extend laterally as small blunt bulgings, which become known as optic vesicles (Fig. 5.29). The vesicles elongate through the loose mesenchyme towards the epidermal covering and remain connected with the brain by a narrow stalk called optic stalk.

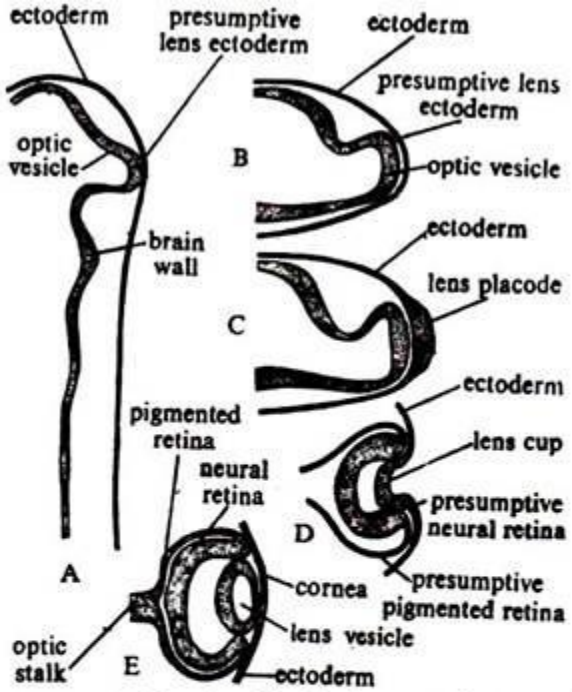


Fig. 5.29. Showing the developmental stages of optic cup, lens and cornea. A. Diagrammatic longitudinal section of the embryo. Note that the optic vesicle originates from the diencephalic region of the brain and makes contact with the overlying ectoderm. B–E. Transverse sectional views. B. Transverse sectional view of A-stage. C. Showing the induction of lens placode by the optic vesicle. D. Invagination of optic vesicle to form optic cup and the lens vesicle. E. Separation of lens vesicle and differentiation of neural and pigmented retina.

Formation of Optic Cup:

As the optic vesicle touches the ectoderm, the ectoderm cells elongate perpendicularly to the region of contact to form the lens placode which invaginates to form lens vesicle. With the invagination of lens placode to form lens vesicle, the optic vesicle reverses its outward bulging

and turns inwards to form the optic cup to accommodate the lens. The optic cup is double-walled.

Such inpushing takes place asymmetrically and continues obliquely into the optic stalk. Near the optic stalk, a slit is left in the ventral side which is called choroid fissure. This fissure acts as an outlet for optic nerve (Fig. 5.30). The blood vessels also find an impasse into the optic cup.

The outer wall of the optic cup remains thin and gives rise to pigmented retina while the inner wall (neural retina) becomes greatly thickened and elaborated to transform into light sensitive retina.

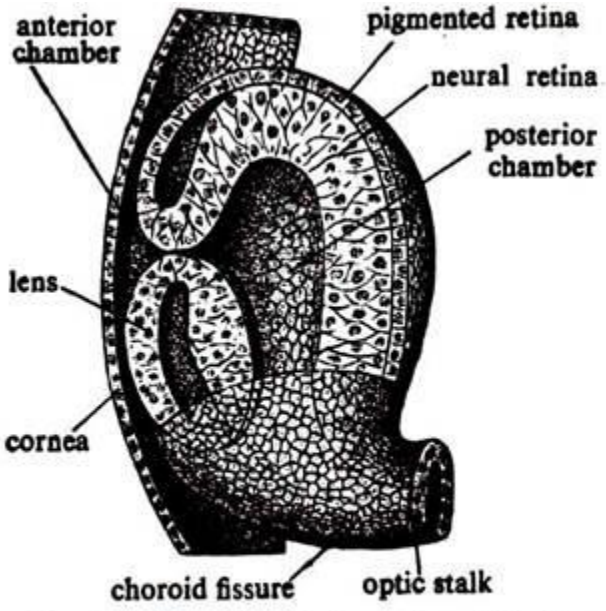


Fig. 5.30. An enlarged schematic model of eye showing the relative position of the structural components.

Formation of Retina and Optic Nerves:

The inner lining of the optic cup transforms into light sensitive retina, which in turn differentiates into seven layers of nervous elements. The neural retina gives rise to rods and cones and some other types of cells with which the visual cells synapse.

The histogenesis of retina is divided into three phases:

(i) A phase of cell multiplication,

(ii) A phase of cellular readjustment and

(iii) A phase of final differentiation.

Fig. 5.31. gives the details of development of retinal cells. Rods and cones are arranged in the outermost part, i.e., towards the pigmented layer of the optic cup. The position of visual cells is due to the migration and stratification of neural layer of the retina. The fibres of rods and cones unite with the fibres of the ganglionated layer of the retina. These fibres converge towards the optic stalk to form the optic nerve.

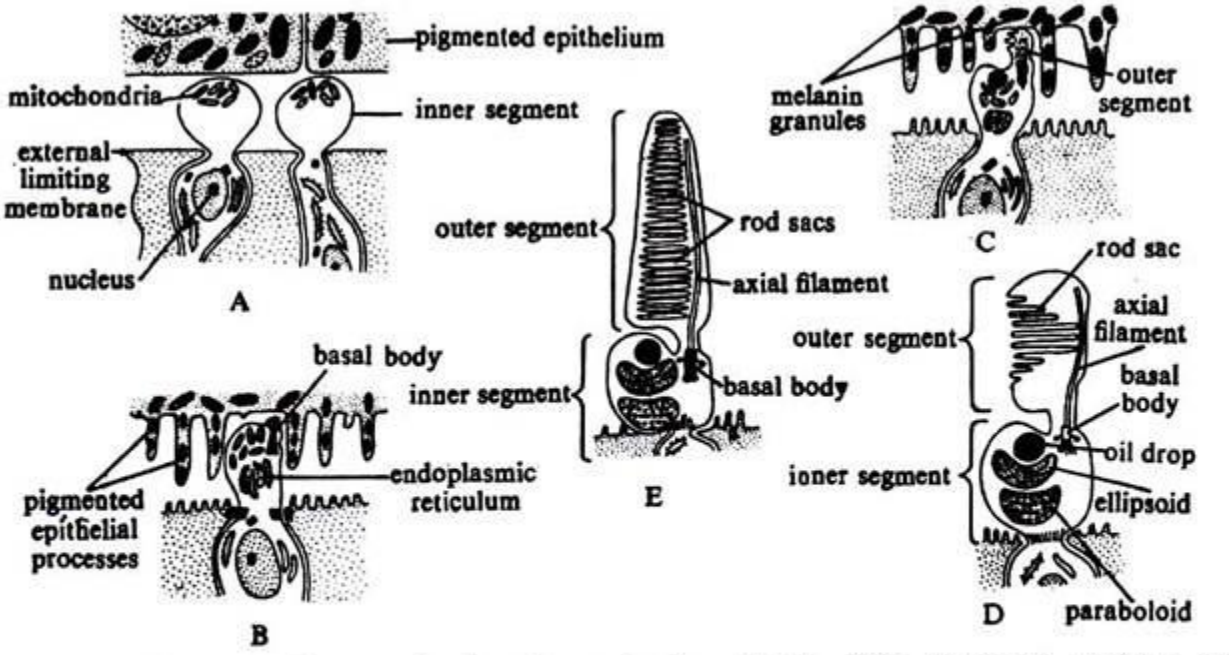


Fig. 5.31. Schematic diagrams showing the maturation of rod. Each visual cell produces a minute cytoplasmic bud which projects through a pore in the external limiting membrane (A). This bud develops into the rod. Inside this bud, several specialised cell organelles develop (B). The bud forms the inner segment of the rod and from this segment the outer segment arises (C). The basal body of the inner segment produces cilia which participate in the differentiation of the outer segment (D). In the outer segment, rod sacs are produced by the folding of the cell surface. These sacs are stacked one above the other and the outer segment elongates with the growth of the sacs (E).

Development of Lens:

The region of the outer ectoderm which comes in contact with the optic vesicle thickens and is known as lens placode. The placode invaginates to form a lens pit or lens cup. The two ends of the lens cup unite and remain within the space between-optic cup and outer ectoderm. It is then called lens vesicle.

The inner cells of the vesicle transform into lens fibres and the cell layer next to ectoderm forms the epithelium of the lens. At about 96 hours in chick embryo, the lens cavity becomes reduced and the cells of the median wall of the lens become elongated to obliterate the lens cavity.

The cytoplasm of the cells becomes clear and these cells transform into lens fibres. The extreme elongation of the cells is evidenced by the placement of their nuclei in the equitorial region of the lens and the cells are stretched extending from one surface of the lens to the other. A lens fibre may reach 10 mm as seen in man.

Development of Associated Structures:

Choroid and Sclera:

The optic cup and optic stalk become invested with a layer of mesenchyme which later forms an outer densely fibrous layer called sclera and an inner pigmented and richly vascularised layer called choroid.

Conjunctiva, Cornea and Aquous Humor:

After the detachment of the lens the ectodermal epithelium forms a cell-free lining. Along the inner border of the ectodermal epithelium lies the mesenchyme, which forms the sclera. The inter epidermal layer is called conjunctiva and inner mesenchymal continuation of sclera is known as cornea. The transparency of cornea is vital for the admission of light into the cavity of eye.

The cornea is composed of an outer epithelial layer (derived from ectoderm) and a postepithelial stroma (derived from immigrating mesenchymal cells). In the stroma, layers of collagen fibres accumulate parallel to the surface. The stioma undergoes significant biochemical changes and undergoes dehydration.

Due to loss of water and suppression of pigmentation, the cornea attains perfect transparency. A space develops between lens and cornea, which is called anterior chamber. A watery fluid, called aqueous humor accumulates within the space.

Iris:

The choroid layer extends in front of the lens to form a circular-curtain known as iris, which has a hole in the centre called pupil. The iris is pigmented and possesses papillary muscles which regulate the diameter of the pupil. The iris develops from the pigmented retina.

Ciliary Muscles:

The pigmented outer layer of the optic cup together with mesenchymal elements extends in front to form ciliary muscles or ciliary bodies.

Vitreous Humor:

The cavity between lens and retina is known as posterior chamber. It becomes filled with a gelatinous matrix called vitreous humor.

Muscles of the Eye:

Several muscles arc formed by the condensation of outer head mesenchyme. These muscles are involved in rotating the eye-ball within the orbit.

Eyelid:

The outermost epidermal layer in front of the eye becomes skin. It splits into two halves to form the eyelids. In different vertebrates the shape of eyelids varies.

Causal Analysis of Eye Development:

The foregoing description reveals that the development of eye is a phasic phenomenon where different component parts appear in sequential order to establish a harmonious functional unit.

A resume of the total events shows that the optic vesicles emerging from the forebrain make intimate contact with the presumptive lens ectoderm to induce lens formation. The lens vesicle induces the optic vesicle to form optic cup and its subsequent differentiation.

The optic cup-lens complex induces the overlying ectoderm with some mesenchyme to form the cornea. Thus a reciprocity of induction occurs in eye morphogenesis—and this type of induction is called the synergistic induction. Fig. 5.32 gives, the schematic representation of the participation of different tissues and the inductive phenomena in the development of eye.

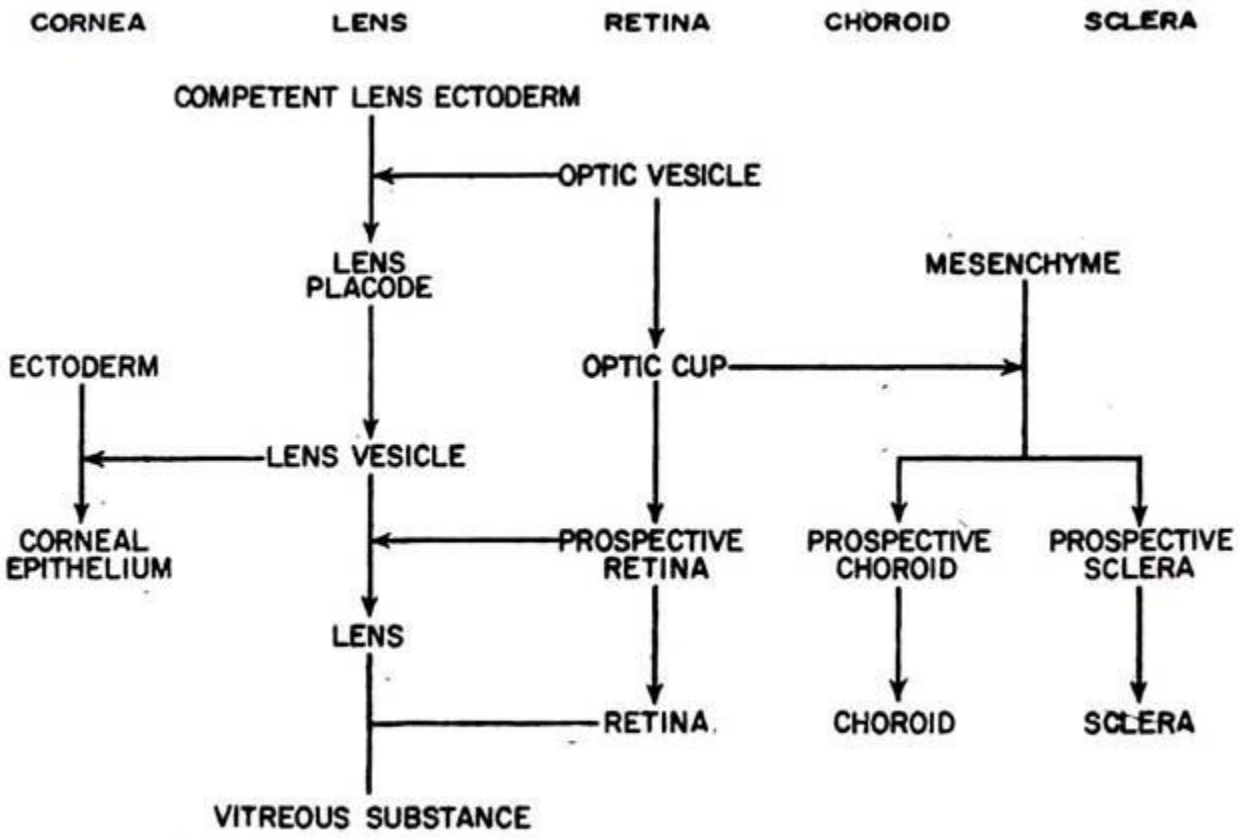


Fig. 5.32. Schematic representation of tissue interactions in eye development.

Events of Eye Development:

Events of eye development may be divided into three phases:

(a) Phase of induction

(b) Phase of cell differentiation and axiation and

(c) Phase of mechanical tension.

The work carried by large number of workers have revealed that no organ is formed if there is any disturbance in the first phase. But at the same time only the occurrence of first phase cannot form the organ.

The disturbance in the second phase, i.e. phase of cell differentiation, produces numerous deviations in the different rudiments of eye. The third phase creates the form and size of the organ and its abnormality affects them considerably.

Some of the important findings to explain the mechanism of eye formation are discussed below:

(i) The lateral extension of the optic vesicles to reach the ectodermal layer is caused by the pressure exerted by the intraventricular fluid of the brain.

(ii) The loose surrounding mesenchyme of the primordial eye rudiment plays important part. Experimental evidences suggest that the mesoderm first contributes most actively to the development of the eye and then exhibits its formation in the middle of the brain.

(iii) In the primary eye rudiment at the beginning, the different layers have the capacity to undergo mutual transformation.

The inner wall of the optic cup transforms into the neural retina while the outer wall develops into the pigmented retina., If the position of these layers is reserved, the original inner layer may form neural retina and vice versa.

The transformation of the inner wall of the optic cup to form neural retina is caused by the inductive influence of the lens vesicle. The outer layer is converted into pigmented retina by the influence of mesenchyme.

(iv) The proportion of pigmented retina and neural retina depends on the extent of tension produced by the fluid which accumulated inside.

(v) The formation of cornea depends upon geometric distribution of different layers and the mechanical tension of the eye.

3. Heart:

The formation of heart (cardiogenesis) in vertebrates is one of the most dynamic events in embryonic development. The heart is a mesodermal organ, which differentiates initially from the ventral edges of the lateral plate mesoderm.

Primarily the cardiac primordia are paired which, however, become fused to form a single organ. The process of cardiogenesis in different vertebrate forms is essentially similar. The events of the development of heart in the chick embryo are discussed below.

Localisation of Cardiac Primordial:

The localisation of heart-forming cells occurs at the onset of gastrulation in the embryos of all vertebrates. Vogt (1929), by using vital staining technique, has localised the prospective heart cells in amphibian embryo. Butler (1935) and Spratt (1942) have shown that heart-forming cells are widespread in the blastodisc of chick (Fig. 5.33A).

With the movement of cells of the epiblast to form the primitive streak, heart- forming cells become restricted to the epiblastic region anterior to the developing primitive streak.

With the migration of epiblastic cells to form the mesodermal layer, the heart-forming cells become concentrated about Hensen's node. The heart- forming cells, then, migrate to join the mesoderm and move laterally. When the definitive primitive streak is formed, the heart-forming cells take lateral position as paired cardiac primordia.

Each primordium is capable of developing a whole heart. If the paired primordia are prevented from fusion, two independently beating hearts ('cardiac bifida') will result in an embryo.

Before the formation of heart, the presumptive heart-forming cells acquire specific biochemical characteristics from their neighbours and have an inherent capacity of undergoing self-differentiation. This is attested by the fact that these bilaterally located cardiac primordia, when transplanted into an indifferent location, are capable of differentiating into cardiac tissue.

The presumptive heart-forming cells are rich in glycogen which is retained throughout its differentiation. So the heart-forming cells become different from other cells by having high glycolytic metabolism.

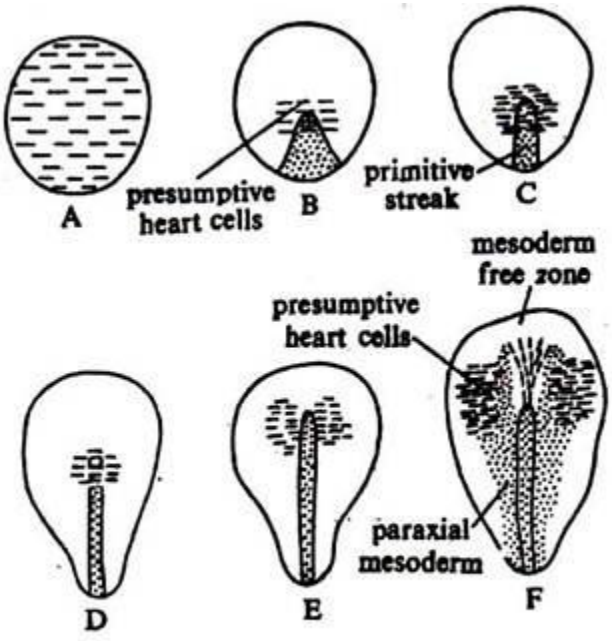


Fig. 5.33. Showing the localisation of presumptive heart cells in the blastodisc of chick.

Stages of Heart Formation:

In course of development of heart, the paired cardiac primordia come together in the midventral line. This is brought about by the action of four types of morphogenetic movements. These are:

(a) Folding Movements of Ectoderm and Endoderm:

This movement of the endoderm to develop into crescentic pouch of the anterior intestinal portal and early foregut is of great importance. The presumptive heart-forming cells use the endodermal layer as the substratum for their migratory activity. The folding movements bring the paired cardiac primordia together in the midline to develop into an unpaired median tube.

(b) Formation of Amniocardiac Vesicles:

This process of development of embryonic coelom or amniocardiac vesicle is also important in heart formation. With the formation of head fold and initiation of foregut, the lateral plate mesoderm in the region of cardiac primordia splits to form a dorsal layer (somatic mesoderm) and a ventral layer (splanchnic mesoderm).

The coelomic space thus enclosed by these two layers is called the early pericardial or amniocardiac vesicles. With the separation of the somatic and splanchnic mesoderm, all the presumptive heart-forming cells move ventrally in the splanchnic mesodermal layer. Because of this reason, this thickened crescentic splanchnic mesoderm is called by Mollier (1906) as the 'cardiogenic plate'.

(c) Cell Movement in the Splanchnic Mesoderm and the Subsequent Emigration of the Mesodermal (Splanchnic) Cells:

Prior to the formation of coelomic space, the precardial mesodermal reticulum consists of a homogeneous loose meshwork of stellate mesenchyme. Within this meshwork small clusters of

tightly packed cells are present which move actively from their lateral position to form the tubular heart.

(d) Formation of Angioblasts:

With the development of cardiogenic plate, angioblasts are formed in the region of the original amniocardiac vesicle. The conversion of the precardial cells to angioblasts is the first sign of histological differentiation in cardiogenesis.

These cells migrate either singly or in small clusters out of the mesoderm and form a loose layer (vascular layer of Pander) in the meso-endo- dermal space. This layer forms the endocardium of the heart and in the posterior region it produces the blood islands. These islands produce the endothelium of the remaining vasculature, erythroblasts and blood plasma.

Formation of Primitive Tubular Heart:

As stated earlier, the primordial endocardial cells begin to differentiate independently as a pair of delicate tubular hearts. These paired tubular hearts are arranged on either side of the anterior intestinal portal (the opening from the yolk into the foregut).

The folding movement of the ectoderm and endoderm to form the head fold and foregut, causes the migration of the paired tubular hearts together when they fuse to form an unpaired tubular heart tube.

The paired rudiments meet and fuse when the foregut is separated from the yolk sac. This process begins at 7 to 8 somite stages in chick and is completed when the embryo comes to 20-somite stage. Each of the paired rudiments has an inner endothelial lining (endocardium) and the outer is the epimyocardium.

Simultaneously with the migration of splanchnic mesoderm anteromedially and separation from the folding endoderm, it becomes thickened to form paired epimyocardia. This layer develops later into the thick myocardium and a thin nonmuscular epicardium (or visceral pericardium).

Fig. 5.34 shows the origin and subsequent fusion of paired cardiac primordia during cardio-genesis. The epimyocardium remains attached ventrally by ventral mesocardium and dorsally by dorsal mesocardium. Both these mesocardia disappear subsequently.

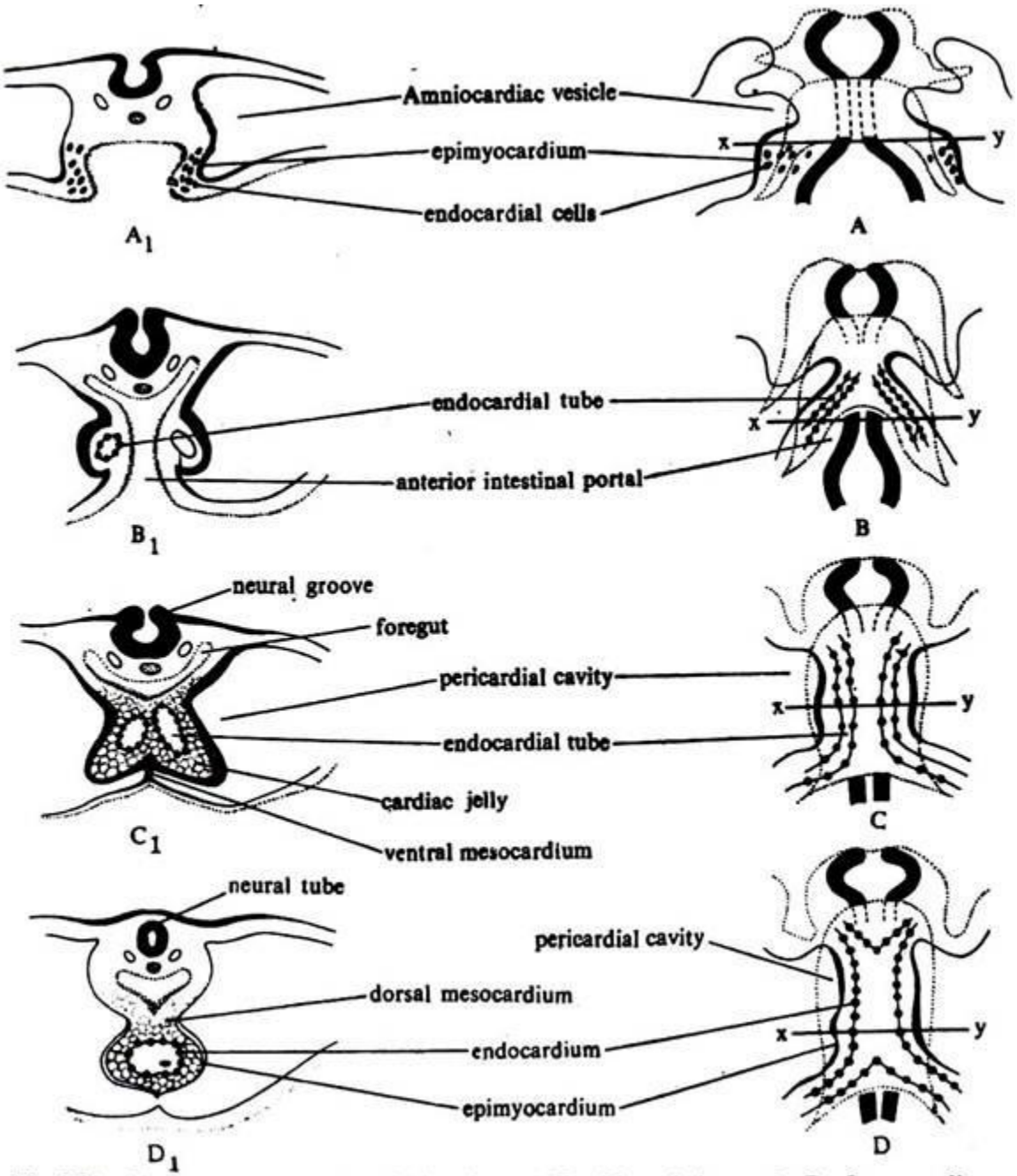


Fig. 5.34. Showing cardiogenesis in chick embryo. A–D. Ventral views. A_1–D_1. Corresponding transverse sections at the levels indicated by *xy* lines.

The fusion of the heart tubes begins at the anterior end and extends gradually to the posterior sides. Fusion starts in the region of the future ventricle and the auricle is still represented by double tubes. Then gradual union occurs in anteroposterior direction and the process of fusion is completed when the embryo becomes 20- somite stage in chick.

Inductive Relationship during Cardio-Genesis:

Many embryologists claim that the development of heart is intimately related to the developing endoderm. In amphibian embryo, the removal of endoderm causes the failure of heart formation. But in the development of chick, the removal of endoderm does not prevent normal cardiogenesis.

Many embryologists have claimed that the migration of cardiac cells and the folding movement of the endoderm is independent processes, normal cardiogenesis is not hampered if such relationship is disturbed. But experimental evidences on this line are unsatisfactory to ascertain the actual role of endoderm on the precardial mesodermal cells.

Histological Differentiation in Cardiogenesis:

The differentiation of angioblasts from the splanchnic mesoderm and the transformation of the splanchnic mesoderm itself to form the myocardium and epimyocardial mantle are the first indication of histogenesis in heart development.

When the first tubular heart tube is produced, the space between the endocardium and myocardium becomes filled by 'cardiac jelly'. It is a thick gelatinous mass containing aldehyde, acid mucopolysaccharides. Many cells from the endocardial and myocardial layers migrate into this gelatinous layer to form a loose meshwork of stellate cells which characterise the early heart tube.

Conflicting views exist as regards the histological nature of heart tissue, nature of fibrillogenesis, the nature of myofibril and the nature of intercalated disc. Electron-microscopic and tissue culture studies have revealed that heart tissue is syncytial in nature and the intercalated disc consists of a pair of apposed cell membranes.

The region is covered with electron-dense granules. The myofibrils do not cross the intercalated discs and there is no protoplasmic continuity across the apposed membranes.

The early tubular heart consists of endocardium and myocardium. The endocardium is composed of a single layer of flattened and granulated cells, while the myocardium is two or three cells deep. Subsequently in course of development the myocardium thickens by mitotic activity.

Myoblasts, composing the myocardium, contain granular materials and scattered loose myofilaments which become grouped to form striated myofibrils shortly before the pulsating of the heart. Mitotic activity is very high in early stage which declines to zero as cardiogenesis is completed.

Structural Differentiation in Cardiogenesis:

One of the important factors, which causes the regional differentiation of heart is the rapid elongation of the primitive heart tube within the lass-rapidly growing pericardial space. The heart is a straight tube when it is formed and does not show any sign of subdivision into chambers.

In course of development, the tube becomes inflected in a characteristic way to assume the adult configuration due to the cellular activities. In chick, the tubular heart becomes 'S'-shaped at the end of 3rd day after incubation. The heart becomes constricted in some regions and dilated at others.

In the 4th day of incubation, the atrial area expands into two lobes—the beginning of the left and right atria. The descending part becomes thickened to form the ventricle. The later development

of heart is the differential growth and subdivision into chambers. Fig. 5.35 shows the twisting and formation of different parts of heart in a chick embryo.

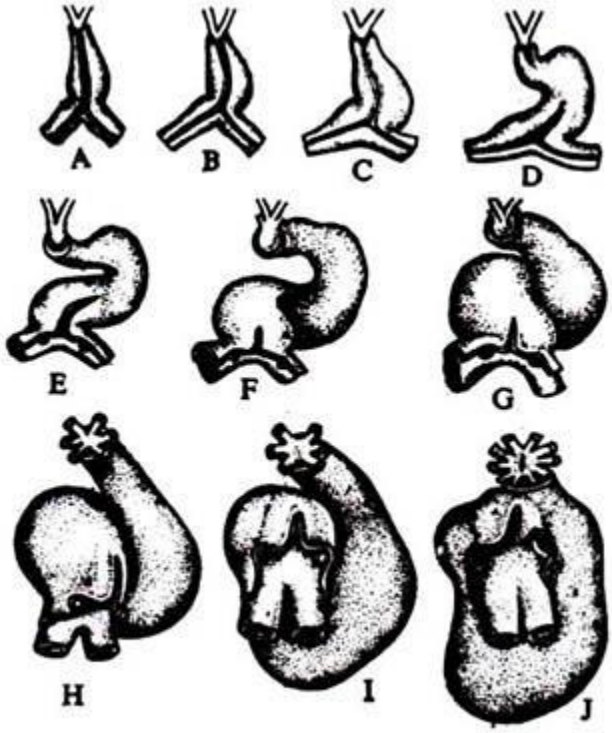


Fig. 5.35. Progressive fusion of paired heart primordia and gradual twisting of heart in chick.

The changes undergone by the tubular heart to form adult heart are essentially:

(i) Constrictions to form chambers.

(ii) Differential growth and thickening of the myocardium resulting in the formation of thin-walled receiving parts and thick-walled forwarding parts.

(iii) Kinking of the chambers—possibly due to rapid growth within crowded quarters.

(iv) Formation of septa, valves, etc.

Functional Changes in Cardiogenesis:

The definitive function of the heart starts as the paired cardiac tubes fuse and the contraction begins as soon as the primitive ventricle is formed. So the heart is the organ which begins its function at an early stage of development. Contraction starts in the myocardium along the right margin of the posterior end of the ventricle.

Gradually the contraction involves the whole ventricular wall which contracts synchronously, i.e. periods of contraction alternating with periods of rest. Meanwhile the atria develop which also contract at a more rapid rate. The atria control the rate of contraction of the heart as a whole.

These contractions set the contained blood in motion. Eventually pacemaker or sinoauricular node develops which takes the controls of the contractility of the heart as a whole.

4. Kidney:

The kidney of vertebrates essentially consists of an aggregation of uriniferous units called nephrons. The kidney develops from the 'intermediate mesoderm' which lies between the somite and the lateral plate mesoderm. The intermediate mesoderm becomes segmented and each segment is called the nephrotome. The nephrotome is transformed into the nephrons which involves significant cellular events.

Mode of Origin:

A nephrotome contains a coelomic space, called the nephrocoel which communicates into the adjacent splanchnocoel by the peritoneal funnel (Fig. 5.36).

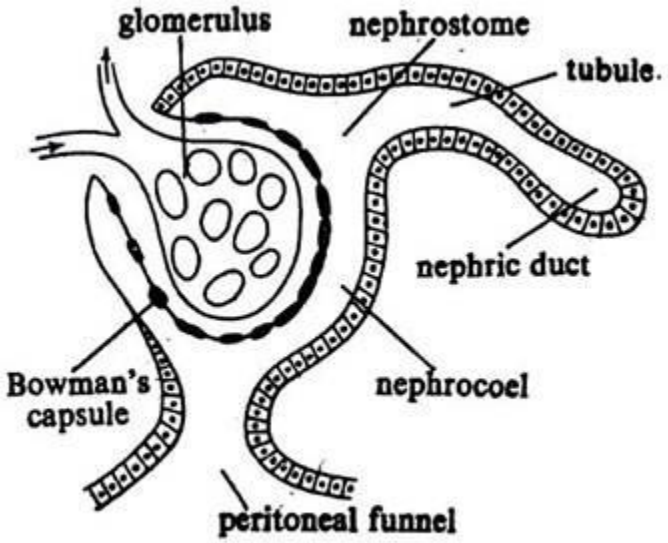


Fig. 5.36. Schematic diagram of a typical nephron.

The nephrotome is converted into a nephron in the following ways:

(i) The nephrotome, prior to its transformation, is a strand of cells between the somite and lateral plate mesoderm.

(ii) A tubular outgrowth develops from the dorsolateral wall of nephrotome.

(iii) The principle tubule originates from the tubular outgrowth which communicates with the nephrocoel through nephros- tome, i.e. the cavity of tubule is actually an extension of the nephrocoel.

(iv) The median wall of the nephrotome invests a tuft of blood vessels (arterial capillaries) to form the renal corpuscle.

(v) The actual mode of origin of renal corpuscle is controversial. It was believed that Bowman's capsule is formed by a process of invagination of the glomerular mass into the wall of the nephrotome.

But the electron-microscopic studies of Kurz (1958) have established that the double- walled Bowman's capsule is not formed as a result of invagination, but due to a cleft within a compact cellular mass. The inner layer becomes reflected over the glomerulus while the outer one forms- the capsular wall.

The basic pattern of the development of nephron becomes greatly modified in different vertebrates.

The deviation is due to:

(i) Typically hollow nephrotomes are not found in embryos of higher vertebrate forms, instead the tubules develop within a continuous nephrogenic cord without exhibiting segmental disposition,

(ii) All the nephrons do not differentiate at a time, rather, the nephrons appear in a sequential order from the anterior to the posterior end.

(iii) The structural organisation of the nephrons also shows gradual complexity progressively from the anterior to the posterior end.

Developmental Events of Nephrons in Vertebrates:

In primitive vertebrates, the distinction between the anterior and posterior nephrons is not well marked but in amniotes (reptiles, birds and mammals) the development of nephric system shows the manifestation of three distinct entities which succeed each other during ontogenic development.

The entities are: Pronephros, Mesonephros and Metanephros. The fishes and amphibians possess first pronephros which gives way to the mesonephros—the final kidney of an adult. In amniotes, besides these two units, a third entity, the metanephros arises as the definitive adult kidney. All the types of nephrons exhibit a striking similarity in their cellular transport mechanism and physiological performances.

Pronephros:

The segmental origin of the pronephric tubules is the characteristic feature in nephric development. In amphibians, the pronephric tubules are developed from the nephrotomes beneath third and fourth somites in salamanders and second, third and fourth somites in frogs.

It is to be noted that the number of the pronephric tubules corresponds directly to the number of segments involved. In chick, the pronephros develops from nephrotomes between fifth to sixteen somites.

The pronephric tubules begin to form when the embryo attains 12 to 13 pairs of somites (40-45 hours of incubation). The tubules become well developed in 16-21 somites stage. At 35-somite stage and at about 65-70 hours of incubation, the pronephros undergoes degeneration.

The sequence of events of transformation of the nephrotomes into the pronephric tubules is clear in lower vertebrates, but in higher tetra- pods the stages are not so clear.

When several pairs of pronephric tubules are developed, they open into the coelomic cavity proximally while the distal ends join the pronephric duct (Fig. 5.37). The nephric duct is called the pronephric duct which not only serves as the drainage channel for the pronephros, but becomes involved with the development of mesonephros.

Typical pronephros is a functional kidney in the larval stages of fishes and amphibians. But in the embryos of reptiles, birds and mammals, the pronephros develops in the anterior nephrotomes and is not functional at any stage. In human embryo, about seven pairs of pronephric tubules develop which start degeneration immediately after the initiation of the nephric duct.

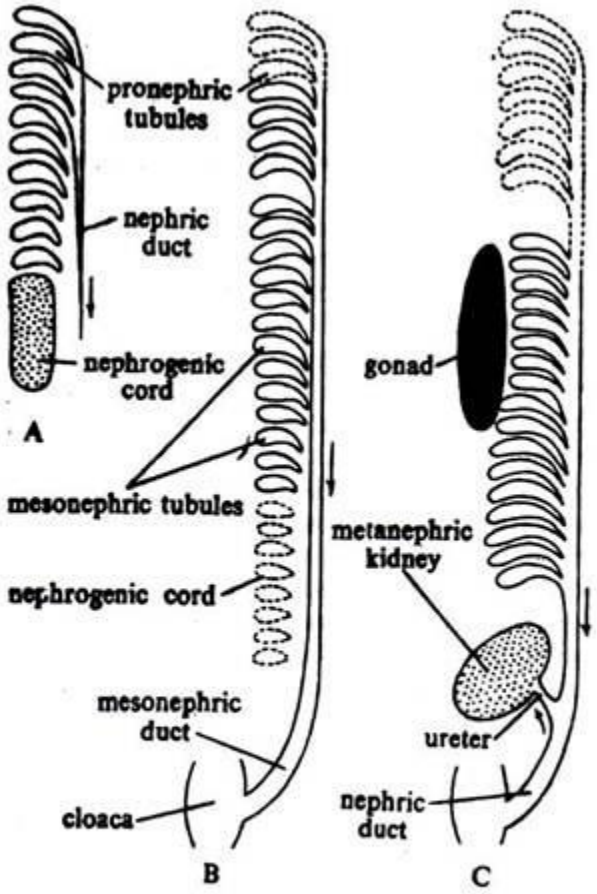


Fig. 5.37. Diagrammatic representation of the development of nephric system (after Torrey). A. Mode of origin of nephric duct. B and C. Showing the relationship between nephric duct and mesonephros and metanephros.

Independency in the Differentiation of Pronephric Tubules and Nephric Duct:

The nephric duct (pronephric duct) starts development from the mesodermal blocks situated more posterior to the segment from which pronephric tubules begin to form. In amphibians, the nephric duct originates from the nephrogenous mesoderm behind that which provides the pronephric tubules.

The somite 5 usually marks the level of the nephric duct primordium in amphibians. O'Connor (1938) has applied vital stain to pronephric swellings below the third and fourth somites in Ambystoma. It was observed that the stain appeared only in the pronephric tubules.

When the stain was applied below the fifth and seventh somites, the stain became confined to the nephric duct. Holtfreter (1943) has bisected the embryo between the levels of fourth and fifth somites and has observed that in the hind piece, though devoid of pronephric tubules, the nephric duct still develops perfectly.

The above experimental fact relates that the pronephric tubules and nephric duct are determined, independently of each other. Once the nephric duct starts development, both the pronephric

tubules and nephric duct elongate at a rapid rate. The pronephric tubules become thrown into loops as a result of elongation and the glomeruli of several segments may join together to form the glomus (Fig. 5.38).

The nephric duct (now designated as pronephric duct) after inauguration, pushes itself backward along the lower ends of the somites and the posterior movement is stopped as it reaches the cloaca. The duct fuses with the wall of the cloaca and its lumen opens into the cloacal cavity.

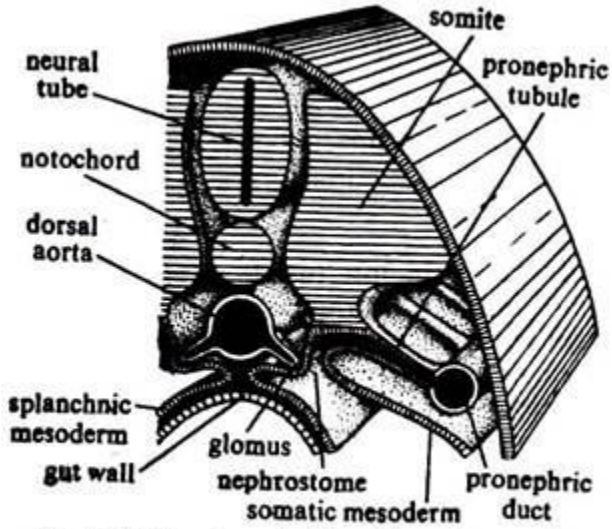


Fig. 5.38. Showing the disposition of pronephros (after Huettner).

The backward elongation of the pronephric duct towards the cloaca is possibly due to either by (i) progressive addition of new material or (ii) due to free terminal growth. Extensive literature exists on this particular issue. Overton (1959) advocated that the duct increases by independent caudal growth.

Holtfreter opined that the growth of pronephric duct towards the cloaca is due to selective cell-adhesions rather than chemotaxis as advanced by many. Holtfreter also suggested the role of blood vessel during the process, but this issue remains open for further investigation.

Mesonephros:

The mesonephros is derived from the nephrotomes posterior to the pronephros. In majority of amphibians and amniotes, the component mesodermal cells of the nephrotomes dissolve into an aggregation of mesenchyme. These aggregated cells stretch on each side of the body along the dorsal margin of the lateral plates.

This mass of mesenchymal cells is called the nephrogenic cord or nephrogenous tissue. The mesonephric tubules develop from the nephrogenic cord extending between 17 to 30 somites in chick embryo.

The proliferation of mesenchymal cells leads to the formation of elongated solid cords. Each suchycord elongates and assumes ‘S’-shaped appearance. It becomes hollow to form a cavity. One end of suph a tubule connects itself to the existing pronephric duct (the mesonephric tubules

do not form a duct of their own), while the proximal end forms a double-walled Bowman's capsule.

The pronephric duct is now designated as the mesonephric or Wolffian duct because it serves as a drainage duct for the mesonephros. The Bowman's capsule is supplied by small blood vessels from the dorsal aorta (Fig. 5.39).

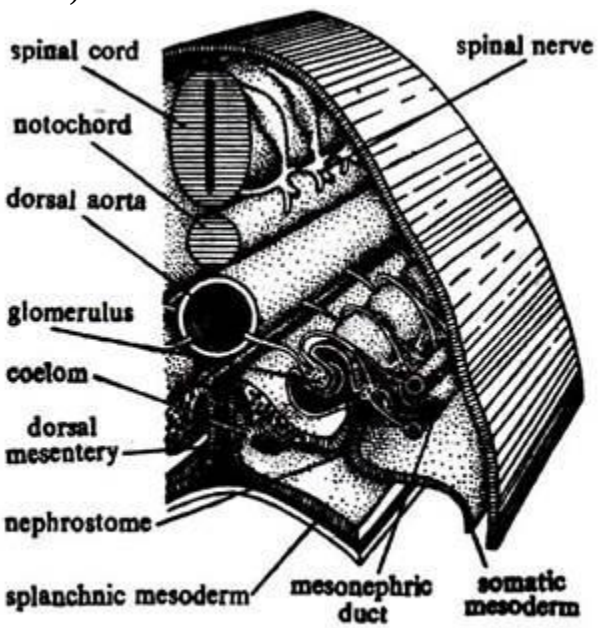


Fig. 5.39. Showing the disposition of meso-nephros (after Huettner).

Several mesoephric tubules are developed in a segment, i.e. the number of mesonephric tubules do not correspond to the number of somites involved in nephrogenesis. When first formed, one mesonephric tubule develops in a segment, but subsequently each tubule gives origin to secondary and tertiary tubules by budding (Fig. 5.40).

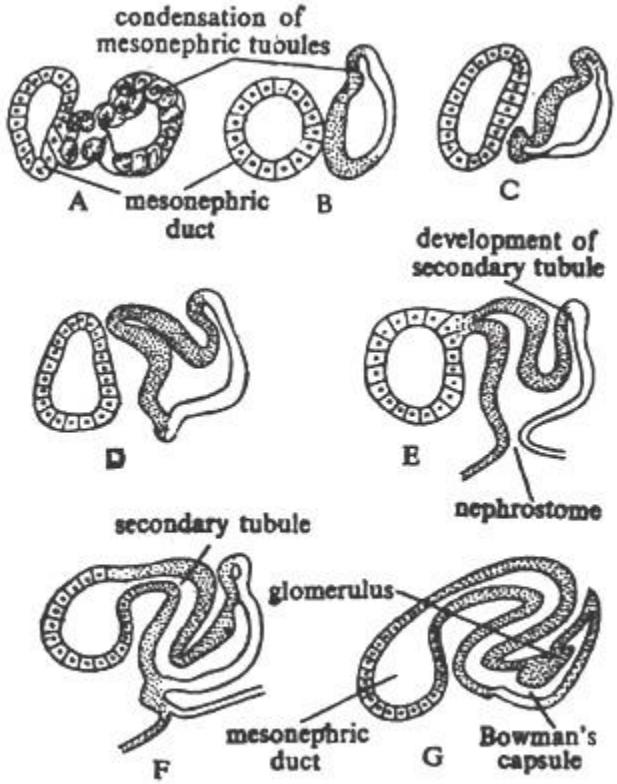


Fig. 5.40. Stages of formation of mesonephric renal unit in frog. Note the origin of secondary tubule.

In case of chick embryo, the mesonephros becomes functional from 5th to 11th days. The tubular system becomes extensively coiled in 8th to 10th days of incubation. After this period the mesonephric tubules start degeneration along the anteroposterior direction and their function is taken over by metanephros which differentiates subsequently in the region posterior to that of mesonephros.

Role of Pronephric Duct in Mesonephros Differentiation:

The mesonephrogenic cord will start differentiation into mesonephric tubules as soon as the pronephric duct is in touch with it. The mesonephrogenic cord develops mesonephric tubules only if stimulated by the pronephric duct.

From this observation it is natural to think that the pronephric duct serves as an inductor for the differentiation of the mesonephros. Extensive experimentations have been done on this issue to ascertain the inductive role of pronephric duct in mesonephros differentiation.

Humphrey (1928), Burns (1938) and Holtfreter (1944) have experimentally obstructed the backward extension of the primordial nephric duct and have found the formation of mere clump of cells in the mesonephrogenic cord. Waddington (1938) and O'Connor (1939) have shown that the mesonephrogenic cord fails to develop renal tubules if the pronephric duct does not reach the specified region.

Local condensation of cells occurs only in the nephrogenic cord. Boyden (1927) by destroying the tip of pronephric duct by cautery and Waddington (1938) by incision of the duct have shown that the differentiation of mesonephrogenic tissue into the mesonephric tubules occurs only when the pronephric duct makes contact with the tissue.

But Gruenwald (1942) and Calame (1962) have cast doubt on such induction and reported that the mesonephrogenic cord is capable of a considerable degree of self-differentiation. Gruenwald (1942) and van Geertruyden (1946) have shown that the nervous tissue, when transplanted into the competent mesonephrogenic cells, can induce mesonephros differentiation.

So it is not unreasonable to think that other tissues (possibly somites) are also involved in this process. So the generalisation that differentiation of mesonephric tubules depends solely upon induction by the pronephric duct appears premature to accept.

Metanephros:

The metanephros is the functional kidney in the postembryonic life of amniotes. It develops from the nephrogenic cord which is derived from the nephrotomes posterior to the mesoriephros adjacent to the cloaca. In chick embryo, the metanephros begins its development at the end of the 4th day of incubation and between the 31-33 somites.

In the embryos of amniotes, the ureteric bud emerges as a diverticulum from the mesonephric duct near its junction with cloaca. This bud develops into the metanephrogenic cord or blastema. The distal end of the bud expands to form the primordial renal pelvis. The metanephrogenic blastema starts condensation around the pelvis (Fig. 5.41).

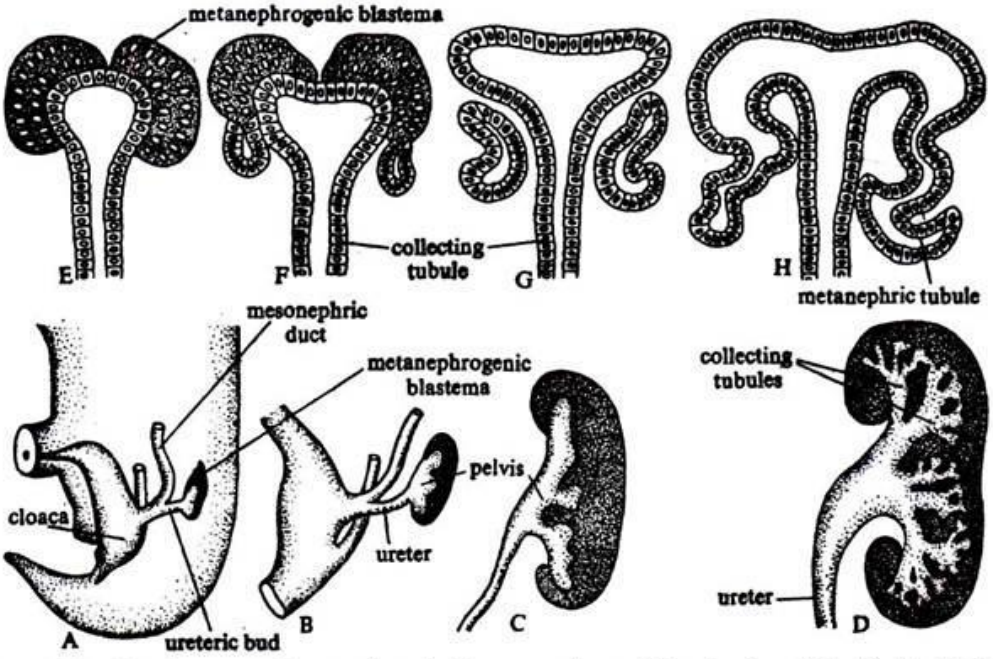


Fig. 5.41. Development of metanephros in human embryos (after Arey) A–F. Sectional views showing the relationship of the metanephrogenic blastema and the ureter.

The pelvis produces subdiverticula, each of which becomes the collecting tubule. The nephrogenic tissue accumulates around the distal end of each collecting tubule and forms 'S'-shaped metanephric tubule (Fig. 5.42).

Each metanephric tubule opens into the collecting tubule at one end and the other end forms a double-walled Bowman's capsule. The metanephros uses the mesonephric duct for the elimination of urine, but the connection between them is not direct, and is established by means of a special outgrowth, the ureteric bud which transforms into the renal pelvis and the ureter.

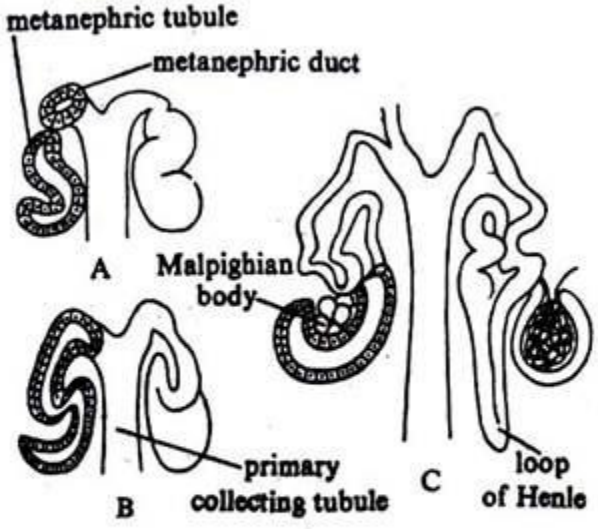


Fig. 5.42. Development of metanephric kidney tubule. A. Condensation of rudiment of renal tubule. B. Renal tubule unites with collecting tubule. C. Subsequent differentiation of renal unit.

Role of Ureteric Bud in Metanephros Differentiation:

The conversion of the metanephrogenic tissue into metanephric tubules is dependent on an induction from the ureteric bud developing from the mesonephric duct. Because the extirpation of either mesonephric duct or ureteric bud causes the failure, of the formation of metanephros. This phenomenon suggests the inductive phenomenon.

It has been experimentally tested that the metanephric primordium undergoes characteristic development when cultured in vitro. The pelvis component develops a system of collecting tubules while the blastema forms coiled tubules. When these two components, after separation with trypsin, are cultured independently, neither of them is able to carry through characteristic morphogenesis.

The subdivision of the renal pelvis is dependent upon the metanephrogenic blastema, while the tubule differentiation in the blastema rests upon an inductive stimulus from the ureteric bud. So the existence of inductive role' played by ureteric bud in metanephric differentiation seems to be positive.

Organogenesis of Human Placenta:

In human females, implantation of the developing embryo occurs in the early luteal phase when the endometrium of the uterus remains in optimum condition. The developing egg reaches the uterus in blastocyst condition with a greatly enlarged blastocoelic space.

The placental organogenesis is described under two broad aspects:

a. Previllous period (6th-13th day).

b. Villous period (14th day to term).

Pre Villous Period:

The implantation of human embryo takes place about 6th to 9th days after fertilization (Figs. 5.44, 5.45).

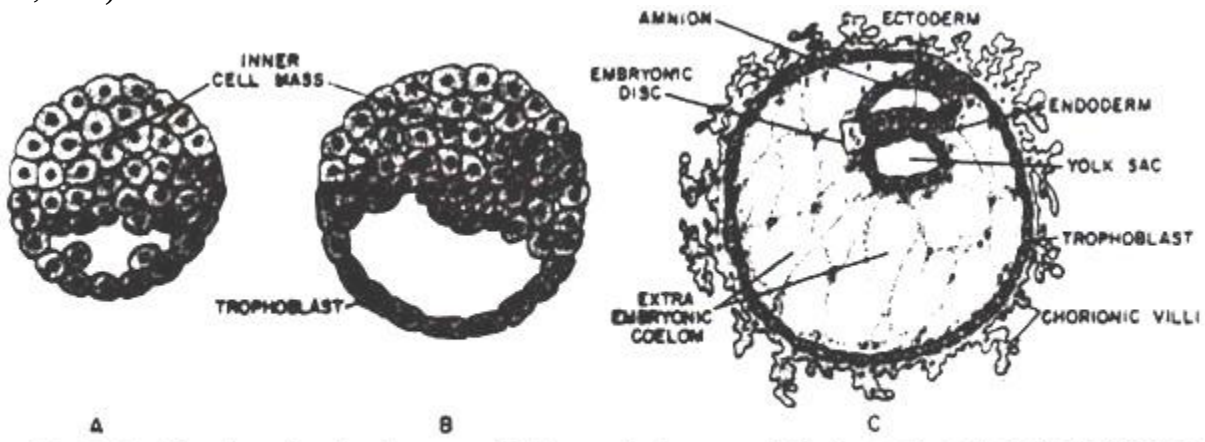


Fig. 5.44. Showing the development of placenta in human. A–C. stages of embryonic development during first week following fertilization (after Patten).

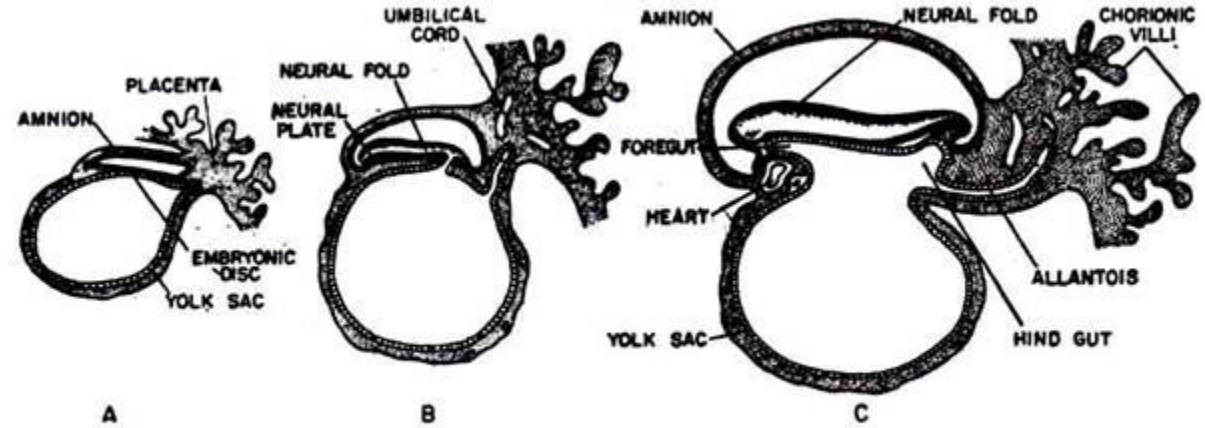


Fig. 5.45. Showing the development of placenta in human (contd.). A–C. Stages of embryonic development during the third week after fertilization (after Arey).

The end of the blastocyst containing the developing germinal disc attaches itself to the uterine wall (Fig. 5.46A). The uterine epithelium is eroded at the region of contact. The trophoblast tissue increases in thickness in this contact area due to the division of epithelial cells of the trophoblast layer.

Prelacunar Stage:

The implanted embryo, consisting of a bilaminar disc, is protruded into a cavity (lecithocoel). This cavity is enclosed by the trophoblast. Profound cytological changes occur in the trophoblast layer. The inner trophoblast cells remain cellular and are designated as the cytotrophoblast while the outer cells fuse together to form a syncytium called the syncytiotrophoblast.

The syncytiotrophoblast serves as the invading tissue of the embryo into the uterine wall. The growth of this syncytiotrophoblast is caused by differentiation of the cytotrophoblast and by amitotic division of the syncytial nuclei.

Lacunar stage (10th to 13th days of Placental development):

As the syncytiotrophoblast invades and increases in quantity, irregular spaces are produced in the syncytiotrophoblast. These spaces are called trophoblastic lacunae. (Fig. 5.46B).

Villous Period:

During the period between 14 and 18 days, the trophoblastic lacunae merge with one another to form large cavities bordered by syncytiotrophoblast. Such a cavity is named as the intervillous space and the primary villi are formed through the proliferation of the cytotrophoblastic elements into the syncytial trabeculae.

The primary villi, when first formed, lack mesodermal core. The mesoderm of the somatopleure invade into them to form the secondary villi (Fig. 5.46D).

With the formation of the blood islands and appearance of blood vessels, the secondary villi transform into the definitive tertiary villi. At this time, some endometrial tissue including blood vessels near to the invading chorionic vesicle break down to produce liquefied areas called the embryotroph. The liquefied

material from the embryotroph is assimilated by the syncytiotrophoblast for the growth of the embryo. This particular type of. nutrition is called the histotrophic nutrition.

From the physiological point of view the foetal villi developing from chorionic plate can be divided into three categories:

(i) Chorionic villi, through which physiological exchange of materials taking place between the foetus and the mother

(ii) Anchoring villi for mechanical anchorage of foetus and

(iii) Free villi.

The developing chorionic vesicle grows and invades the endometrium of the uterus. The uterine mucosa extends over the invading vesicle. The endometrial tissue covering the chorionic vesicle is called the decidua capsularis while the endometrial portion which is not concerned with the closure of such vesicle is called the decidua parietalis or decidua vera.

The endometrial portion lying between the musculature of the uterine wall and the invading villi is called decidua basalts.

Although the chorionic villi are formed over the entire chorionic vesicle, only the villi in relation to decidua basalis are retained and those grown to decidua parietalis are reabsorbed to form a smooth area named as the chorion leave. The villi within decidua basalis become greatly enlarged to serve the main role of physiological interchange (Fig. 5.46E).

This area of chorionic vesicle with the villi is the chorion frondosum which together with the tissue of decidua basalis forms the actual placenta. Thus the placenta is composed of decidua basalis (maternal placenta) and chorion frondosum (foetal placenta) (Fig. 5.46F).

The villi, at the early phase of development, consist of blood capillaries within mesodermal core covered over by cytotrophoblast and syncytiotrophoblast on the outer side. As development goes on the blood capillaries grow enormously and the cytotrophoblast layer is extremely reduced to few scattered cells below syncytiotrophoblast.

The villi are aggregated into groups called the cotyledons which are separated by incomplete placental septa. The villi in each cotyledon remain surrounded by a pool of maternal blood and by this way a hemochorial type of placenta is established.

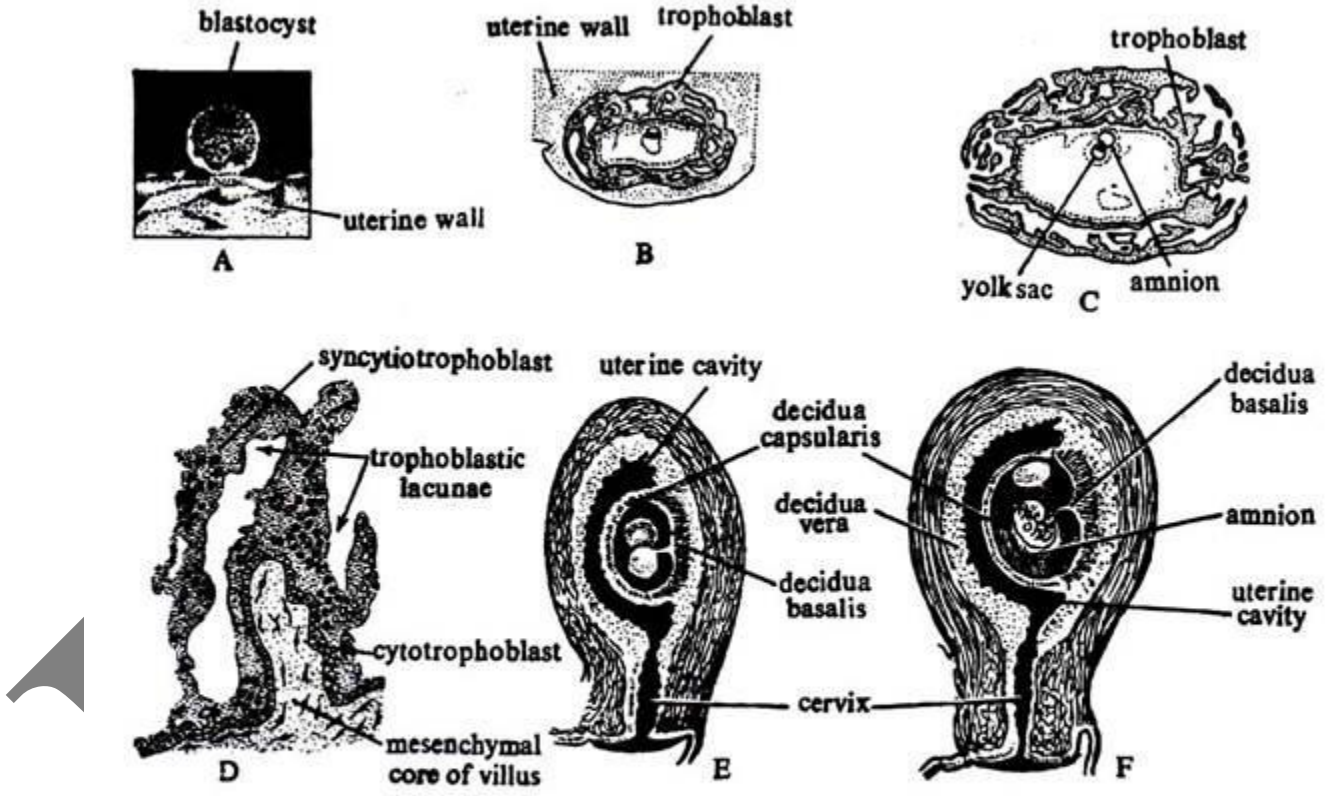


Fig. 5.46. Showing the development of placenta in human (contd.). A. Surface view of the implantation site of a human blastocyst. B. Sectional view of blastocyst of about 12 days which is engulfed completely inside the endometrium. C. Sectional view of embryonic vesicle of about 13–15 days. Note the presence of trophoblastic lacunae. D. Formation of secondary villus. E. Sectional view of uterus along with the developing embryo of 4 weeks old. F. Sectional view of six weeks old embryo. Note the disappearance of villi at one side and elaboration of villi on chorion frondosum (after various sources).

Development of Immune system

Development of the immune system will also link to cardiovascular development notes (blood and vessel) and bone marrow development. Two organs which also relate to this system are the thymus and spleen, which have in the past been included in endocrine and gastrointestinal tract

development respectively. There are now also movies showing lymphocyte (B and T cells) traffic within adult lymph nodes.

The maternal immune system also has specialised functions and cells associated with implantation, placenta development and pregnancy.

- **During prenatal development**, maternal IgG antibodies are transferred from about **GA** week 13 from maternal blood across the placenta. Placenta syncytiotrophoblast cell endosomes bind IgG, in maternal blood lacunae, through neonatal Fc receptors.
- **During early postnatal development**, maternal IgA, IgM, IgG antibodies are transferred from breast milk into the infant gastrointestinal tract.
- **During postnatal development**, the immune system matures

Spleen Development

The human spleen arises in week 5 within the dorsal mesogastrium as proliferating mesenchyme overlying the dorsal pancreatic endoderm. Cells required for its hemopoietic function arise from the yolk sac wall and near dorsal aorta.

The spleen generates both red and white cells in the 2nd trimester. Note that many embryonic RBCs remain nucleated.

Thymus Development

The thymus has a key role in the development of an effective immune system as well as an endocrine function.

The thymus has two origins for the lymphoid thymocytes and the thymic epithelial cells. The thymic epithelium begins as two flask-shape endodermal diverticula that form from the third pharyngeal pouch and extend lateralward and backward into the surrounding mesoderm and neural crest-derived mesenchyme in front of the ventral aorta.

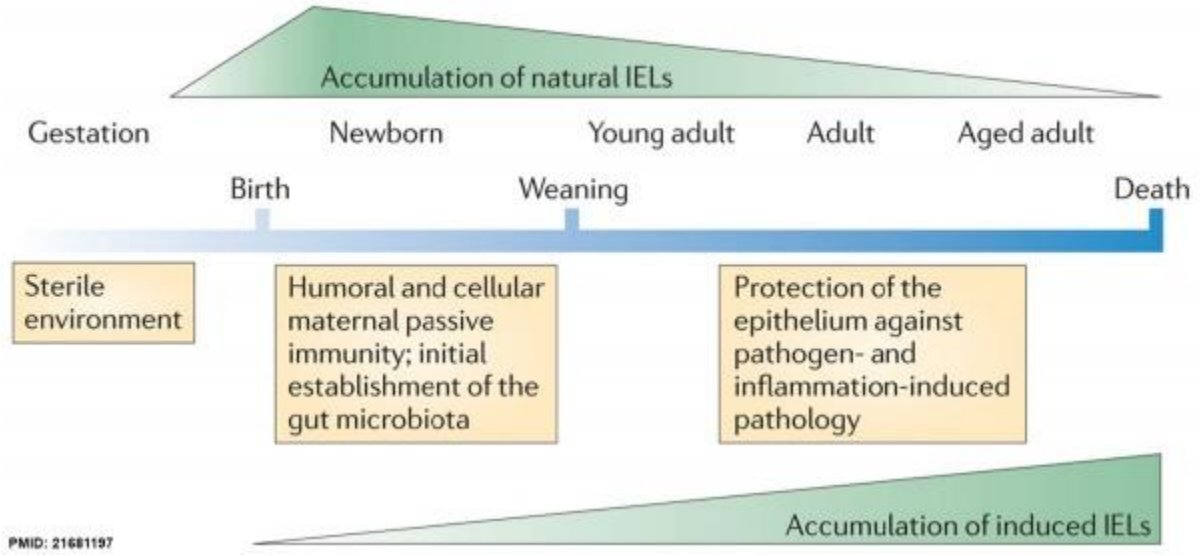
T Lymphocyte Development

A study of cord blood from 19 early second and third trimester fetuses (GA 18-36 weeks) and 16 term newborns (GA 37-42 weeks).

- Percentage of lymphocytes in fetal white blood cells was 79.3%, reducing to 40% by term birth
- higher than that of adults.
- Mononuclear cells (cord blood mononuclear cells (CBMC)
- fetal mononuclear cells were unable to produce IL-2, IL-4 or IFN-gamma.
- spontaneously secreted IL-10, IL-6 and TNF-alpha in vitro.
- fail to respond to mitogen (PHA) or allogeneic stimulation in vitro.
- Stimulation with PHA up-regulated the production of IL-10, IL-6 and TNF-alpha substantially.
- CD3+ T cells in fetal (40.1%) and neonatal (42.4%)
- lower than that of men (59.6%) and pregnant women (53.6%).
- CD8+ T cells (9.5%)
- gamma delta - T cells (0.5%)
- NK cells (4.8%)

Gastrointestinal Tract

Intraepithelial Lymphocytes



Thymic and peripheral differentiation of natural and induced intraepithelial lymphocyte.

Peyer's Patches

Peyer's patches appear and are continuously formed from 13.5 weeks (**GA** 15.5 wk) through to about 36 weeks in the gastrointestinal tract. Then between 15.5 and 20 weeks (**GA**17.5 wk to 22 wk) they develop the surface markers of lymphocytes in the mucosal immune system of the gut. This developmental change is also occurring within the appendix lymphocyte markers: HLA - DR, CD19 (B cell population), CD9 (pre-B cells), CD3 T lymphocytes, CD4 helper / inducer lymphocytes, the CD8 suppressor / cytotoxic cells and lastly, the CD57 Natural Killer cells.

Other Organs

- **Liver** - The adult liver is a lymphoid organ with a predominantly innate immune system. NK cells are abundant in the normal liver (about one-third of intrahepatic lymphocytes), differs from other lymphoid organs and peripheral blood.

Maternal Antibodies

During prenatal development, maternal antibodies are transferred across the placenta to the fetus. Immunoglobulin G (IgG) is transferred across the syncytiotrophoblast cell layer is mediated by the Neonatal Fc receptor (FcRn). Once inside placental villi, immunoglobulins then need to enter fetal circulation by crossing the second cellular endothelial cell layer by an as yet unknown mechanism.

During postnatal development, maternal antibodies are transferred by maternal milk across the neonatal gastrointestinal tract epithelium by the Neonatal Fc receptor (FcRn).

Genetic basis of differentiation

The development of a complex, multicellular organism such as a human being is accompanied by changes in which a single, unspecialized cell (the fertilized egg or zygote) ultimately gives rise to cells and tissues that are highly specialized in structure and function.

This progressive transition is characterized by four component processes: determination, differentiation, growth, and morphogenesis.

New cells are usually not "committed" to a specific function. For example, following fertilization of an egg cell or ovum by a sperm cell, the resulting zygote divides mitotically many items to produce a ball of cells called a blastula.

Each cell or group of cells in the blastula subsequently gives rise to specific tissues and organs of the developing embryo.

Although a blastula gives rise to a single embryo, should one cell separate from the others at an early blastula stage, that cell may also develop into a complete embryo. In humans, it is this phenomenon that results in identical twins, identical triplets, and so on. At later stages of embryo development, cells that normally become epidermal tissue (i.e., ectoderm) can be surgically transplanted to another part of the embryo and there develop into mesodermal tissue (e.g., muscle) or endodermal tissue (e.g., intestinal epithelium).

At some early stages of an organism's development, all cells have the potential to develop into any of the variety of different tissue and cell types that characterize that organism. This potential is called totipotency. Generally, at some point in development, cells become committed to a specific course of differentiation, that is, they differentiate into a specific type of cell or tissue. The process that establishes the fate of a cell is called determination. During determination, certain genes become permanently "turned off" and others are sequentially expressed, further and further narrowing the course of differentiation of the cell.

During differentiation cells take on new and specific properties. These can be structural (such as the formation of organized arrays of actin and myosin filaments in muscle cells) or biochemical (as in the appearance of enzymes of a new metabolic pathway). Differentiation can also take the form of loss of existing structures or biochemical processes. For example, in the differentiation of mammalian red blood cells, the nucleus and other cellular organelles are lost, together with the biochemical processes that were provided by these structures.

The determination and differentiation of the cells of a developing organism are accompanied by growth, that is, an increase in the size and number of cells comprising the organism. In humans, for example, embryonic and fetal development proceeds from the hundreds of cells comprising the small blastula to a fetus weighing several kilograms and containing hundreds of millions of cells.

Growth, in turn, is accompanied by morphogenesis—the generation of form and shape in the developing organism. In this process, differentiating and growing cells give rise to the characteristic organizational pattern of the organism. Small masses of cells take on the form and shape of specific and identifiable structures, such as bones, appendages, the brain, and other organs.

The pattern of differentiation of a cell is founded on the nature of the DNA in the cell nucleus. Before a cell can develop into a hair cell of a mammal, a feather cell of a bird, or a scale cell of a reptile, its nucleus must contain a genome whose transcription and translation into enzymes and other proteins allow the cell to differentiate in a specific direction.

Moreover, given the appropriate genetic complement, conditions must allow these genes to be expressed. Gene expression is regulated at three levels. The first level involves molecular or metabolic interactions, such as mass action and allosteric enzyme function. The second level of control is effected through the interaction between the cell nucleus, the cytoplasm, and the

cytoplasmic organelles. The third level of control involves the interactions between the cell as a whole and its environment.

Nuclear transplantation

Nuclear transplantation is a method in which the nucleus of a donor cell is relocated to a target cell that has had its nucleus removed (enucleated). Nuclear transplantation has allowed experimental embryologists to manipulate the development of an organism and to study the potential of the nucleus to direct development. Nuclear transplantation, as it was first called, was later referred to as somatic nuclear transfer or cloning.

Apoptosis during development

This single term "apoptosis" describes the way in which the majority of cells die within our adult body are removed every day, "Programmed Cell Death". In development, apoptosis begins in the early blastocyst and is a developmental mechanism found throughout tissues in the embryo and fetus developmental stages. In addition to the many developmental roles this process is used in multicellular organisms to remove cells that are: aged, superfluous, infected, contain genetic errors or are transformed.

Blastocyst development

Removing cells from the developing inner cell mass.

Digit development

Removing cells between digits (fingers and toes) of the upper and lower limbs.

Neural development

Removing excess or inappropriately connected neurons.

Ovary Development

Removing excess primordial follicles from the ovary cortex.

Apoptotic Cell Morphology

The following cellular changes occur in sequence during apoptosis.

- loss of cell membrane phospholipid asymmetry
- Condensation of chromatin
- Reduction in nuclear size
- Internucleosomal DNA cleavage
- DNA ladder
- shrinkage of the cell
- Cleavage of cytoskeletal proteins
- actin also binds DNase 1, cleavage may release this enzyme to further cleave DNA
- membrane blebbing
- breakdown of the cell into membrane-bound apoptotic bodies (apoptosomes)
- bodies then phagocytosed by other cells

Terotogens and Terotogenesis

Teratogenesis is a specific term for abnormal process (processes) during the prenatal development that leads to developmental errors (in general called birth defects or congenital malformations - see question 100: Inborn errors of development in human, examples, classification).

Teratogenesis is quite complex process which involves different factors - called teratogens - that affect the normal process of prenatal development. Not each of the teratogen exposures actually leads to formation of some defect, there are many other factors that will influence the final result of the exposure:

Type of teratogenic factor (its "potency").

- Genotype of the mother and embryo/fetus.
- Species of the particular organism.
- Time factor - length and timing of teratogen exposure (mind so called "preteratogenic period".
- Dose/intensity of teratogenic factor.
- Other/random factors.

The word, teratogen, is derived from "terato" meaning monster and "gen, " to give rise to, so teratogens give rise to monsters (not really). Teratogens are non-genetic factors that interfere with normal embryonic and fetal differentiation and morphogenesis. They are not mutagens. Mutagens act randomly on all DNA and do not produce one specific genotype. Children who have been exposed to teratogens *in utero* will not pass their defect on to their children. Because the effects of teratogens are seen at birth and are therefore a congenital defect, they are often thought to be genetic and can mimic genetic disorders. But we know that congenital does not mean genetic and genetic does not mean congenital.

The study of teratogens is part of training in human genetics because it is important to distinguish the problems they cause from genetic disorders. They are also important because they can be prevented by education of the community. Fetal death, prematurity, growth retardation, and unexplained dysmorphology are all suggestive of teratogenic effects.

The four important factors in teratogenicity are

1. **Time.** The gestational age of the fetus at the time of the exposure to the teratogen. Different organs of the body are forming at different times and therefore the sensitivity to the teratogen and the affected organ will vary. There is an "all or none" period in the first two weeks where the fetus is generally not susceptible to teratogens.
2. **Dosage**. To how much of the teratogen was the fetus exposed.
3. **The genotype of the fetus**. The fetus may be more or less resistant to the teratogen because of inactivation of the teratogen.
4. **The genotype of the mother**. Mothers also differ in their ability to detoxify the teratogen.

Common teratogenic agents and their syndromes include alcohol (most common) and fetal alcohol syndrome (FAS), cocaine and central nervous system (CNS) defects, smoking and miscarriage, low birth weight, mental retardation, prematurity and sudden infant death (SIDs), uncontrolled diabetes and caudal regression, cardiac and neural tube defects (NTD), isotretinoin (retinine for acne) and fetal loss, head, ear, CNS abnormalities, rubella and deafness, varicella and limb defects and skin scarring, PKU and mental retardation, seizure medications and the fetal hydantoin syndrome (FHS), thalidomide and limb defects. There are teratogen registries and all street drugs and medications have been classified into categories A,B,C,D,X where A and B show no evidence of risk and C, D, and X show evidence of risk in increasing certainty. The

information often comes from retrospective and uncontrolled studies so information is usually not complete although a few are well documented.

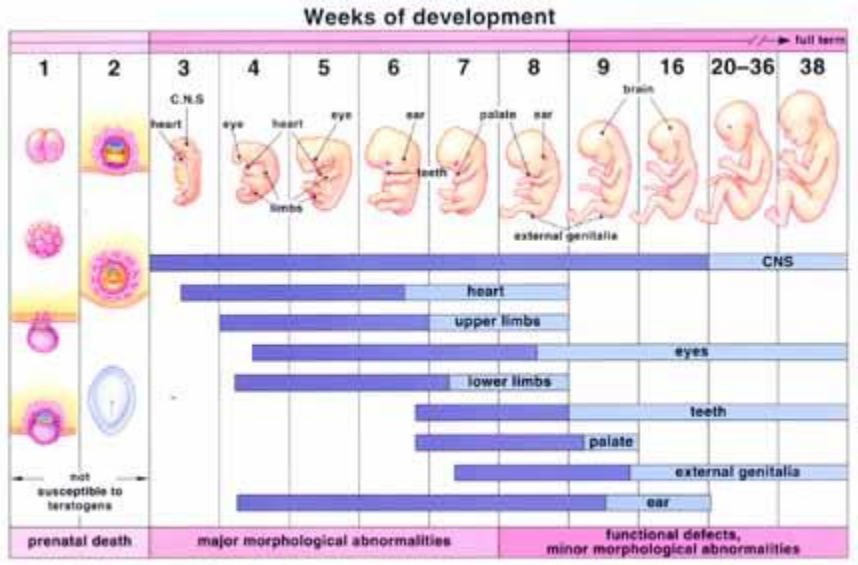


Fig. 34.24 Sensitivity to Teratogens during pregnancy.

Possible questions

1. What is organogenesis? Explain with induction and differentiation of organs?
2. Discuss about the development of immune system.
3. Write about differentiation of brain, eye, ear and limb during organogenesis.
4. Comment on genetic basis of differentiation.
5. Give a brief account on genetic basis of differentiation.
6. Write about nuclear transplantation in brief.
7. Discuss about the development of immune system.
8. Write about the teratogens and teratogenesis.
9. What is organogenesis? Explain with induction and differentiation of organs?
10. Explain in detail about aging process.

KARPAGAM ACADEMY OF HIGHER EDUCATION
DEPARTMENT OF BIOCHEMISTRY
I-M.Sc., BIOCHEMISTRY
DEVELOPMENTAL GENETICS (18BCP203)
MULTIPLE CHOICE QUESTIONS

UNIT V

S.No	Questions	Option A	Option B	Option C	Option D	Answer
1	The vertebrate nervous system is derived from	ectoderm	endoderm	epiderm	mesoderm	ectoderm
2	Vertebrate lungs are derived from	ectoderm	endoderm	epiderm	mesoderm	endoderm
3	Vertebrates develop bones from	ectoderm	endoderm	epiderm	mesoderm	mesoderm
4	What is the estimated average rate at which the human brain generates ne	250 per day	250,000 per minute	25 million per minute	25,000 per second	250,000 per minute
5	A newborn infant obtains maternal antibodies from the mother's	lanugo	colostrum	rubella	afterbirth	colostrum
6	What chemical messenger causes the uterus to contract during labor and birth?	oxytocin	prostaglandins	both oxytocin and prostaglandins	none of the options	both oxytocin and prostaglandins
7	When does neurulation occur during human pregnancy?	first week	second week	third week	fourth week	third week
8	The first truly vertebrate phase of development is marked by the formation of the	archenteron	primitive streak	notochord	neural crest	neural crest
9	The gut or digestive tract of a vertebrate arises from the	vegetal pole	primitive streak	archenteron	blastocoel	archenteron
10	In humans, organogenesis in the embryo is essentially complete by the end of which stage of the pregnancy?	first week	first month	first trimester	second trimester	first trimester
11	The allantois is _____	occurs in birds but not in mammals	is an outgrowth of the amnion in all animals producing it	contributes blood vessels to the umbilical cord in mammals	becomes the placenta in mammals	contributes blood vessels to the umbilical cord in mammals
12	Contact of the optic stalk with ectoderm induces the formation of the	retina	lens	cornea	iris	lens
13	Stage of development in which body organs are formed, cells interact and differentiate is called	gastrulation	fertilization	organogenesis	growth	organogenesis
14	Stage of development in which organs size increase and adult body is attained, is known as	gamete formation	gastrulation	fertilization	growth	growth
15	The digestive system develops from the	ectoderm	mesoderm	endoderm	C) archenteron	endoderm
16	The oral membrane that breaks through to form the mouth is the	stomodeum	proctodeum	endodeum	mesodeum	stomodeum
17	Face develops from facial processes that are proliferating mesenchyme covered by epithelium, where the mesenchyme ossifies to become facial bones. The two medial nasal processes grow down toward the midline and fuse to form the	Nasolacrimal duct	Upper edge of mouth	Lower mandibular process		Upper edge of mouth
18	Fusion of two lower mandibular process produce	Nasolacrimal duct	Lower edge of mouth	Upper edge of mouth	middle of mouth	Lower edge of mouth
19	A pocket like depression forms where anterior end of gut cavity (endodermis) touches and fuses with the epidermis. This membrane thins and breaks through forming the	Nares	Mouth	Lower jaw	Upper jaw	Mouth
20	Place the events of the formation of Teeth in the correct order A. enamel organs form at the in base B. oral epithelium bifurcates at the base C. "Bell Stage" becomes detached from surface D. "Bell Stage" calcification of bone begins E. invagination of oral epithelium	C D E A B	E B A D C	A B C D E	E A B D C	E B A D C
21	The primordium of which organ begins as a midventral outpocking of the pharynx	Liver	Thyroid	Pancreas	Parathyroid glands	Thyroid
22	Which of the following derivatives of the Pharyngeal Pouches does not migrate from where it was produced	Parathyroid glands	Thyroid gland	Tonsils	Post branchial bodies	Tonsils
23	The Foregut of the Endodermal Tube produces evaginations or outpocketing that produce the	Respiratory system-Trachea, Lungs	Allantois	Yolk sac	stomach	Respiratory system-Trachea, Lungs
24	The Hindgut of the Endodermal Tube produces evaginations or outpocketing that produce the	Respiratory system-Trachea, Lungs	Allantois	Yolk sac	stomach	Allantois
25	Place the events in the development of the Lower Respiratory Tract in order A. gives rise to bronchioles and alveoli B. bifurcates into two branches that grow laterally C. pocket-like evagination from the midventral floor of gut D. grows downward and elongates E. continues to branch dichotomously	D A B C E	C D B E A	A B C D E	C D A B E	C D B E A
26	The hepatic diverticulum produces all the following EXCEPT	Liver	Gall bladder	Pancreatic duct	Proximal hepatic ducts	Pancreatic duct
27	Which developing organ in the fetus carries out glycogen storage and urea synthesis	Pancreas	Liver	Thyroid gland	Allantois	Liver
28	The narrow portion of allantois between bladder region and urogenital sinus become	Urethra	Rectum	Bursa Fabricii (Birds)	anal	Urethra
29	Immunity acquired after an infection is	active immunity	Passive immunity	Innate immunity	Passive immunity and Innate immunity	active immunity
30	Innate immunity is	Active acquired immunity	Passive acquired immunity	Inborn immunity	Passive acquired and Inborn immunity	Inborn immunity
31	Which one helps in differentiation of cells of immune system	Cortiosol	Thymosin	Steroid	Thyroxine.	Thymosin

32	Rh- mother carries Rh+ foetus. The foetus is at a risk of disease called	Haemophilia	Haemolytic disease	Tuberculosis	Syphilis	Haemolytic disease
33	In developmental biology, what is meant by the concept of "growth"?	Growth occurs through increases in cell size.	Growth occurs through increases in cell number.	Growth can result from increases in the volume of extracellular matrix between cells.	all options	all options
34	Adult human bone:	grows only at the extreme ends (epiphysis)	grows only in the center (diaphysis)	grows only at the growth plates between the epiphysis and the diaphysis	grows throughout its length	grows only at the growth plates between the epiphysis and the diaphysis
35	Nucleosomes inhibit	activators	RNA polymerase	translation	assembly of transcription factors	assembly of transcription factors
36	Unlike prokaryotes, the control of transcription by eukaryotes is designed to react to change by	changing	ignoring change	remaining constant	changing the environment	remaining constant
37	All of the following can be found in a human transcription complex except	activator	RNA	enhancer	silencer	RNA
38	In many animals, the genes that regulate the development of stem cells are activated	once	only twice	up to 10 times	over a hundred times	once
39	The most common form of gene expression regulation in both bacteria and eukaryotes is	translational control	transcriptional control	post-transcriptional control	post-translational control	transcriptional control
40	Change in frequency of allele in a population due to random sampling of organisms is	gene expression	gene linkage	genetic drift	gene mutation	genetic drift
41	Which cellular organelles are involved in the initiation of the intrinsic pathway of apoptosis?	endoplasmic reticulum	lysosomes	mitochondria	peroxisomes	mitochondria
42	Which of the following are killed by the extrinsic apoptosis pathway?	Cells with damaged DNA.	Developing nerve cells that fail to make profitable connections.	Irradiated cells.	Virus infected cells.	Virus infected cells.
43	Excessive rate of apoptosis causes	AIDS	fever	sneezing	atrophy	atrophy
44	In average human adult, rate of apoptosis per day is	100 billion cells	50-70 billion cells	30 billion cells	10 billion cells	50-70 billion cells
45	Programmed cell death is termed as	oxidative stress	apoptosis	cell cycle	cell division	apoptosis
46	The type of programmed cell death that is a part is normal development is called _____.	mitosis	apoptosis	cytokinesis	angiogenesis	apoptosis
47	Apoptosis begins when _____.	the cell is injured	a “death receptor” on the cell membrane receives the signal to die	caspases become activated	killer enzymes tear up the cytoskeleton	a “death receptor” on the cell membrane receives the signal to die
48	What is the function of apoptosis?	to weed out cells that grow uncontrollably	to allow the immune system to distinguish self from non-self .	to shape organs during development	All options	All options
49	Signs of aging include	loss of hair pigment	dryness and wrinkling of skin	forgetfulness	All options	All options
50	Changes is intracellular substances during aging includes	increased cross linkages of collagen	loss of elasticity in elastic tissues	loss of resilience in connective tissue	All options	All options
51	Process of aging can be slowed by	better nutrition	improved living conditions	adequate sleep	All options	All options
52	Study of aging is called	geronotology	histology	physiology	anthropology	geronotology
53	Which of these substances is/are not teratogenic?	viruses	certain drugs	most water-soluble vitamins	certain fat-soluble vitamins	most water-soluble vitamins
54	Aging appears to result from each of these causes except _____.	genetics	apoptosis	accumulation of free-radicals	adequate nutrition and exercise	adequate nutrition and exercise

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STAFF NAME: Dr. Rajesh Pandiyan

SUBJECT NAME: DEVELOPMENTAL GENETICS

SUB.CODE: 19BCP203

SEMESTER: II **CLASS: III B.Sc., BIOCHEMISTRY**

POSSIBLE QUESTIONS

UNIT – I

1. Differentiate incomplete dominance and codominance with suitable examples.
2. What are the methods employed for sex determination? Write a brief note on it.
3. Discuss about the penetrance and expressivity.
4. Write a brief note on epistasis.
5. Discuss on Mendelian principles and his experiments.
6. Write a short note on multiple allele with examples.
7. Write a note on complementary gene and duplicate gene.
8. Comment a brief note on environmental effects and twin studies.
9. Comment on Mendelian inheritance and his experiments. Explain with monohybrid, dihybrid and trihybrid crosses.

POSSIBLE QUESTIONS

UNIT – II

1. Give a short note on amniocentesis.
2. Comment on molecular probes diagnosis in genetic diseases (Down syndrome and sickle cell anemia).
3. What is karyotyping? Explain the method in detail.
4. Comment on HIV and HPV infections during development.
5. Write in brief about prenatal diagnosis of genetic diseases.
6. Discuss about DNA and RNA probes.
7. Comment about mycobacterial and plasmodial infections during development.
8. Explain in detail how the molecular probes are used in diagnosis of genetic diseases.

POSSIBLE QUESTIONS

UNIT – III

1. Discuss on the significance of different stages of gametogenesis.
2. Discuss in brief about fertilization.
3. Write a note on the essence of activation upon fertilization.
4. Discuss about maturation of oocyte and egg envelopes.
5. Explain the changes in egg cytoplasm during fertilization.
6. Write a note on sperm structure and its types.
7. Comment on spermatogenesis in detail.

POSSIBLE QUESTIONS

UNIT – IV

1. Give a brief note on patterns of embroyonic cleavage.
2. Explain the morphogenetic movements in detail.
3. Write a short note on morphogenetic gradients.
4. Explain in brief about gastrulation.
5. Give a note on role of egg cortex.
6. Explain in detail about primary organs and rudimental organs.
7. Write a short note on morula and blastula.
8. Explain in brief about fate map.

POSSIBLE QUESTIONS

UNIT – V

1. What is organogenesis? Explain with induction and differentiation of organs?
2. Discuss about the development of immune system.
3. Write about differentiation of brain, eye, ear and limb during organogenesis.
4. Comment on genetic basis of differentiation.
5. Give a brief account on genetic basis of differentiation.
6. Write about nuclear transplantation in brief.
7. Discuss about the development of immune system.
8. Write about the teratogens and teratogenesis.
9. What is organogenesis? Explain with induction and differentiation of organs?
10. Explain in detail about aging process.

UNIT-I
SYLLABUS

Mendelian Principle and experiments: Mendelian inheritance-principles; Mendel‟s experiments-monohybrid, dihybrid trihybrid and multihybrid crosses. Interaction of genes: incomplete dominance, codominance, epistasis, complementary genes, duplicate genes, polymeric genes, modifying genes; lethal genes. Environmental influence of gene expression: penetrance and expressivity; temperature, light, phenocopies. Environmental effects and twin studies; human intelligence. Quantitative or polygenic inheritance: Inheritance of kernel color in wheat; corolla length in tobacco skin color inheritance in man, transgressive and regressive variation. Multiple alleles; Sex determination; Extra chromosomal inheritance. Genetic abnormalities

Mendelian inheritance-principles

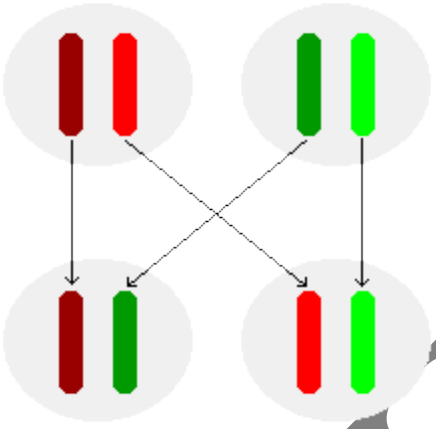
For thousands of years farmers and herders have been selectively breeding their plants and animals to produce more useful hybrids . It was somewhat of a hit or miss process since the actual mechanisms governing inheritance were unknown. Knowledge of these genetic mechanisms finally came as a result of careful laboratory breeding experiments carried out over the last century and a half.

By the 1890's, the invention of better microscopes allowed biologists to discover the basic facts of cell division and sexual reproduction. The focus of genetics research then shifted to understanding what really happens in the transmission of hereditary traits from parents to children. A number of hypotheses were suggested to explain heredity, but Gregor Mendel , a little known Central European monk, was the only one who got it more or less right. His ideas had been published in 1866 but largely went unrecognized until 1900, which was long after his death. His early adult life was spent in relative obscurity doing basic genetics research and teaching high school mathematics, physics, and Greek in Brno (now in the Czech Republic). In his later years, he became the abbot of his monastery and put aside his scientific work.

Mendel's observations from the experiments can be summarized in two principles:

1. the principle of segregation
2. the principle of independent assortment

The **principle of segregation**, for any particular trait, the pair of alleles of each parent separate and only one allele passes from each parent on to an offspring. Which allele in a parent's pair of alleles is inherited is a matter of chance. i.e., segregation of alleles occurs during the process of sex cell formation (i.e., meiosis).



Segregation of alleles in the production of sex cells

The **principle of independent assortment**, different pairs of alleles are passed to offspring independently of each other. The result is that new combinations of genes present in neither parent are possible.

Though the experiments are: (1) Segregation and Dominance: Monohybrid Experiments and (2) Independent Assortment: Dihybrid and Trihybrid Experiments.

Experiment # 1. Segregation and Dominance: Monohybrid Experiments:

After several preliminary trials, Mendel selected the edible pea (Pisum sativum) for his subject. Following his idea that the heredity of each character must be separately investigated in order to arrive at a clear and uncomplicated conclusion, he chose a number of pairs of characters and made separate crosses for each pair between varieties differing markedly in respect of the particular characters.

As the hybrids involve single pairs of characters these are known as Monohybrid Experiments. He chose seven such character pairs, namely, (i) form of ripe seed—round or wrinkled; (ii) colour of cotyledons—yellow or green; (iii) colour of seed-coats and flowers—coloured or white; (iv) form of pod—ripe pod simply inflated or constricted between pods; (v) colour of pod—unripe pod green or yellow; (vi) position of flowers—arranged along axis or bunched at top; and (vii) length of stem (i.e., height of plant)—tall (6 to 7ft.) or dwarf (3/4to 1½ ft.). It will be found out later that Mendel was specially lucky in getting perfect dominance in all these cases and in having the seven characters located in seven different chromosomes. Otherwise, his results could not have been so clear.

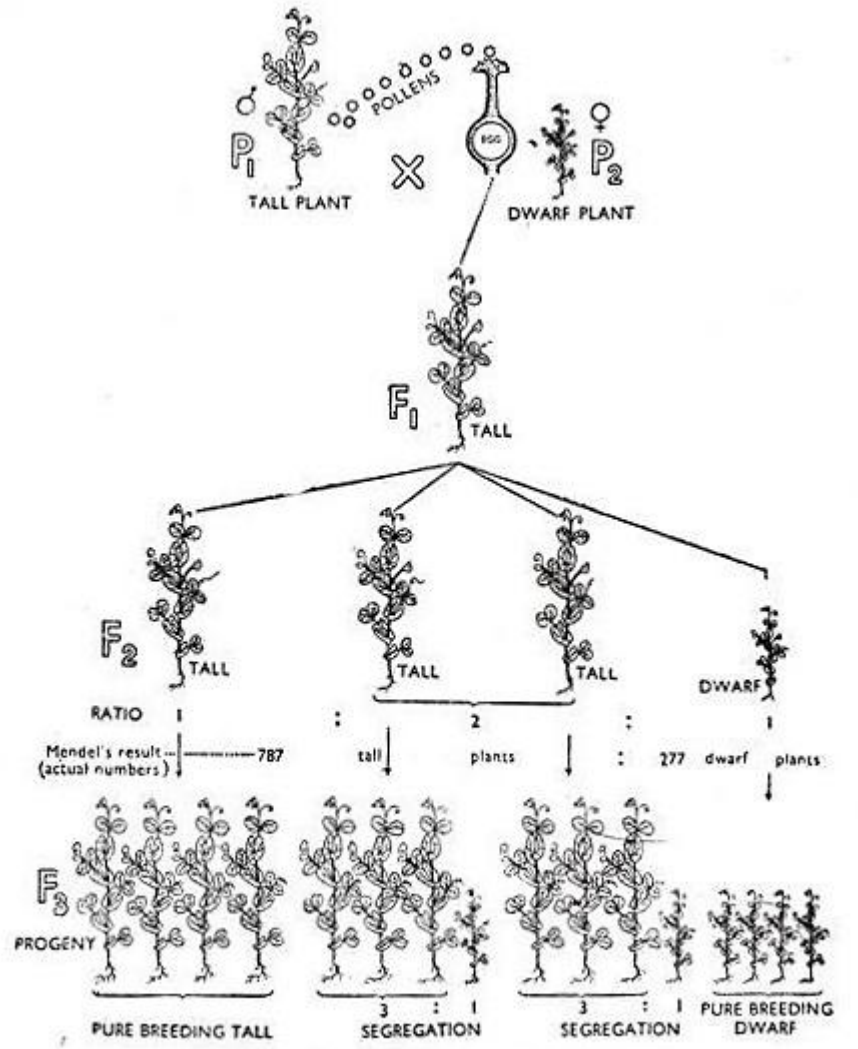


Fig. 817. Diagram illustrating the result obtained by Mendel in his Tall × Dwarf cross.

The pea plants are naturally self-fertilised. Mendel first carried on his experiment separately for each pair of characters. For each pair he crossed between two suitable plants of the proper varieties and grew the hybrid seed. The hybrid plant represents the first filial generation or the F_1 The F_1 plant self-fertilised itself, all its seed was collected and planted to form the second filial generation or F_2. Then the seed of each F_2 plant was planted separately to form a number of separate F_2populations.

The result obtained by Mendel in the Tall x Dwarf cross is diagrammatically represented in Fig. 817.

The results were exactly similar in all the seven different crosses.

Mendel found that in the case of each cross:

1. In the F_1, one character of each pair disappeared. The character that showed itself in the F_1 he signified as dominant while the character that did not show was termed recessive. In the list of the seven character pairs given above, the first character is dominant and the second recessive.

2. In the F_2, the parental characters reappeared. Thus, the dominant F, plant segregated into the parental types. In all these cases he found that in the F_2, the dominant and the recessive types occurred in the ratio 3:1.

3. In the F_3, the recessive plants bred true. But, of the dominant plants only one- third bred true while the other two-thirds again segregated in the ratio 3: 1. So, the F_2 ratio, instead of being stated as 3 dominants: 1 recessive, is more correctly stated as 1 true or pure breeding dominant: 2 segregating dominant: 1 true breeding recessive.

The results obtained by Mendel are shown in the table below:

A glance at the results will show that the F_2 ratios obtained were not exactly 3:1 in most cases. But, when a result is dependent on chance (any plant may die accidentally) such deviations are bound to occur. It will be shown that the results obtained by Mendel are sufficiently correct to conform to the 3:1 ratio.

Mendel explained the phenomena by assuming these principles:

1. Existence of unit character. Each character is represented by a unit in the gamete. In the zygote the two units of characters or factors from the two gametes come to lie side by side but they remain independent and do not lose their individual identities.

2. Occurrence of characters in pairs and Dominance of characters. There are pairs of all types of characters, e.g., tallness and dwarfness for height, round and wrinkled for seed, etc. In a hybrid zygote one such unit comes from each parent and the two units lie together. When both the character units are present, only one of them shows itself and is called dominant while the other lies latent and is called recessive. Such pairs of characters are called allelomorphs or alleles.

TABLE II

SUMMARY OF F_2 RESULTS OF MENDEL'S MONOHYBRID EXPERIMENTS WITH PEAS

Character	Dominant character	No. obtained	Recessive character	No. obtained	Total plants	Phenotypic percentage and ratio
1. Form of seed	Round	5474	Wrinkled	1850	7324	74·75:25·25 2·96:1
2. Colour of cotyledon	Yellow	6022	Green	2001	8023	75.00:25·00 3·00:1
3. Colour of seedcoat	Coloured	705	White	224	929	76·00:24·00 3·15:1
4. Form of pod	Simply inflated	882	Constricted	299	1181	74·75:25·25 2·95:1
5. Colour of pod	Green	428	Yellow	152	580	73·75:26·25 2·82:1
6. Position of flowers	Axial	651	Terminal	207	858	75·75:24·25 3·14:1
7. Height of plant	Tall	787	Dwarf	277	1064	74·00:26·00 2·84:1
Total		14949		5010	19959	75·00:25·00 3·00:1

3. Eventual segregation of units contributed by respective parents. The hybrid plants will again form gametes of two types and these gametes will pair to form zygotes of the next generation. The zygotes will be different as gametes may associate in different ways. So, the F_2plants will be of parental as well as of hybrid types.

This breaking down of the hybrid type into parental and hybrid types are called segregation which is sometimes called the First Law of Mendel. Mendel declared **"it is now clear that the hybrids (F_1) form seeds having one or other of the two differentiating characters and of these one half develop again the hybrid form, while the other half yields plants which remain constant and receive the dominant or the recessive characters (respectively) in equal numbers."**

Segregation can be explained easily if we consider the association of the gametes. A gamete can contain only one unit character of each allelomorphic pair. Thus, if T and d represent the unit characters for tallness and dwarfness respectively, there can be male gametes (formed out of pollens) and female gametes (eggs) of either T or d types. Thus, when the first cross is made, one of the parents gives rise to only T gametes (within pollens) and the other to only d gametes (eggs). The F_1 zygote is Td.

This Td plant now gives rise to two types of pollens resulting in two types of gametes T and d in approximately equal numbers. The same parent will also give rise to equal numbers of T and d eggs. These two types of gametes are now capable of fertilising one another freely. The above facts are diagrammatically represented in Fig. 818.

The diagram explains that the F_2 segregation ratio is

1 TT: 2 Td: 1 dd

or, 1 true breeding tall : 2 segregaing tall : 1 true breeding dwarf.

The TT plants, when self-fertilised, can form only T gametes—so they will be true breeding tails. Similarly dd plants will be true breeding dwarfs. But, the Td plants will again give rise to T and d male and female gametes so that the descendants of these plants will again segregate. Hence, the Td plants are segregating tails.

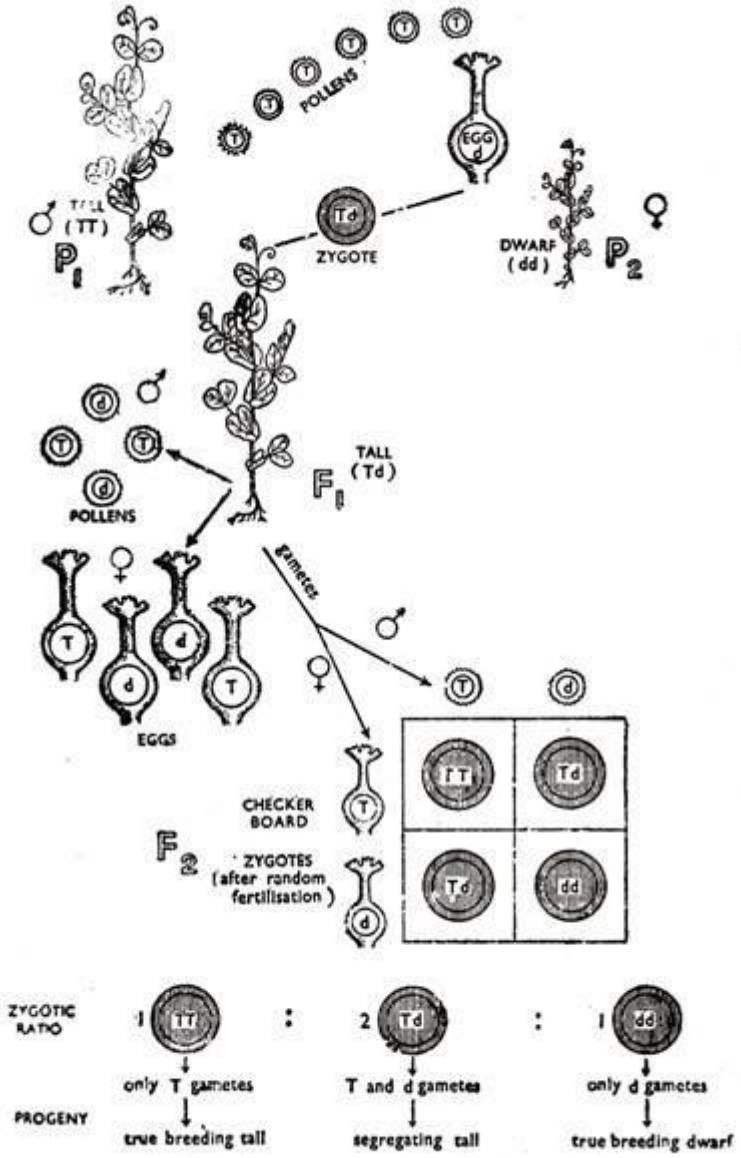


FIG. 818. Diagram explaining monohybrid segregation.

We find that among the tall F_2 plants there are two types, TT and Td. These are constitutionally different as their future segregation shows but, outwardly, their appearances are the same—i.e., tall. When a group of organisms are similar in appearance but not necessarily similar in their genetic constitution, they are said to form a phenotype.

On the other hand, organisms of the same genetic constitution form a genotype. Thus, the F_1 segregation shows two phenotypes—tall and dwarf, but three genotypes—TT, Td and dd.

The zygote formed by the union of two gametes always contains two factors of an allelomorphic pair. If the two factors are the same, e.g., TT or dd, then the zygote is called a homozygote and the plant from it is homozygous. On the other hand, if the two factors are different, e.g., Td, then it is a heterozygote and

the plant from it is called heterozygous. Homozygous plants are always true breeding while heterozygous ones always segregate.

In order to find out the segregation ratio, the gametes and the zygotes have been represented in the diagram in the form of a checker-board or chess-board. This method of finding out the segregation ratio is known as the Checker-board or Chess-board Method.

Experiment # 2. Independent Assortment: Dihybrid & Trihybrid Experiments:

After considering the character pairs singly, Mendel now began his experiments with two pairs of characters simultaneously and thus obtained the Dihybrid Ratio. Here again Mendel had another bit of luck in experiencing no linkage.

For a dihybrid experiment, Mendel crossed a variety having yellow cotyledons and round seeds with one having green cotyledons and wrinkled seeds. The result obtained is shown in Fig. 819.

The F_2 ratio can be explained in the same way as was done in the case of the mono- hybrid ratio. Let Y represent yellow cotyledon, g green cotyledon, R round seed and w wrinkled seed. Thus, a gamete will contain either Y or g and, at the same time either R or w. The eventual segregation is explained by Fig. 820. The checker-board shows that only four phenotypes in the ratio 9 Y-R-: 3 Y-ww: 3 ggR: 1 ggww are possible because Y is dominant over g and R over w. The actual ratio obtained by Mendel is very close to its expected ratios.

It should also be noted that the four phenotypes are composed of the following genotypes:

The 9 of **Y-R-** phenotype include	1 of **YYRR** genotype
	2 of **YYRw** "
	2 of **YgRR** "
	4 of **YgRw** "
3 of **Y-ww** phenotype include	1 of **YYww** genotype:
	2 of **Ygww** "
3 of **ggR-** phenotype include	1 of **ggRR** genotype:
	2 of **ggRw** "

The ggww phenotype is a genotype by itself.

Next, Mendel worked with three pairs of characters and thus obtained a Trihybrid Ratio. He considered the same Yg and Rw alleles along with the characters coloured or white seed-coat. Putting C and c for coloured and white seed-coat respectively, his results may be shown as in Fig. 821.

The F_2 segregation can be easily explained as has been done in the cases of mono- hybrid and dihybrid ratios. Naturally, the checker-board will be a little more complicated, there being 8 possible types of gametes so that the checker-board will have 8X8 = 64 squares.

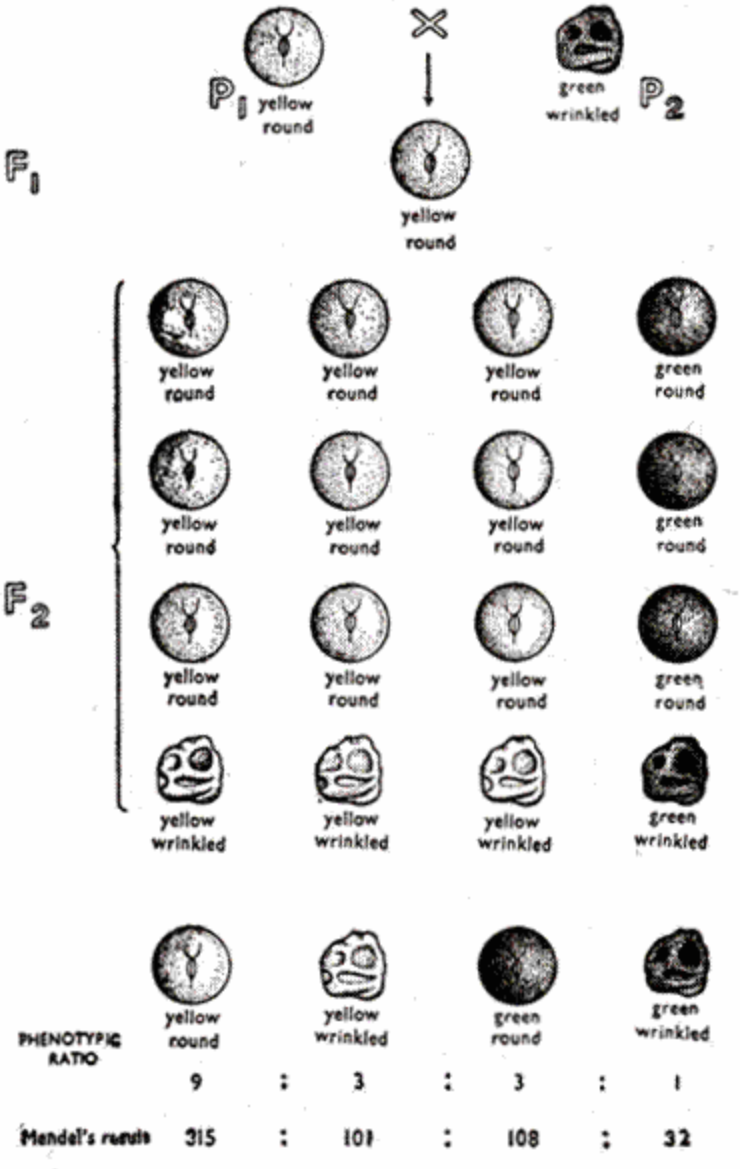


FIG. 819. Dihybrid ratio obtained by Mendel.

Form the dihybrid and trihybrid ratios obtained, Mendel concluded his principle of independent assortment of characters which is sometimes known as Mendel's Second Law. This states that the different factors or allelomorphic pairs in the gametes and zygotes assort themselves and segregate independently of one another.

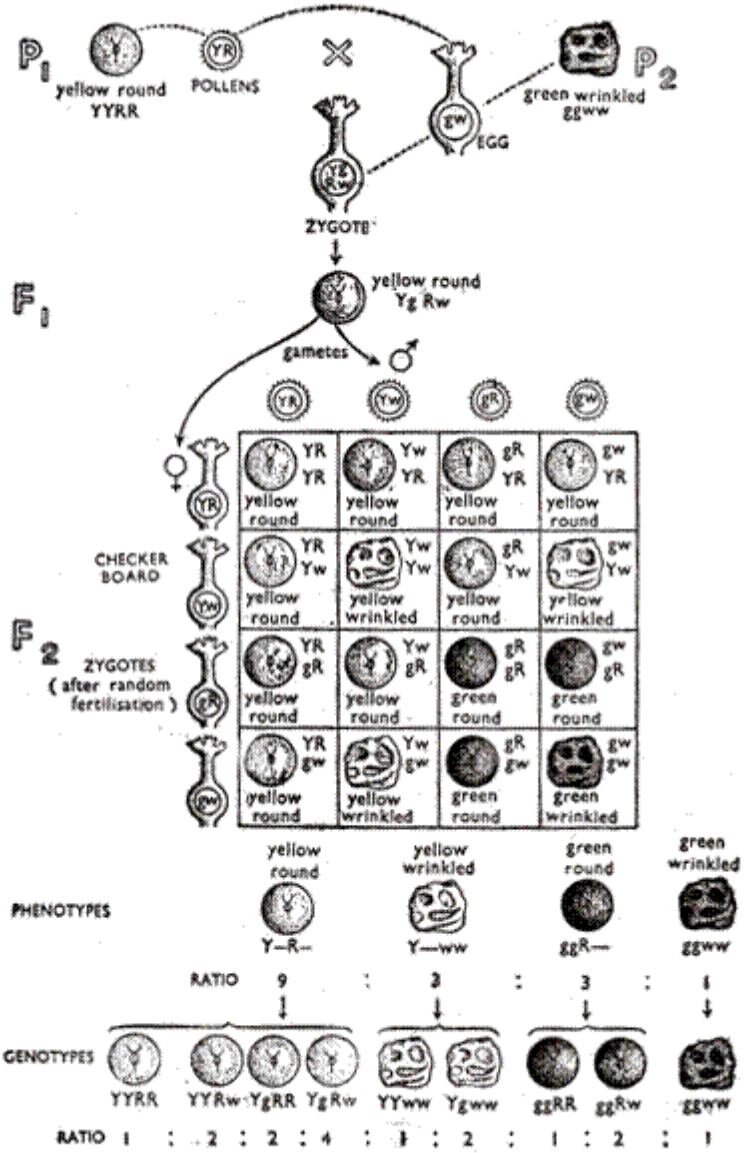


Fig. 820. Explanation of dihybrid ratio by checker-board.

Mendel stated the off-springs of the hybrids in which several essentially different characters are combined exhibit the terms of a series of combinations, in which the developmental series for each pair of differentiating characters are united. It is demonstrated at the same time that the relation of each pair of different characters in hybrid union is independent of the other differences in the two original parental stocks.

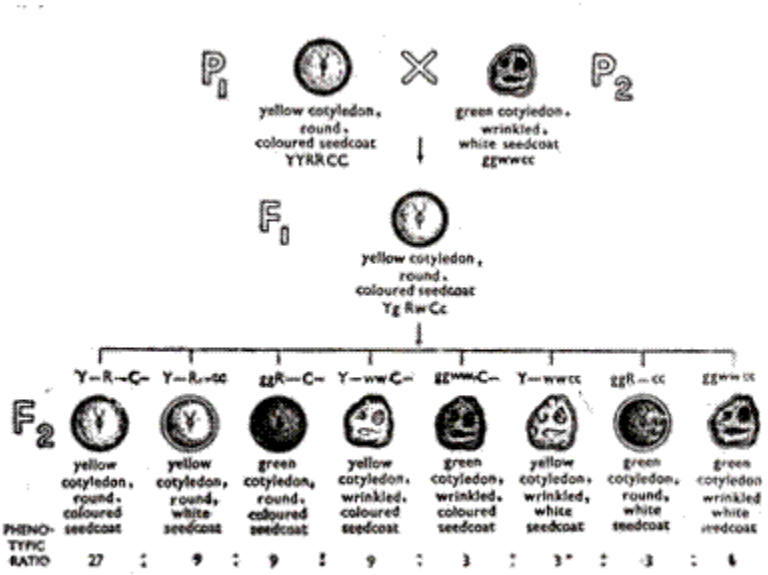


Fig. 821. Trihybrid segregation.

Polyhybrid:

If the principle of independent assortment of factors is followed, it will be found that it is possible to calculate the segregation ratio when more than three pairs of factors are considered together. Naturally, such calculation will be much more complicated. There will be a greater number of phenotypes showing a gradation almost as in fluctuating variations and a still greater number of genotypes.

The great variation of segregates will naturally cause any experimentation very difficult. Such a ratio is known as Polyhybrid Ratio and the hybrid, which is heterozygous for a large number of characters is called a Polyhybrid. Although Mendel did not work directly on this, a number of such experiments have been performed since the rediscovery of Mendel's paper.

INTERACTION OF GENES

GENETIC DOMINANCE

Why you have that particular eye color or hair type? It's all due to gene transmission. As discovered by Gregor Mendel, traits are inherited by the transmission of genes from parents to their offspring. Genes are segments of DNA located on our chromosomes. They are passed on from one generation to the next through sexual reproduction. The gene for a specific trait can exist in more than one form or allele. For each characteristic or trait, animals cells typically inherit two alleles. Paired alleles can be homozygous (having identical alleles) or heterozygous(having different alleles) for a given trait.

When the allele pairs are the same, the genotype for that trait is identical and the phenotype or characteristic that is observed is determined by the homozygous alleles. When the paired alleles for a trait are different or heterozygous, several possibilities may occur. Heterozygous dominance

relationships that are typically seen in animal cells include complete dominance, incomplete dominance, and co-dominance.

- Complete Dominance
- Incomplete Dominance
- Co-dominance
- Differences Between Incomplete Dominance and Co-dominance

COMPLETE DOMINANCE

In **complete dominance** relationships, one allele is dominant and the other is recessive. The dominant allele for a trait completely masks the recessive allele for that trait. The phenotype is determined by the dominant allele. For example, the genes for seed shape in pea plants exists in two forms, one form or allele for round seed shape **(R)** and the other for wrinkled seed shape **(r)**. In pea plants that are heterozygous for seed shape, the round seed shape is dominant over the wrinkled seed shape and the genotype is **(Rr)**.

- Genetic Dominance
- Incomplete Dominance
- Co-dominance
- Differences Between Incomplete Dominance and Co-dominance

INCOMPLETE DOMINANCE

In incomplete dominance relationships, one allele for a specific trait is not completely dominant over the other allele. This results in a third phenotype in which the observed characteristics are a mixture of the dominant and recessive phenotypes. An example of incomplete dominance is seen in hair type inheritance. Curly hair type **(CC)** is dominant to straight hair type **(cc)**. An individual who is heterozygous for this trait will have wavy hair **(Cc)**. The dominant curly characteristic is not fully expressed over the straight characteristic, producing the intermediate characteristic of wavy hair. In incomplete dominance, one characteristic may be slightly more observable than another for a given trait. For example, an individual with wavy hair may have more or fewer waves than another with wavy hair. This indicates that the allele for one phenotype is expressed slightly more than the allele for the other phenotype.

- Genetic Dominance
- Complete Dominance
- Co-dominance
- Differences Between Incomplete Dominance and Co-dominance

CO-DOMINANCE

In **co-dominance** relationships, neither allele is dominant, but both alleles for a specific trait are completely expressed. This results in a third phenotype in which more than one phenotype is observed. An example of co-dominance is seen in individuals with the sickle cell trait. Sickle cell disorder results

from the development of abnormally shaped red blood cells. Normal red blood cells have a biconcave, disc-like shape and contain enormous amounts of a protein called hemoglobin. Hemoglobin helps red blood cells bind to and transport oxygen to cells and tissues of the body. Sickle cell is a result of a mutation in the hemoglobin gene. This hemoglobin is abnormal and causes blood cells to take on a sickle shape. Sickle-shaped cells often become stuck in blood vessels blocking normal blood flow. Those that carry the sickle cell trait are heterozygous for the sickle hemoglobin gene, inheriting one normal hemoglobin gene and one sickle hemoglobin gene. They do not have the disease because the sickle hemoglobin allele and normal hemoglobin allele are co-dominant with regard to cell shape. This means that both normal red blood cells and sickle-shaped cells are produced in carriers of the sickle cell trait. Individuals with sickle cell anemia are homozygous recessive for the sickle hemoglobin gene and have the disease.

- Genetic Dominance
- Complete Dominance
- Incomplete Dominance
- Differences Between Incomplete Dominance and Co-dominance

DIFFERENCES BETWEEN INCOMPLETE DOMINANCE AND CO-DOMINANCE

The pink tulip color is a mixture of the expression of both alleles (red and white), resulting in an intermediate phenotype (pink). This is incomplete dominance. In the red and white tulip, both alleles are completely expressed. This shows co-dominance. Pink / Peter Chadwick LRPS/Moment/Getty Images - Red and white / Sven Robbe/EyeEm/Getty Images

INCOMPLETE DOMINANCE VS CO-DOMINANCE

People tend to confuse incomplete dominance and co-dominance relationships. While they are both patterns of inheritance, they differ in gene expression. Some differences between the two are listed below:

1. ALLELE EXPRESSION

- **Incomplete Dominance:** One allele for a specific trait is not completely expressed over its paired allele. Using flower color in tulips as an example, the allele for red color **(R)** does not totally mask the allele for white color **(r)**.
- **Co-dominance:** Both alleles for a specific trait are completely expressed. The allele for red color **(R)** and the allele for white color **(r)** are both expressed and seen in the hybrid.

2. ALLELE DEPENDENCE

- **Incomplete Dominance:** The effect of one allele is dependent upon its paired allele for a given trait.
- **Co-dominance:** The effect of one allele is independent of its paired allele for a given trait.

3. PHENOTYPE

- **Incomplete Dominance:** The hybrid phenotype is a mixture of the expression of both alleles, resulting in a third intermediate phenotype. Example: Red flower **(RR)** X White flower **(rr)** = Pink flower **(Rr)**
- **Co-dominance:** The hybrid phenotype is a combination of the expressed alleles, resulting in a third phenotype that includes both phenotypes. (Example: Red flower **(RR)** X White flower **(rr)** = Red and white flower **(Rr)**

4. OBSERVABLE CHARACTERISTICS

- **Incomplete Dominance:** The phenotype may be expressed to varying degrees in the hybrid. (Example: A pink flower may have lighter or darker coloration depending on the quantitative expression of one allele versus the other.)
- **Co-dominance:** Both phenotypes are fully expressed in the hybrid genotype.

EXAMPLES

In **incomplete dominance** relationships, one allele for a specific trait is not completely dominant over the other allele. This results in a third phenotype in which the observed characteristics are a mixture of the dominant and recessive phenotypes. In **co-dominance** relationships, neither allele is dominant but both alleles for a specific trait are completely expressed. This results in a third phenotype in which more than one phenotype is observed.

- Genetic Dominance
- Complete Dominance
- Incomplete Dominance
- Co-dominance

Additional input

In **codominance**, both alleles are expressed and appear in the heterozygote. Human ABO blood types are one example of where this occurs. Blood type in this case is determined by a single locus, where three different alleles are possible (I^A, I^B, I^O). I^O is completely recessive to both I^A and I^B, so an I^AI^O individual is phenotypically A and an IBIO individual is phenotypically B. However, an individual whose genotype is I^AI^B, is phenotypically AB, as the I^A and I^B alleles are codominant.

To understand why this is the case, its necessary to look at ABO gene function. It may all sound a bit "Old MacDonald's Farm" with all these I^As and I^Os, but bear with us on this one.

What are blood types about anyway? Vampire lore says different blood types have different flavors—well, maybe, if you're a vampire with a particularly sensitive palate. But for us regular, everyday mortals, the important point is this: the different blood groups have different kinds of proteins, called antigens, on the surface of their blood cells.

The I^A allele codes for the production of "A antigens," I^B for "B antigens," while I^O doesn't produce any kind of antigen at all (the slacker).

Also, the immune systems of individuals with different blood groups produce different antibodies (proteins that identify and help neutralize foreign objects and cells). Individuals with blood type A produce antibodies against B antigens, individuals with blood type B produce antibodies against A antigens, but individuals with blood type AB don't produce antibodies against either A or B antigens (because it would be a bit of a disaster all round for them if they did). Individuals with blood type O produce antibodies against both A and B antigens.

This, by the way, is why people with AB blood type are universal receivers; they can receive blood transfusions from any blood group precisely because they produce no anti-blood group antibodies. On the other hand, people with O blood types are universal donors: anyone can receive their blood because it can't provoke an immune response, thanks to having no antigens on the surface of its blood cells.

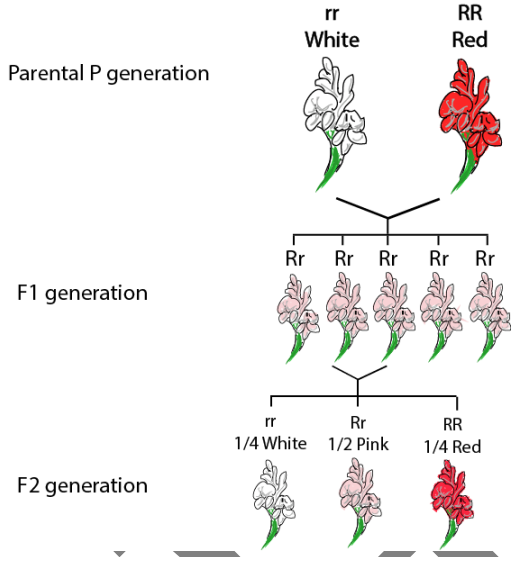
When alleles show **incomplete dominance**, the phenotype of the heterozygote is intermediate in nature between those produced by the homozygous genotypes. This is because the heterozygote has only one functional copy of the gene, and thus produces half the protein. Think of it as a dosage problem: instead of two teaspoons of cough syrup (homozygote for cough syrup dose), you get a teaspoon of cough syrup and a teaspoon of water (heterozygote for cough syrup dose), or two teaspoons of water (no cough syrup at all—ouch!).

Let's look at a real-world example: if a true-breeding red snapdragon (*RR*) is crossed to a true-breeding white snapdragon (*WW*), all of the resulting F1 are pink (*RW*). The pink simply results from producing half the amount of red pigment.

It's tempting, however, to think it works just like paint—red paint mixed with some white paint produces pink paint. In fact, before Mendel's discoveries, many believed in such "blending inheritance": that the phenotype of an offspring resulted from the mixing of the parent's phenotypes, and so is usually in between their traits.

Back it up, though. Taking a closer look at flower color inheritance reveals it's actually consistent with Mendelian principles. If the pink F1 individuals (*RW*) are interbred to produce an F2 generation, 50% of the resulting plants have pink flowers (*RW*), 25% have red flowers (*RR*), and 25% have white flowers (*WW*). The F1 plants produced gametes carrying *R* and *W* in equal numbers that, when mixed up to produce new plants, generate the same genotypic ratios as with the traditional Mendelian traits.

Just like it was in the case of codominance, however, because the heterozygote produces a different phenotype than the homozygous dominant, the phenotypic ratios exactly match the genotypic ratios (which they don't do in a standard dominant/recessive relationship).



EPISTASIS

Due to the phenomenon of dominance a recessive allele remains obscure in the hybrid. But when two different genes which are not alleles, both affect the same character in such a way that the expression of one masks, inhibits or suppresses the expression of the other gene, it is called epistasis. The gene that suppresses is said to be epistatic, and the gene which remains obscure is hypostatic.

Types of Epistasis:

i. Dominant Epistasis:

In poultry white birds belong to two different varieties namely white leghorns or white Wyandotte's. Experiments reveal that the gene for white plumage of white leghorns is dominant over the gene for coloured plumage of coloured varieties. But the gene for white plumage of white Wyandotte's is recessive to the gene for coloured plumage of coloured varieties.

Therefore the gene which produces white plumage in white leghorns is different from the gene for white plumage in white Wyandotte's. A cross between a white leghorn and a white Wyandotte gives an F_1 of white birds with small dark flecks. When such birds are inbred, the F_2 progeny segregates in the ratio of 13 white to 3 coloured birds.

The experiment is explained below by postulating two genes C and I for the white leghorns:

P:	White Leghorn	×	White Wyandotte	
	IICC		*iicc*	
Gametes:	*IC*		*ic*	
	White with small			
F_1:	dark flecks	×	Inbred	
	IiCc			
F_2:		White	:	Coloured
		13		3

A checkerboard for the 16 phenotypes and genotypes of the F_2 birds indicates that only three out of sixteen genotypes, that is iiCC, iiCc, iiCc produce coloured birds. The white leghorns obviously contain

a gene I, which in the dominant state inhibits or suppresses the expression of the dominant colour gene C, resulting in white plumage.

The recessive alleles of the inhibitor gene (ii) produce coloured birds due to expression of gene C. In other words gene I is epistatic to gene C. This is a case of dominant epistasis because even one dominant allele of gene I is able to express itself.

ii. Recessive Epistasis:

Epistasis due to recessive genes is called recessive epistasis. In mice albinism (white coat) is produced by a recessive gene aa. There is a different gene B which in the dominant state (BB and Bb) produces grey coat colour called agouti, and when recessive (bb) leads to black coat colour.

The recessive gene for albinism (aa) is found to be epistatic to the gene for agouti (BB and Bb), and also to its recessive, homozygous allele (bb) for black. The presence of the dominant allele (AA) of the epistatic gene allows expression of gene B so that agouti (BB and Bb) and black (bb) coat colours can be produced (Fig. 2.2).

P: Agouti *AABB* × Albino *aabb*

Gametes: *AB* *ab*

F_1: Agouti *AaBb* × Agouti *AaBb*

Gametes: *AB*, *Ab*, *aB*, *ab*

	AB	*Ab*	*aB*	*ab*
A B	*AABB* Agouti	*AABb* Agouti	*AaBB* Agouti	*AaBb* Agouti
Ab	*AABb* Agouti	*AAbb* Black	*AaBb* Agouti	*Aabb* Black
aB	*AaBB* Agouti	*AaBb* Agouti	*aaBB* Albino	*aaBb* Albino
ab	*AaBb* Agouti	*Aabb* Black	*aaBb* Albino	*aabb* Albino

9 Agouti : 3 Black : 4 Albino

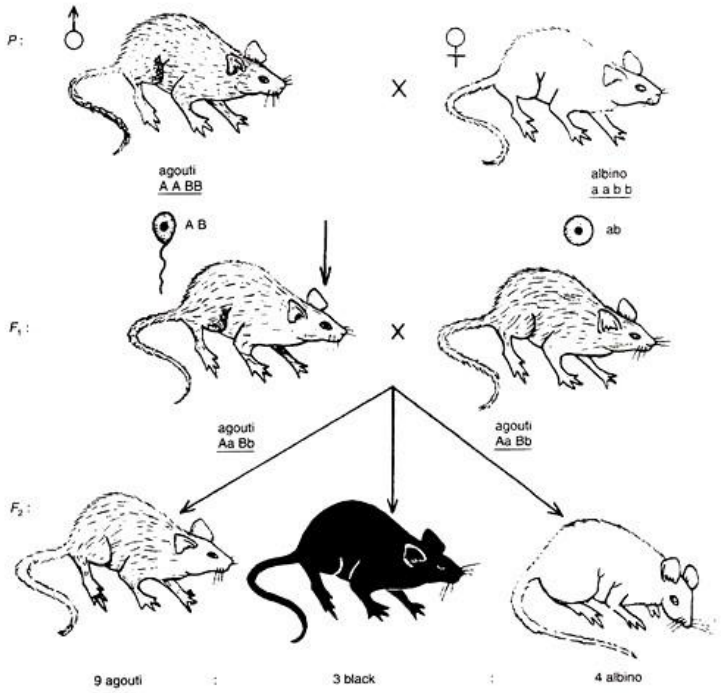


Fig. 2.2 Inheritance of the coat colour gene in mouse illustrating recessive epistasis.

The 9: 3: 4 ratio obtained is a modification of the classical 9: 3: 3 : 1 in which the last two classes (3: 1) are phenotypically identical and are therefore added up together. In human beings also the recessive gene for albinism shows epistasis in a similar manner. Epistatic effect is usually only in one direction, from one particular gene pair to another.

Epistasis in Drosophila:

There are two recessive wing mutants in Drosophila: apterous (ap) which produces small stubby wings instead of the normal transparent ones; the other called cubitus interrupts (ci) which causes a small interruption in the fourth longitudinal vein (Fig. 2.3). When the two mutants are used in a dihybrid cross, the F_2 progeny segregates in the ratio of 9 normal: 3 interrupted vein: 4 apterous wing.

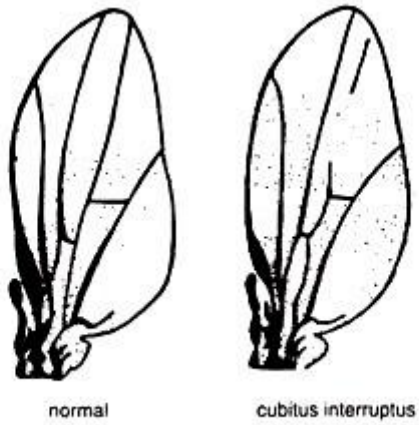


Fig. 2.3 Normal and interrupted veins in lateral wings of *Drosophila*.

P:	apterous	×		interrupted vein
	ap/ap+/+			*+/+Ci/Ci*
Gametes:	*ap+*			*+Ci*
F_1:		Wild type	×	Inbred
		ap/+Ci/+		
F_2:	9 Wild type	: 3 interrupted vein	:	4 apterous wing.

In the cross above, the presence of the homozygous recessive mutant gene for apterous wing (ap/ap) marks the expression of the gene for interrupted vein.

Epistasis and Blood Groups in Man:

In the ABO blood group system, a person with blood group A has antigen A on the surface of red blood cells; a blood group B person has B antigen: an AB person has both A and B antigens, whereas type O has neither A nor B antigen. The antigens are controlled by an autosomal gene I (iso-haemaglutinin) which has multiple alleles. Thus I^A controls antigen A, I^B controls antigen B. The recessive allele i produces no antigen and results in phenotype O.

Certain proteins present in the blood serum of a person show a precise relationship with the red cell antigen. These proteins are the agglutinins or antibodies. Thus the serum of an individual with blood group A has antibodies against antigen B, whereas the serum of a blood group B person is anti-A. In persons with blood group AB where both antigens A and B are present, the serum has neither antibody A nor B; individuals with blood group O have both types of antibodies.

It has been found that there is another gene H which controls production of a precursor substance H. In persons of blood type A, having gene I^A, precursor H gets converted into antigen A under control of gene I^A. Similarly when gene I^B is present, H gets converted to antigen B.

In the presence of the recessive allele i in type O persons, precursor H is not converted and remains as such in the blood. It can be agglutinated by anti-H or by such substances as an extract of the seeds of a plant Ulex europeus or blood of eel. In persons of heterozygous blood groups such as I^A/j or I^B/i only part of the H substance which is unconverted is agglutinated.

In AB persons there is likewise no agglutination of H substance. It is found that most human beings are homozygous (HH) for gene H, few are heterozygous (Hh), and very rarely a homozygous recessive (hh) individual is met with that cannot produce any H substance at all.

Such a person may have any blood group phenotype from the ABO system, but his red cells are not agglutinated by anti-A, anti-S, anti-H. First discovered in Bombay, India, a homozygous recessive (hh) person is said to show the Bombay phenotype. It demonstrates epistatic action of hh gene over I gene.

The secretor trait in humans also shows epistatic effect of genes. Just as red blood cells carry antigens on their surface, most persons have these antigens in a water soluble form in some body secretions such as saliva, gastric juice, fluids from nose, eyes and mammary glands of females. Such individuals are known as secretors. In a few persons regardless of blood group present, there are no antigens in body secretions. They are known as non-secretors.

The secretor trait is due to a dominant autosomal gene Se, whose recessive allele sd results in a non-secretor. The dihybrid inheritance pattern of secretor trait and blood groups has been studied in a large number of families. Marriages between two double heterozygotes for both blood groups and secretor trait, i.e., I^AiSese x I^AiSese or I^BiSese x I^BiSese show progeny phenotypes in the ratio 9:7 indicating epistatic interaction of genes.

$I^Ai Sese \times I^Ai Sese$

Gametes: I^ASe, I^Ase, iSe, ise

	I^ASe	I^Ase	iSe	ise
I^ASe	I^AI^ASeSe	I^AI^ASese	I^AiSeSe	I^AiSese
I^Ase	I^AI^ASese	I^AI^Asese	I^AiSese	I^Aisese
iSe	I^AiSeSe	I^AiSese	$iiSeSe$	$iiSese$
ise	I^AiSese	I^Aisese	$iiSese$	$iisese$

The checkerboard shows that there are 9 individuals showing both dominant phenotypes (blood group A secretors), 3 type 0 secretors and 4 non-secretors. The data indicate epistatic interaction of genes.

Complementary gene

Complementary genes are genes that both contribute to a single characteristic, where both genes can mask the effect of the other. You may also think of complementary genes as any instance in which dominant forms of both genes are required for the dominant characteristic to be seen.

They are those non-allelic genes which independently show a similar effect but produce a new trait when present together in the dominant form. Complementary genes were first studied by Bateson and Punnet (1906) in case of flower colour of Sweet Pea (Lathyrus odoratus).

The latter is also an example of recessive epistasis where the recessive homozygous alleles of one type suppress the effect of dominant alleles of the other type. Here, the flower colour is purple if dominant alleles of two genes are present together (C—P—). The colour is white if the double dominant condition is absent (ccP—, C—pp, ccpp).

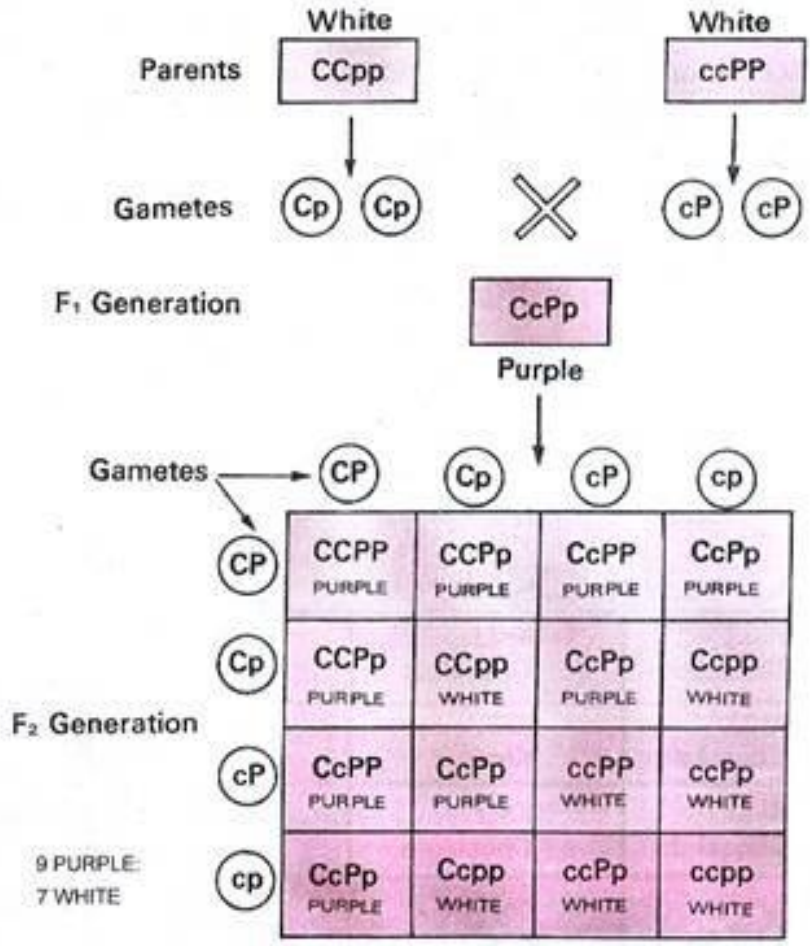
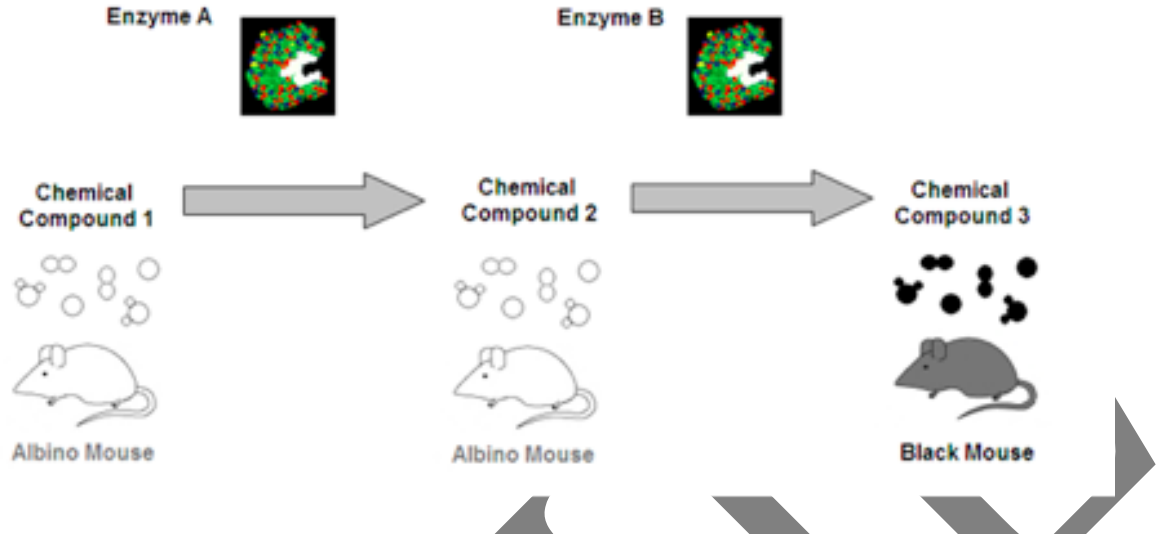


Fig. 5. 32. Inheritance of purple colour in Sweet Pea (*Lathyrus odoratus*) due to complementary and epistatic interactions of two nonallelic gene pairs.

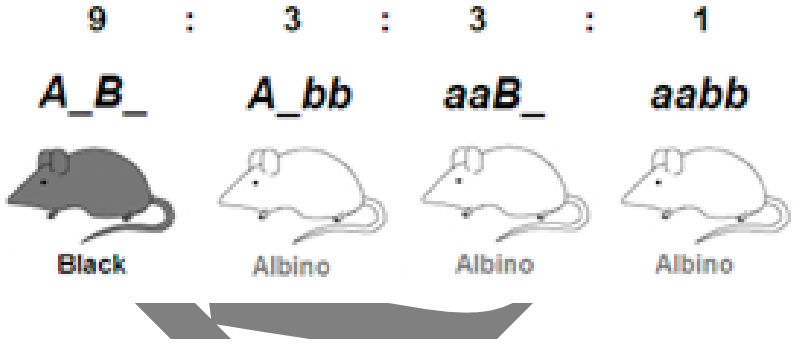
Bateson and Punnet (1906) crossed two white flowered strains (CCpp, ccPP) of Sweet Pea and obtained purple flowered plants (CcPp) in the F_1 generation. Clearly both the parents have contributed a gene or factor for the synthesis of this purple colour. The purple flowered plants of F_1 generation were then allowed to self-breed.

Both purple and white flowered plants appear in the F_2 generation in the ratio of 9: 7. It is a modification of the di-hybrid ratio of 9: 3: 3: 1. The appearance of purple colour in 9/16 population shows that the colour is determined by two dominant genes (C and P). When either of the two is absent (ccPP or CCpp, ccPp or Ccpp), the pigment does not appear (Fig. 5.32).

It is believed that the dominant gene C produces an enzyme which converts the raw material into chromagen. The dominant gene P gives rise to an oxidase enzyme that changes chromagen into purple anthocyanin pigment. This is confirmed by mixing the extract of the two types of flowers when purple colour is formed. Thus purple colour formation is two- step reaction and the two genes cooperate to form the ultimate product.



At least one dominant allele for both Gene A and Gene B is required if any colour is to be produced; thus an individual must be A_B_ to have the black phenotype. If the individual is missing a dominant allele for either one of the two genes, they will be albino. As a result, there are only two possible phenotypes; Black and Albino. In a heterozygous cross (AaBb x AaBb) the following offspring are produced:



Note that although six of the albino individuals have a dominant allele, for one of the two genes, they lack a dominant version of the other gene which is also required for any colour to be produced. Thus the final phenotypic ratio for Complementary genes is always:

9 : 7

Duplicate Genes

Duplicate genes or factors are two or more independent genes present on different chromosomes which determine the same or nearly same phenotype so that either of them can produce the same character.

The independent genes do not have cumulative effect. They produce the same phenotype whether present in homozygous or heterozygous state. As a result dominant phenotype is more abundant. F_2 ratio is 15: 1.

Polymeric Genes:

(Duplicate Genes with Cumulative Effect)

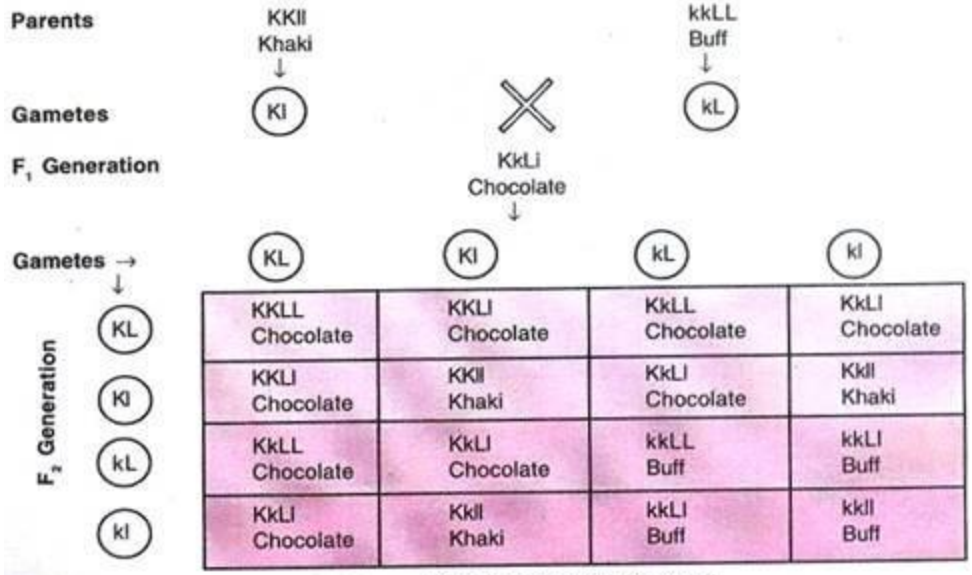
Two independent dominant genes, whether present in homozygous or heterozygous condition, have similar phenotypic effect when present individually but produce a cumulative or double effect when found together. A dihybrid ratio of 9: 6: 1 is obtained in the F_2 generation as phenotypes produced by single gene dominance of the two different genes are similar.

Supplementary Genes:

Supplementary genes are a pair of non-allelic genes, one of which produces its effect independently in the dominant state while the dominant allele of the second gene (supplementary gene) needs the presence of other gene for its expression.

Example Lablab:

Lablab has two genes, K and L. In the recessive state the second or supplementary gene (11) has no effect on seed coat colour. Dominant K independently produces Khaki colour while its recessive allele gives rise to buff colour irrespective of the supplementary gene being dominant or recessive. In the dominant state the supplementary gene (L—) changes the effect of dominant allele of pigment forming gene (K) into chocolate colour. F_2 ratio is 9: 3: 4 (Fig. 5.33).



9 Chocolate : 3 Khaki : 4 Buff

Fig. 5.33. Inheritance of seed coat colour in Lablab showing effect of supplementary gene.

Modifying Genes

They are two nonallelic genes which not only are able to produce their own effects independently when present in the dominant state but can also interact to form a new trait. Comb types in poultry is an example of collaborative supplementary genes, P and R.

When none of these genes is present in the dominant state (pprr), single comb is formed. In case P alone is dominant, a pea comb is formed (Pprr, PPrr). If R alone is dominant, a rose comb is obtained (ppRr,

ppRR). A walnut comb is formed when both P and R occur together in dominant state (P — R —) to produce supplementary effect.

When pure pea combed and pure rose combed birds are crossed, all the offspring of F, individuals have walnut comb. On inbreeding the walnut combed birds, the F_2 generation comes to have all the four types of combs in the ratio of 9 walnut: 3 pea: 3 rose: 1 single (Fig. 5.34).

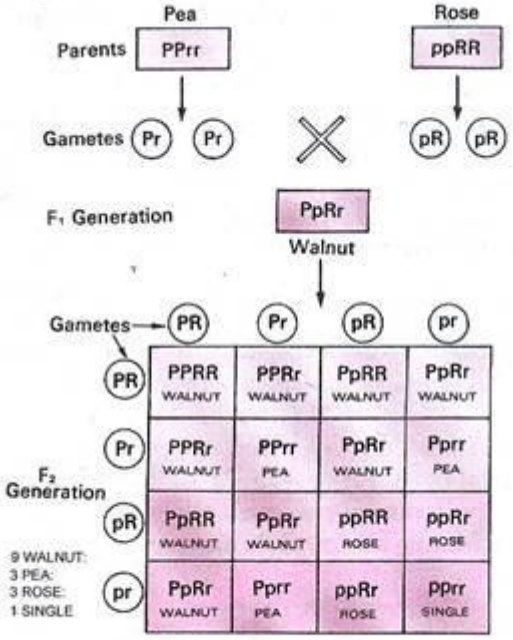


Fig. 5.34. Cross between pure Pea comb and pure Rose comb chicken to demonstrate collaborative interaction of genes or collaborative supplementary genes.

Lethal Allele

Alleles that cause an organism to die are called lethal alleles, where the gene involved is an essential gene. When Mendel's Laws were rediscovered, geneticists believed that mutations would only alter the appearance of a living organism. However, it was discovered that a mutant allele could cause death. When an essential gene is mutated, it can result in a lethal phenotype. If the mutation is caused by a dominant lethal allele, the homozygote and heterozygote for the allele will show the lethal phenotype. If the mutation is caused by a recessive lethal allele, the homozygote for the allele will have the lethal phenotype. Most lethal genes are recessive.

Examples of diseases caused by recessive lethal alleles are cystic fibrosis, Tay-Sachs disease, sickle-cell anemia, and brachydactyly. Huntington's disease is caused by a dominant lethal allele and even though it is not described as lethal, it is invariably lethal in that the victim experiences gradual neural degeneration and mental deterioration for some years before death occurs. One coat color of ranch foxes is caused by a recessive lethal gene. This gene causes a death if both recessive alleles are possessed by the same individual.

Recessive lethal alleles do not cause death in the heterozygous form because a certain threshold of protein output is maintained. In the homozygous form, the protein output does not meet the threshold, causing death.

Environmental influence of gene expression

Most common diseases are a result of both your genes and your environment. Your environment can include personal choices, such as what foods you eat and how much you exercise, and external factors, such as stress, clean water, and air quality. Only a small number of diseases are a result of just a single mutation in a gene. Examples of these single-gene disorders are Huntington disease and Tay Sachs . Most diseases, especially common diseases, are a combination of your genetic risk and your environment. It is becoming difficult to group diseases into either purely 'genetic' or 'environmental' because most diseases are a little bit of both. For example, emphysema can be the result of both smoking and a disorder called alpha-1-AT deficiency . The field of research looking at gene-environment interactions (GxE) is growing.

It is important to understand that most times your genes do not determine your health. Small differences in your genetic makeup mean that two people can respond differently to the same environmental exposure. Here are some ways that your genes and your environment can interact:

- **Mutagens** – Mutagens are pollutants in the environment that enter the body and directly change your DNA sequence. Example: The chemicals in cigarette smoke can cause cancer.
- **Gene-gene interactions** – Gene-gene interactions occur when pollutants in the environment do not change your DNA sequence, but rather cause a chain reaction that affects the functioning of one gene that then affects the functioning of another gene. Example: Regularly drinking way too much alcohol can cause a specific gene, TACE, not to produce enough of its protein. TACE protein is supposed to help the MTHFR gene make enough of its protein. Too little MTHFR protein changes the level of folate (another protein) in our blood, and low folate levels may cause depression.
- **Transcription factors** – Pollutants in the environment can indirectly affect the DNA sequence by altering transcription factors, which are responsible for starting the process of using genes to make proteins that are needed for different functions in the body. Example: Stress can change the amount of proteins made by genes involved in your immune system and therefore, you may get sick more easily when you're stressed.
- **Epigenetics** – The environment can alter your health by affecting the proteins that turn genes on or off. Continue reading for more information on epigenetics. Example: half the genes that cause familial or inherited cancer are turned off when pollutants in the environment affect these proteins. Because they are turned off, these genes cannot suppress tumor formation or repair DNA.

Penetrance and Expressivity:

The presence of a gene does not always bear an absolute relationship with the appearance or absence of a trait. In the ABO blood group system, the genes are expressed in an absolute way. But in many other instances the gene is expressed in a variable manner, i.e. the visible phenotype shows varying intensities. The terms penetrance and expressivity are used to describe variable gene expression. Penetrance is the proportion of individuals that show an expected phenotype. When a gene is completely penetrant it is

always expressed; when incompletely penetrant, the gene is expressed in some individuals, not in others, the proportions depending upon the degree of penetrance.

For example in the recessive traits which Mendel studied, the phenotype was expressed fully when the gene was in homozygous condition; this is due to 100 per cent penetrance. Suppose instead that in a hypothetical cross, only 60 per cent of individuals show the expected trait when all 100 are carrying the gene; we say that in this case penetrance is 60 per cent.

Expressivity is the degree to which a gene is expressed in the same or in different individuals. Thus the gene for lobe eye in Drosophila may show a complete range of phenotypic expression in different individuals. Some flies may have a normal sized eye, in others the eye is smaller, in still others the eye is absent.

Temperature:

The earliest studies related to the effect of temperature on genetic constitution were done on the Himalayan breed of rabbits and Siamese cats. Coat colour in rabbits is controlled by multiple alleles of a gene.

When one of the recessive alleles c^h is present in the homozygous condition (c^hc^h), the Himalayan coat colour results. Such a rabbit is a mosaic with white fur all over the body except the nose, paws, ears and tail which are black (Fig. 5.1).

Fig. 5.1 The Himalayan rabbit.

The black extremities are the portions which have lower temperature (less than 34°C) than the rest of the body. If the extremities are exposed to higher temperature artificially, the new hair which starts growing is white.

Similarly, if some portion of the body bearing albino fur is artificially kept at a lower temperature, the new hair formed is black. These observations explain the temperature sensitive behaviour of the allele (c^h) which controls Himalayan trait in the homozygous state.

The allele codes for an enzyme used in pigment formation which is temperature sensitive and is inactivated by temperatures above 34°C resulting in albino phenotype; if temperature is lower the same alleles promote synthesis of pigment and the phenotype is black.

When rabbits of this genotype are grown at cold temperatures, they are completely black. The Siamese cat shows the same pigmentation pattern as the Himalayan rabbit due to the presence of similar type of temperature-sensitive allele.

In Drosophila temperature changes the penetrance of the gene known as tetraptera which controls wing development. At 25°C the gene has 35 per cent penetrance so that the corresponding number of flies develop wings whereas 65 per cent do not. At 17°C penetrance is much reduced so that only one per cent of flies show the winged phenotype.

The recessive gene vg/vg which produces vestigeal wings in Drosophila is also influenced by temperature. At 32°F the wings are feebly developed and extend very little from the body (Fig. 3.1). At

40°F the wings are better developed and have some venation. At 88°F wings are well developed with conspicuous venation.

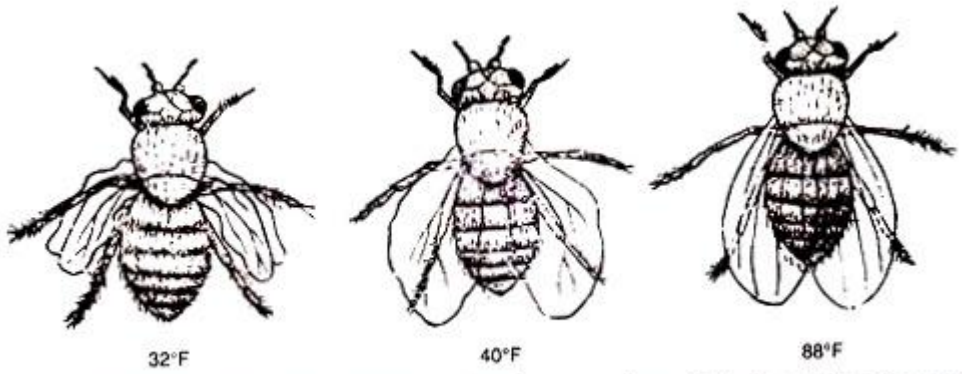


Fig. 3.1 Influence of temperature on the expression of the gene for vestigial wings (*vg*) in *Drosophila*.

Some temperature-sensitive mutations are exhibited in bacteriophages. In general the temperature at which normal phenotypes are produced is referred to as permissive temperature: that which produces mutant phenotypes is called restrictive temperature. Some lethal mutations in viruses and in Drosophila are temperature sensitive. Among plants, colour of flower in primrose changes from red to white when temperature is raised above 86°F.

Light:

There is a gene in maize plants which controls anthocyanin pigment formation. When ears of plants carrying the homozygous gene are exposed to sunlight by removing the green leafy coverings on the young cobs, the kernels become bright red in color ("sunred").

If however, the blue violet rays of the light spectrum are prevented from reaching ears of maize plants (by wrapping red cellophane paper around them so that only red rays penetrate the cells) the sunred phenotype is not visible.

In this case sunlight interferes with one or more chemical reactions leading to pigment formation. The reddish freckles on the sensitive skin of white skinned human races are also caused by sunlight in a similar way.

In human beings a skin cancer known as xeroderma pigmentosum is caused by a homozygous recessive gene. The skin becomes extremely sensitive to sunlight so that even a minor exposure to faint light gives rise to pigmented spots on the facial skin. The spots can become cancerous and if they spread to other parts of the body, death results. If an individual homozygous for the recessive gene is not exposed to light, the gene is not able to express itself.

Phenocopies:

Depending upon the extent to which the environment influences the genotype, the changes in the phenotype may be subtle or dramatic. Sometimes the phenotype becomes altered by the environment in such a way that the new phenotype resembles another phenotype produced by known genes. The induced phenotype is not inherited and is called a phenocopy.

In many instances phenocopies result from application of specific treatments like radiation, chemicals poisons, temperature shocks etc. The Himalayan rabbit described develops a coat that is all black if the rabbit is made to live in a cold environment.

The Himalayan rabbit is a phenocopy of the genetically black rabbit. If both rabbits live together at moderately high temperature, the Himalayan rabbit has a phenotype very different from the genetically black rabbit.

One of the most striking examples of phenocopies could be observed in what were known as thalidomide babies in the early 1960's. A number of deformed children were born in West Germany and Great Britain to mothers who had taken the tranquilizing drug thalidomide in their sixth week of pregnancy.

The abnormal children showed deformities in limbs; some had one, two or three limbs, others had no limbs at all. The abnormalities showed a great resemblance to another phenotype known as phocomelia caused by a recessive gene.

Diabetes mellitus is a heritable human trait associated with reduced amounts of the hormone insulin that is secreted by the pancreas. In the presence of insulin glucose is absorbed by the cell membranes. When the hormone is not produced in sufficient quantity, the unabsorbed glucose passes into the blood and urine. The exact mode of inheritance of diabetes is not properly understood.

There are different types of diabetes arising from different causes; it therefore seems likely that there are several gene pairs controlling the trait. On the other hand the study of a pair of genetically identical twins, one of whom had diabetes the other not, indicates that the condition is due to a recessive gene with low penetrance.

If proper doses of insulin are administered to a diabetic person, he reverts to the normal phenotype. In other words, control of diabetes produces a phenocopy of the normal individual. There are many other examples in human beings where, by giving drugs, the mutant genotype produces a phenocopy of the normal phenotype.

In haemophiliac patients, a specific protein required for blood clotting is either defective or deficient. If however, an anti-haemophiliac factor isolated from humans is injected into a patient, a phenocopy of the normal individual results. Similarly, if thyroxine is administered to a child whose thyroid gland does not secrete this substance in adequate quantities, the normal phenotype is produced.

The creeper trait in chickens is observed sometimes in domestic fowl when the newly hatched chickens have the legs drawn up under the body. The affected chicken is not able to walk but creeps along the ground.

The creeper trait (Fig. 3.2) is expressed by the heterozygous condition of a dominant gene which is lethal when homozygous. Creeper chickens can also be produced if incubating eggs of normal fowls are treated with boric acid or insulin. Such induced creepers are phenocopies of the genetically controlled heterozygous creeper chickens.

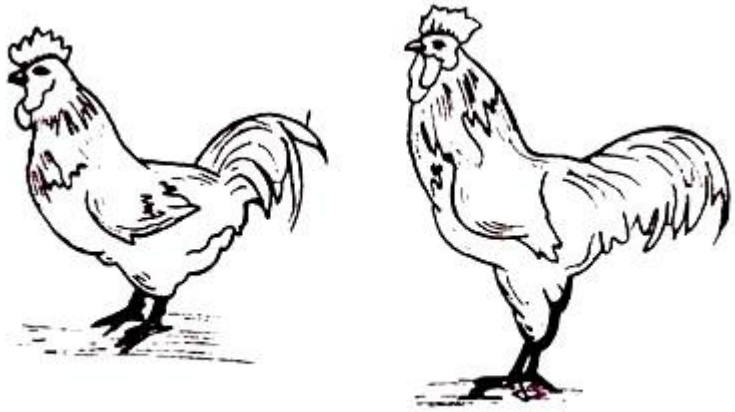


Fig. 3.2 Chicken expressing creeper trait (left) compared with a normal chicken (right).

Due to a recessive gene, maize plants become dwarfed, because they are deficient in the plant growth hormone known as gibberellic acid. But if the hormone is supplied to the dwarf plants they grow to normal height producing phenocopies of normal plants.

Environmental Effects and Twin Studies:

In human beings it is not possible to perform controlled breeding experiments. Twin studies are perhaps the best way of determining as to whether the observed differences between individuals are due to heredity.

Twins are of two types—monozygotic or identical twins that arise from a single fertilised egg and have identical genotypes; dizygotic or fraternal twins which arise from two fertilised eggs and are therefore no more genetically alike than siblings (brothers and sisters).

The correct identification of twin types is difficult and unreliable unless done by a physician. For assessing the role of environment in heredity, the percentage of concordance (both twins showing identical phenotype) and discordance (different phenotypes) for a given trait must be determined for twins of both types.

In general if concordance percentage for a trait is high in the case of monozygotic twins, and much less in dizygotic twins, one can conclude that heredity has played a role. If the concordance rate is similar in monozygotic and dizygotic twins, it suggests that the environment is determining the phenotype. From studies of a large number of twins it has been found that measles (caused by infection with Rubelia virus in early pregnancy) is largely controlled by the environment.

On the other hand conditions like diabetes, schizophrenia, Rickets and tuberculosis appear to be controlled by the genotype. Another useful aspect of twin studies is to determine the effects of different environments on identical genotypes by analysing those rare cases of monozygotic twins that have been separated from birth and reared apart. However in absence of adequate data it is not possible to conclude much on this aspect as yet.

Human Intelligence:

A number of studies have been done to determine how much of human intelligence and I.Q. are controlled by the genotype and how much by the environment. Both clarifications and complications have been revealed. The differences in intelligence among different racial groups have been extensively studied by Arthur Jensen in 1969. This work is highly controversial and has been much debated.

Nevertheless, it is generally agreed that intelligence is under the control of several gene pairs interacting with the environment. From twin studies it has been further estimated that about one-half to three-fourths of human intelligence is determined genetically; the remainder is controlled by the environment.

ADD ON FOR YOUR UNDERSTANDING

Environment and Sex Determination:

The marine worm Bonellia demonstrates the effect of environment on sex. In this sexually dimorphic organism the female is very large, about 10 cm in length; the male is 3 mm long and lives inside the cloaca of the female.

If the free swimming larvae that have arisen from fertilised eggs remain in the sea bed away from the females, they develop into female worms. But if females are available, the larva settles on the female proboscis, draws nourishment from it, and develops into a male.

Of the many experiments performed with Bonellia, one is most interesting and relevant here. If Bonellia are raised in the laboratory in a tank containing artificial sea water, the free-swimming larvae settle down at the bottom of the tank and develop into females. But if the artificial sea water is agitated by some mechanical device, the larvae develop into males.

Drug Resistance:

It is fairly well established that mosquitoes develop resistance to DDT and other insecticides used for eradicating malaria. The resistance develops due to change in the genotype in response to the environment, and is inherited.

Similar resistance is reported also in insects which carry the causal agent for some other diseases like dengue fever, yellow fever, filariasis and river blindness. A number of pests which are harmful to major crops such as rice, maize, cotton, wheat and potato are also known to have become resistant to a wide range of insecticides.

Quantitative or polygenic inheritance

Mendel believed that a factor or gene is responsible for the expression of one character. This factor or gene has two alternate forms or alleles.Laws of heredity by Mendel offer a simple and correct explanation of qualitative difference among plants and animals such as the flower colour, red or white and the seed colour, either yellow or green. But certain characters are quantitative instead of being qualitative such as weight, height, intelligence in man.

These qualitative characters permitted Mendel to make accurate counts of individuals showing one or the other of these alternative traits. For instance, if tall and dwarf plants were crossed, in F_1 only tall plants and in F_2, only tall and dwarf plants were obtained. No plants of intermediate types were ever obtained. Such type of inheritance based on Mendelian ratio is known as qualitative inheritance.

On the other hand, when two or more genes at different loci are responsible for the expression of one character, such genes are known as polygenes or multiple genes and the character or trait they produce is called a polygenic trait.

Some other important characters in cultivated plants and domestic animals such as yield of seeds, fruits, eggs and amount of milk or meat produced, do not fall into clear cut classes and all gradations come between the two extremes between large and small, heavy and light etc.

Such quantitative characters show a continuous variation. Mendel's method of analysis is hard to apply in such continuously varying characters because they seem to mix or blend instead of segregating in the offspring of hybrids.

The problem of the inheritance of quantitative character was taken up by the Swedish botanist. H. Nilsson-Ehle (1908) and American E.N. East (1910, 1916). These investigators showed that this apparent 'blending inheritance' can be explained by supposing that a continuously varying characters are due to the combined action of several genes, each of which has a small effect on the same character. Such genes are called the cumulative or additive or polygenes.

A cumulative gene is one which if added to another identical or similar gene affects the intensity or the degree of expression of a quantitative character. In other words, a character is governed by several genes (=polygenes) and their effects or actions are cumulative or additive in nature.

This is the essence of the multiple factor hypothesis. As quantitative inheritance it is controlled by many genes. Therefore, it is also known as polygenic inheritance.

The inheritance pattern involving multiple genes is known as polygenic or quantitative inheritance. The quantitative traits in man include its height, weight, intelligence and colour and those of plants include their size, shape, number of seeds and fruits etc.

The quantitative traits are also called metric traits. In quantitative inheritance, the offspring's do not show clear cut differences among them, rather show intermediate character of two parents. The gradations in characters are determined by a number of genes and all the genes have additive effect or are cumulative. It means that each gene has a certain amount of effect, and more is the number of dominant genes, the more is the degree of expression of the character.

A few common examples of polygenic inheritance are described as below:

Inheritance of kernel color in wheat

Nilsson-Ehle, crossed two varieties of wheat, red and white in colour and found that all the F_1 offsprings were intermediate between red and white i.e., light red colour, showing that red is incompletely dominant over white.

When the F_1 hybrids were self-fertilized the F_2 progenies or offsprings showed a ratio of 15 red to 1 white. The red progenies, however, varied in shade from pure red to pink. The ratio 15:1 clarify that this was di-hybrid cross in which two identical genes were involved for producing the red colour.

Parents : Red Wheat $R_1R_1R_2R_2$ × White Wheat $r_1r_1r_2r_2$

Gametes R_1R_2 ↓ r_1r_2

F_1 Medium $R_1r_1R_2r_2$

Gametes R_1R_2 R_1r_2 r_1R_2 r_1r_2

Male gametes

Female Gametes	R_1R_2	R_1r_2	r_1R_2	r_1r_2
R_1R_2	$R_1R_1R_2R_2$ Red	$R_1R_1R_2r_2$ Dark	$R_1r_1R_2R_2$ Dark	$R_1r_1R_2r_2$ Medium
R_1r_2	$R_1R_1R_2r_2$ Dark	$R_1R_1r_2r_2$ Medium	$R_1r_1R_2r_2$ Medium	$R_1r_1r_2r_2$ Light
r_1R_2	$R_1r_1R_2R_2$ Dark	$R_1r_1R_2r_2$ Medium	$r_1r_1R_2R_2$ Medium	$r_1r_1R_2r_2$ Light
r_1r_2	$R_1r_1R_2r_2$ Medium	$R_1r_1r_2r_2$ Light	$r_1r_1R_2r_2$ Light	$r_1r_1r_2r_2$ White

F_2

Summary of F_2 : 1/16 Red, 4/16 Dark, 6/16 Medium, 4/16 Light, 1/16 White.

(Member of several gene pairs which act in a cumulative way on a trait or character are known as multiple factor-Altenburg.)

In some other examples, it is found that 63 out of 64 of the F_2contains red colour and only 1 of 64 is white, suggesting that three genes are involved in this case, each producing red colour, the red parent will be represented then by the genotypes $R_1R_1R_2R_2R_3R_3$ and the white parents by $r_1r_1r_2r_2r_3r_3$.

The F_1 hybrids will have only three colour genes $R_1r_1R_2r_2R_3r_3$ and will show light red shade. In F_2 1/64 will be completely like the red grand parent having six colour genes, 6/64 will have 5, 15/64 will have four, 20/64 will have three, 15/64 will have two, 6/64 will have one colour genes while 1/64 will be completely white without any colour gene.

Nilsson-Ehle (1908) made a interesting study on the colour of grain in wheat. He crossed red kerneled variety with white kerneled strain and obtained F_1 plants whose grains were uniformly red but intermediate between red and white parental generations. When the members of F_1 were self-crossed five different phenotypic classes appeared in F_2 in the ratio of 1: 4: 6: 4: 1.

(i) Red (extreme) – 1/16
(ii) Dark red – 4/16
(iii) Medium red – 6/16
(iv) Light red – 4/16
(v) White – 1/16

Later it was found that the kernel colour in wheat is determined by two pairs of genes Aa and Bb. Genes A and B are dominant genes which determine the red colour whereas a and b are recessive alleles. Results of this polygenic inheritance is depicted in Fig. 5.8.

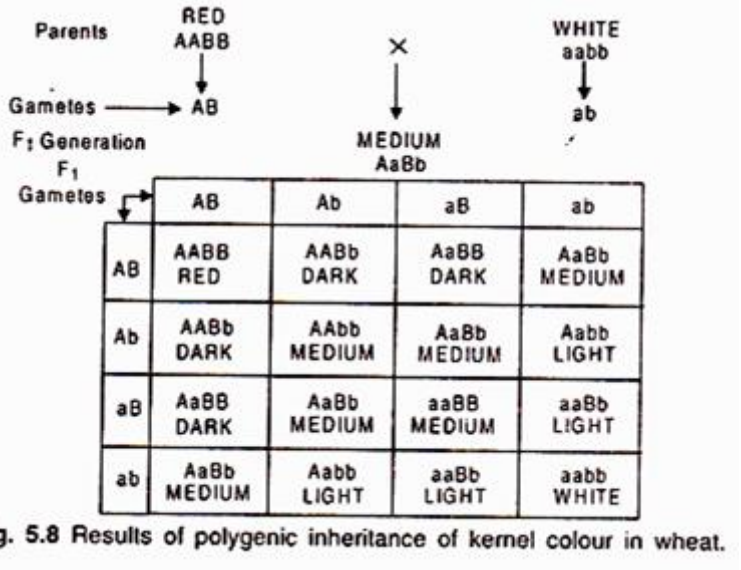


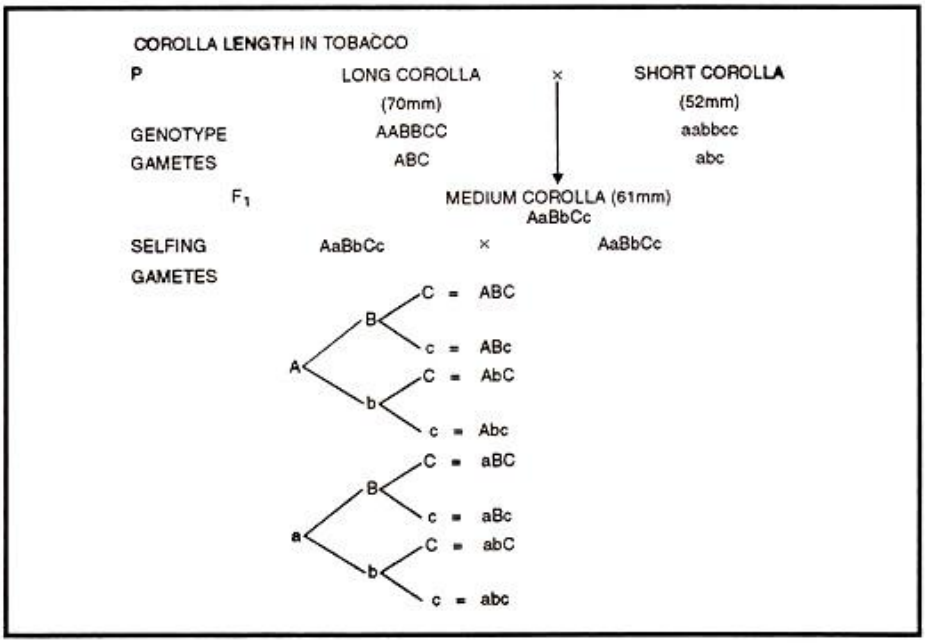
Fig. 5.8 Results of polygenic inheritance of kernel colour in wheat.

Corolla length in tobacco (Nicotiana):

East (1916) reported his studies on the inheritance of corolla length in Nicotiana longiflora, a self-pollinated variety of tobacco. This character is governed by multiple genes. He crossed a variety, the corolla of which had an average length of 52 mm, to a variety, the corolla of which had an average length of 70 mm. Both these varieties had long been inbred and therefore were homozygous.

The marked differences in corolla lengths were heritable pointing out that they are controlled by genes rather than environmental. East found that F_1 was intermediate with mean corolla length of 61 mm.

In F_2 a much larger variation for corolla length than F_1 was observed. The variation was continuous as well. East raised 444 F_2 plants and failed to get even a single plant like either of the parents. This pointed out that more than 4 pair of genes are involved in determining the length of corolla in Nicotiana longiflora.



ABC, ABc, AbC, Abe, aBC, aBc, abC, abc X ABC, ABc, AbC, Abc, aBC, aBc, abC, abc

F_2 generation.

Genotype	Frequency	Number of dominant factors	Length	Frequency
AABBCC	1	6	70mm.	1
AABBCc AABbCC AaBBCC	2 2 2	5	67mm.	6
AABbCc AaBBCc AaBbCC AABBcc AAbbCC aaBBCC	4 4 4 1 1 1	4	64mm.	15
AaBbCc AABbcc AaBBcc AAbbCc AabbCC aaBBCc aaBbCC	8 2 2 2 2 2 2	3	61mm.	20
AaBbcc AaBbCc aaBbCc AAbbcc aaBBcc aabbCC	4 4 4 1 1 1	2	58mm.	15
Aabbcc aaBbcc aabbCc	2 2 2	1	55mm.	6
aabbcc	1	0	52mm.	1

Number of Active alleles	0	1	2	3	4	5	6
Length value (mm)	52	55	58	61	64	67	70
Frequency (Phenotypic ratio)	1	6	15	20	15	6	1

Skin colour in Man:

It was idea of Davenport (1913) that the multiple factor hypotheses explains the mode of inheritance of skin colour in man. His assumption was that the Negroes differ from the whites in having 2 pairs of colour forming genes that do not show complete dominance. He carried on his studies in Jamaica and Bermuda where intermarriages between coloured and white people were very common.

A marriage between a Negro ($P_1P_1P_2P_2$) and a white ($p_1p_1p_2p_2$) results in children having intermediate shade and have only 2 colour forming genes ($P_1p_1P_2p_2$). These are mulattoes. When 2 mulattoes marry they may have children showing different degrees of colouration ranging from pure black to white.

In the F_2 1/16 will be as dark as the negro grandparent having 4 colour genes. The rest 14/16 will show intermediate shades depending upon the number of colour genes contained by them. But all these gradations could be seen only when a large number of children are born. In a small family the mulattoes parents will produce a completely black or white child.

Parents Negro × White
$P_1P_1P_2P_2$ × $p_1p_1p_2p_2$
Gametes P_1P_2 ↓ p_1p_2
F_1 Mulattoes
$P_1p_1P_2p_2$

♂ gametes

F_2		P_1P_2	P_1p_2	p_1P_2	p_1p_2	
	P_1P_2	$P_1P_1P_2P_2$	$P_1P_1P_2p_2$	$P_1p_1P_2P_2$	$P_1P_1P_2p_2$	Summary :
♀ gametes	P_1p_2	$P_1P_1P_2p_2$	$P_1P_1p_2p_2$	$P_1p_1P_2p_2$	$P_1p_1p_2p_2$	15/16 Dark
	p_1P_2	$P_1p_1P_2P_2$	$P_1p_1P_2p_2$	$p_1p_1P_2P_2$	$p_1p_1P_2p_2$	1/16 White
	p_1p_2	$P_1p_1P_2p_2$	$P_1p_1p_2p_2$	$p_1p_1P_2p_2$	$p_1p_1p_2p_2$	

It has been proposed that the difference in the skin colour between Negros and whites is due to the presence of more than two pairs of colour forming genes bringing about a considerable variation in the skin colour. Gates has suggested for three where as Stern's for four, five or six pairs of genes. Other geneticists have estimated the colour genes number from two to twenty pairs but the exact number involved is till unknown.

Quantitative inheritance is based on the following facts:

(i) Continuous variation.
(ii) A marked effect of the environment on their expression.
(iii) Governed by multiple or polygenes.
(iv) Each gene produces unit or individual effect. The effects of genes are additive or cumulative.
(v) Dominance is absent or partial. F_1 hybrids show blending in characters or in other words the F_1 hybrid is intermediate.
(vi) Segregation and independent assortment of genes in F_2 is according to Mendelian inheritance but the phenotype is in continuous range between the extreme limits of the parents. The phenotypic proportion of F_2 is modified according to the number and nature of genes.
(vii) Some times polygenic characters are governed by single gene too. i.e., single gene mutation may have the same effect as changes in many cumulative genes. For example, in sweet peas tallness is controlled by polygenes. Variations in the size of tall plants is partly environmental and partly polygenic but single mutation as well can result in to dwarf plants.
(viii) For statistical analysis of polygenic inheritance we owe a great deal to Mather, Haldane & Fisher etc. Biological samples are infinite and therefore, statistical parameters are not well defined. Sampling is essential and this can lead us only near the truth but never to the truth or reality.

Difference between Qualitative and Quantitative characters:

Qualitative characters:

(i) It shows discontinuous variation. It may be put in to clear cut classes.
(ii) Governed by major genes. Their effect is definite.
(iii) Not usually affected by environment.

Quantitative characters:

(i) It shows continuous variation. It may not be put up in to clear cut classes.
(ii) Governed by minor genes. Their effect is additive.
(iii) Well affected by environment.

Transgressive and regressive variation

The range of variation in F_2 progeny remains normally well within the limits of both the parents involved in a cross. But some times the extremes of F_2 exceed those of the parents. This type of variation is called Transgressive variation. K.Mather firstly used the term polygene in 1941.

Clearly speaking, transgressive segregants surpass the parental limits for a quantitative character and they are the result or effect of segregation. This is the reason for their name.

A classical example of transgressive variation was found in the experiments carried out by Punnett in chicken. He made a cross between a large Hamburg chicken with a small Sebright Bantam and found that the F_1 were of intermediate size.

But the F_2 progeny, however, contained some birds which were larger and some which were smaller than the parental varieties. Such results are obtained if the parents do not represent the extreme genotypes.

For example, 4 pairs of genes are responsible for determining the size of the chicken and in Hamburg variety only 3 are recessive and 1 dominant. A cross between the genotypes AABBCCdd X aabbccDD will produce only one type of F_1 which will be heterozygous for all the 4 genes (AaBbCcDd) thereby determining intermediate size. In the F_2generation the offspring with the genotype AABBCCDD will be larger or heavier than the original Hamburg parent.

Likewise, those with the genotype (aabbccdd) will be smaller than the Sebright Bantam parent. However, these extreme types would be very few or limited in number. Thus, new and desirable types in plants and animals may be produced through proper crossings.

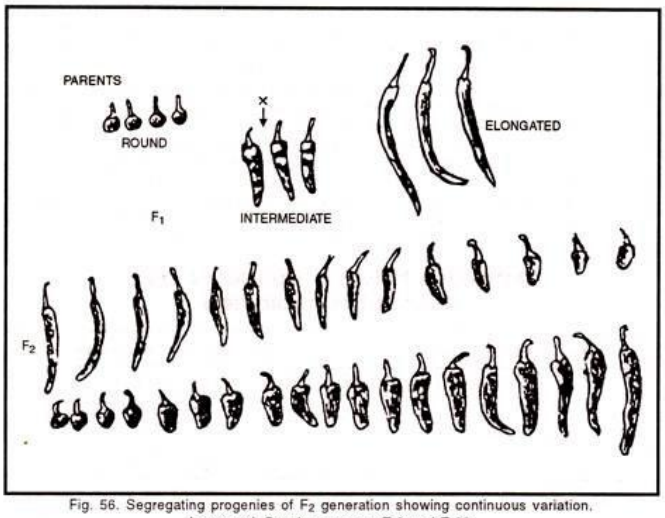


Fig. 56. Segregating progenies of F_2 generation showing continuous variation. A cross of *Capsicum annum* T-3 and T-29.

Multiple Alleles

The word allele is a general term to denote the alternative forms of a gene or contrasting gene pair that denote the alternative form of a gene is called allele. These alleles were previously considered by Bateson as hypothetical partner in Mendelian segregation.

In Mendelian inheritance a given locus of chromosome was occupied by 2 kinds of genes, i.e., a normal gene (for round seed shape) and other its mutant recessive gene (wrinkled seed shape). But it may be

possible that normal gene may show still many mutations in pea besides the one for wrinkledness. Here the locus will be occupied by normal allele and its two or more mutant genes.

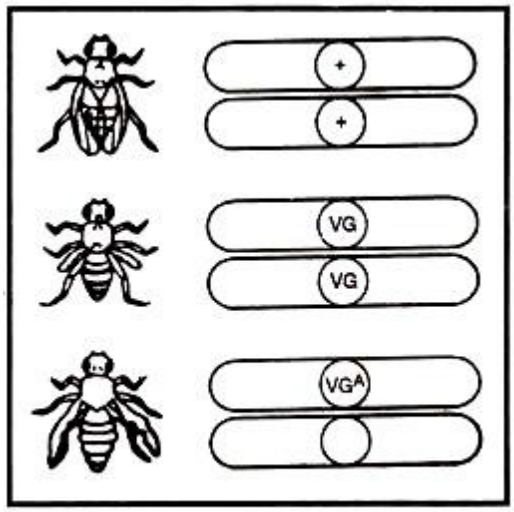


Fig. 57. Multiple alleles. Different form of wings in *Drosophila*.

Thus, three or more kinds of genes occupying the same locus in individual chromosome are referred to as multiple alleles. In short many alleles of a single gene are called multiple alleles. The concept of multiple alleles is described under the term "multiple allelism".

Dawson and Whitehouse in England proposed the term panallele for all the gene mutations at a given locus in a chromosome. These differ from the multiple factor in one respect that multiple factors occupy different loci while alleles occupy same locus.

"Three or more kinds of gene which occupy the same locus are referred to as multiple alleles." Altenburg

Characteristics of Multiple Alleles:

1. The study of multiple alleles may be done in population.
2. Multiple alleles are situated on homologous chromosomes at the same locus.
3. There is no crossing over between the members of multiple alleles. Crossing over takes place between two different genes only (inter-generic recombination) and does not occur within a gene (intragenic recombination).
4. Multiple alleles influence one or the same character only.
5. Multiple alleles never show complementation with each other. By complementation test the allelic and non-allelic genes may be differentiated well. The production of wild type phenotype in a trans-heterozygote for 2 mutant alleles is known as complementation test.
6. The wild type (normal) allele is nearly always dominant while the other mutant alleles in the series may show dominance or there may be an intermediate phenotypic effect.

7. When any two of the multiple alleles are crossed, the phenotype is of a mutant type and not the wild type.

8. Further, F_2 generations from such crosses show typical monohybrid ratio for the concerned character.

Examples of Multiple Alleles:

Wings of Drosophila:

In Drosophila wings are normally long. There occurred two mutations at the same locus in different flies, one causing vestigial (reduced) wings and other mutation causing antlered (less developed) wings. Both vestigial and antlered are alleles of the same normal gene and also of each other and are recessive to the normal gene.

Suppose vestigial is represented by the symbol 'vg' and antlered wing by 'vg^a'. The normal allele is represented by the symbol +.

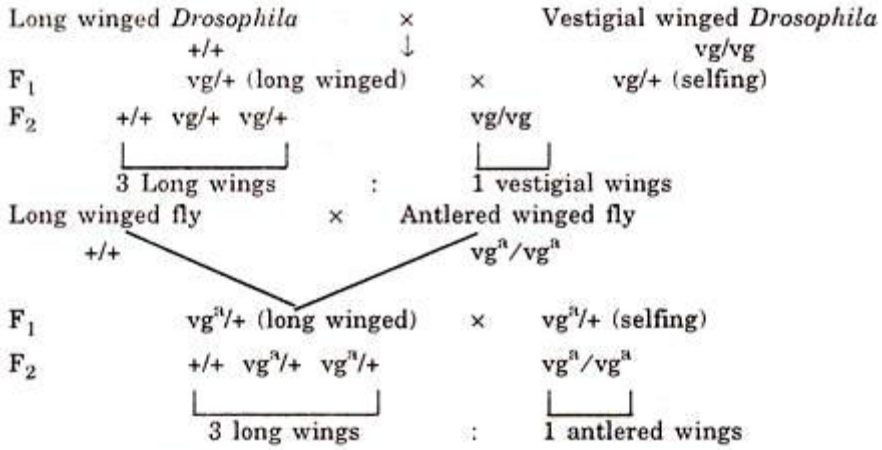
Thus, there are three races of Drosophila:

(i) Long ++ (+/+)

(ii) Vestigial vg vg (vg/vg)

(iii) Antlered vg^a vg^a (vg^a/vg^a)

A cross between a long winged normal fly and another having vestigial wings or antlered wings is represented below:



(*Inheritance presentation of multiple alleles in Drosophila*)

When a fly with vestigial wing is crossed with another fly having antlered wings, the F_1 hybrids are intermediate in wing length showing that none of the mutated gene is dominant over the other. This hybrid is some times said as the vestigial antlered compound and contains two mutated genes at the same locus. They show Mendelian segregation and recombination.

	Vestigial winged fly	×	Antlered winged fly
	vg/vg		vg^{a}/vg^{a}
F_1	vg/vg^{a} (intermediate)	×	vg/vg^{a} (selfing)
F_2	vg/vg,	vg/vg^{a}, vg/vg^{a}	vg^{a}/vg^{a}
	(vestigial)	(intermediate)	(antlered)
	1 :	2 :	1

(Cross representation between a vestigial and an antlered winged fly)

Besides the vestigial and antlered wing described above there are several other mutations occurring at the same locus and resulting in nicked wings, strap wings or no wings etc. These are all multiple alleles.

Close Linkage Versus Allelism:

If we assume that these mutant genes, vestigial and antlered are not allelic located at different loci in place of locating at same locus in different chromosomes so closely linked that there is no crossing over between them, the mutant gene will suppress the expression of adjacent normal allele to certain extent. These closely linked genes are called pseudo alleles and this suppression is the result of position effect. Thus, visible or apparent cases of allelism may be explained on the assumption of close linkage.

Another example of multiple alleles is the eye colour in Drosophila. The normal colour of the eye is red. Mutation changed this red eye colour to white. Other mutations at white locus took place changing the red eye colour to various lighter shades like cherry, apricot, eosin, creamy, ivory, blood etc., are also visible and are due to multiple alleles.

A cross between the two mutant forms, produces intermediate type in the F_1 except white and apricot races which are not alleles but closely linked genes.

Coat Colour in Rabbit:

The colour of the skin in rabbits is influenced by a series of multiple alleles. The normal colour of the skin is brown. Besides it there are white races called albino and Himalayan as the mutant races. The Himalayan is similar to albino but has darker nose, ear, feet and tail. The mutant genes albino (a) and Himalayan (a^{h}) occupy the same locus and are allelic. Both albino and Himalayan are recessive to their normal allele (+).

A cross between an albino and Himalayan produces a Himalayan in the F_1 and not intermediate as is usual in the case of other multiple alleles.

	Albino	×	Himalayan				
	a/a	↓	a^h/a^h				
F_1		a^h/a (Himalayan)					
	a^h/a	×	a^h/a	(Selfing)			
F_2	a^h/a^h	:	a^h/a	:	a^h/a	:	a/a
			3	:			1
			Himalayan	:			Albino

(*Representing a cross between an albino and Himalayan races of rabbits*)

Blood Groups in Man:

Several genes in man produce multiple allelic series which affect an interesting and important physiological characteristic of the human red blood cells. The red blood cells have special antigens properties by which they respond to certain specific components (antibodies) of the blood serum.

The antigen-antibody relationship is one of the great specificity like that between lock and key. Each antigen and its associated antibody has a peculiar chemical configuration. Landsteiner discovered in 1900 that when the red cells of one person are placed in the blood serum of another person, the cells become clumped or agglutinated.

If blood transfusions were made between persons of two such incompatible blood groups, the transfused cells were likely to clump and shut out the capillaries in the recipient, sometimes resulting in death.

However, such reactions occurred only when the cells of certain individuals were placed in serum from certain other persons. It was found that all persons could be classified in to four groups with regard to the antigen property of the blood cells.

Large number of persons have been classified in to these four groups by means of the agglutination test and the distribution of blood groups in the offspring of parents of known blood groups has been studied. The evidence shows that these blood properties are determined by a series of three allelic genes I^A, I^B and i, as follows:

Blood groups	Genotype
AB	I^AI^B
B	I^BI^B or I^Bi
A	I^AI^A or I^Ai
O	ii

I^A is a gene for the production of the anti-gin A. I^B for antigen B, and i for neither antigen. The existence of these alleles in man and the case with which the blood groups can be identified have obvious practical applications in blood transfusion, cases of disputed percentage and description of human populations.

The alleles of these genes which affect a variety of biochemical properties of the blood, act in such a way that in the heterozygous compound I^AI^B, each allele exhibits its own characteristics and specific

effect. The cells of the heterozygote contain both antigens A and B. On the other hand, I^A and I^B both show complete dominance over i, which lacks both antigens.

PARENTS		CHILDREN	
Phenotypes	Genotypes	Phenotypes	Genotypes
O X O	ii X ii	O	ii
O X A	ii X I^AI^A or I^Ai	O, A	ii, I^Ai
O X B	ii X I^BI^B or I^Bi	O, B	ii, I^Bi
O X AB	ii X I^AI^B	A, B	I^Ai, I^Bi
A X A	I^AI^A or I^Ai X I^AI^A or I^Ai	A, O	I^AI^A, ii
A X B	I^AI^A or I^Ai X I^BI^B or I^Bi	A, AB, O, B	I^Ai, I^AI^B, ii, I^Bi
A X AB	I^AI^A or I^Ai X I^AI^B	A, B, AB	I^AI^A, I^Bi, I^AI^B
B X B	I^BI^B or I^Bi X I^BI^B or I^Bi	B, O	I^BI^B, ii
B X AB	I^BI^B or I^Bi X I^AI^B	A, B, AB	I^AI^A, I^BI^B, I^AI^B
AB X AB	I^AI^B X I^AI^B	A, B, AB	I^AI^A, I^BI^B, I^AI^B

Table showing possible blood types of children from parents of various blood groups.

Table showing possible blood types of children from parents of various blood groups.

The 'Rhesus' Blood Group in Man:

A very interesting series of alleles affecting the antigens of human blood has been discovered through the work of Landsteiner, Wiener, Race, Levine, Sanger, Mourant & several others.

The original discovery was that the red cells are agglutinated by a serum prepared by immunizing rabbits against the blood of Rhesus monkey. The antigen responsible for this reaction was consequently called as Rhesus factor and the gene that causes this property was denoted as R-r or Rh-rh.

Interest in this factor was stimulated by Levine's study of a characteristic form of anemia, known as Erythroblastosis foetalis, which occurs occasionally in new born infants.

It was found that the infants suffering from this anemia are usually Rh-positive and so are their fathers; but their mothers are Rh-negative. The origin of the disease was explained as follows: The Rh^+foetus developing in the uterus of an Rh^- mother causes the formation of mother's blood stream of anti Rh antibodies.

These antibodies, especially as a result of a succession of several Rh^+pregnancies, gain sufficient strength in the mother's blood so that they may attack the red blood cells of the foetus. The reaction between these antibodies of the mother and the red cells of her unborn child provokes haemolysis and anemia; this may be serious enough to cause the death of the newborn infant or abortion of the fetus.

The blood stream of a mother who has had an erythroblastotic infant is a much more potent and convenient reagent than sera of rabbits, immunized by blood of rhesus monkeys for testing the blood of other persons to distinguish Rh^+ from Rh^- individuals using such sera from woman who had erythroblastotic infants, it was discovered that: there exist not one but several kinds of Rh^+ and Rh- persons. There are several different Rh antigens which are detected by specific antisera.

Thus, an Rh^- woman immunized during pregnancy by the Rh^+children may have in her blood serum antibodies, that agglutinate not only Rh+ red cells but also cells from a few persons known to be Rh^-.

By selective absorption two kinds of antibodies may be separated from such a serum, one known as anti-D which agglutinates (= coagulates) only Rh^+ cells, the other known as anti-C which agglutinates particular rare types of Rh^-. Another specific antibody, known as anti-c agglutinates all cells that lack C.

With these three antisera, six types of blood can be recognized. Studies of parent and children show that persons of type Cc are heterozygous for an allele C determining C anti-gena. CC persons are homozygous for C and cc are homozygous for c. There is obviously no dominance, each allele producing its own antigen in the heterozygote as in the AB blood type.

No anti serum is available for detecting d, the alternative to D. D^+persons may be heterozygous or homozygous. However, the genotypes of such persons may be diagnosis from their progeny; for example D^+person who has a d^- child is thereby shown to be Dd.

Two other specific antibodies, anti-E and anti-c have been found. These detect the antigens E and e determined by a pair of alleles E and e. The three elementary types of antigens C-c, D and E-e, occur in fixed combinations that are always inherited together as alleles of a single gene. Wiener and Fisher showed the existence of a series of eight different alternative arrangements of these three types of Rh antigens and expressed them by means of following symbols.

The Rh System of Alleles:

Fisher's symbols	Wiener's symbols	
CDE	R^z	Rh-Positive
CDe	R^1	
cDE	R^2	
cDe	R^0	
CdE	r^Y	Rh-Negative
Cde	r'	
cdE	r''	
cde	r	

Thus, allelism is determined by cross-breeding experiments. If one gene behaves as dominant to another the conclusion is that they are alleles and that they occupy identical loci in homologous chromosomes when two genes behave as dominant to other gene. They should occupy identical loci in the chromosome. When more than a pair of alleles occur in respect of any character in inheritance the phenomenon is known as multiple allelism.

There is not much difference between the two theories of Wiener and Fisher. Wiener opinion is that there are multiple variations of one gene whereas according to the view of Fisher three different genes lying very close together are responsible for differences.

Sex determination

Sex chromosomes

The sex chromosomes include the X and Y chromosomes, which have a crucial role in sex determination. Males are defined by XY chromosomes and females by XX.

X chromosome

- Has more than 153 million base pairs
- Represents about 5% of the total DNA in women's cells, 2.5% in men's
- Contains about 2000 genes compared to the Y chromosome containing 78 genes, out of the estimated 20,000 to 25,000 total genes in the human genome
- Genetic disorders that are due to mutations in genes on the X chromosome are described as X linked.
- X chromosome inactivation is when most genes on one of the two X chromosomes in females are inactivated and do not produce any product. In somatic cells in normal females (but not in normal males), one X chromosome is inactivated early in development, thus equalizing the expression of X-linked genes in the 2 sexes. In normal female cells, the choice of which X chromosome is to be inactivated is a random one. Thus females are mosaic with respect to X-linked gene expression. In patients with extra X chromosomes, any X chromosome in excess of one is inactivated, but not all genes on that chromosome are inactivated.

Y chromosome

- Much smaller than X
- Carries only a few genes of functional importance, e.g. the TESTIS-DETERMINING FACTOR (also SRY protein – Sex-determining Region Y): certain genes that cause the male sex organs to develop. It is located on the short arm of the Y chromosome close to the pseudoautosomal region (see below).

In male meiosis, the X and Y chromosomes normally pair by segments at the ends of their short arms and undergo recombination in that region. The pairing segment includes the PSEUDOAUTOSOMAL region of the X and Y chromosomes so called because the X and Y-linked copies of this region are homologue to one another like autosomes.

Sex determination

Most body cells contain chromosomes in matched pairs. The number of pairs of chromosomes varies between species. Human body cells have 23 pairs of chromosomes in the nucleus. One of these pairs controls the inheritance of gender - whether offspring are male or female,

- in males, the two sex chromosomes are different. They are XY
- in females, the two sex chromosomes are the same. They are XX.

A set of human chromosomes can be separated from its cell, spread out on a microscope slide and magnified many thousands of times. When stained and photographed, they look like this:

Chromosomes from a male

Chromosomes from a female

Dosage compensation of X-linked gene

Dosage compensation is the process by which organisms equalize the expression of genes between members of different biological sexes. Across species, different sexes are often characterized by different types and numbers of sex chromosomes. In order to account for varying numbers of sex chromosomes, different organisms have acquired unique methods to equalize gene expression amongst the sexes. Because sex chromosomes contain different numbers of genes, different species of organisms have developed different mechanisms to cope with this inequality. Replicating the actual *gene* is impossible; thus organisms instead equalize the *expression* from each gene. For example, in humans, females (XX) silence the transcription of one X chromosome of each pair, and transcribe all information from the other, expressed X chromosome. Thus, human females have the same number of expressed X-linked genes as do human males (XY), both sexes having essentially one X chromosome per cell, from which to transcribe and express genes.

There are three main mechanisms of achieving dosage compensation which are widely documented in the literature and which are common to most species. These include random inactivation of one female X chromosome (as observed in *Mus musculus*), a two-fold increase in the transcription of a single male X chromosome (as observed in *Drosophila melanogaster*), and decreased transcription by half in both of

the X chromosomes of a hermaphroditic organism (as observed in *Caenorhabditis elegans*). These mechanisms have been widely studied and manipulated in model organisms commonly used in the laboratory research setting. A summary of these forms of dosage compensation is illustrated below. However, there are also other less common forms of dosage compensation, which are not as widely researched and are sometimes specific to only one species (as observed in certain bird and monotreme species).

As human females have two X chromosomes, this means they have the ability to produce twice as much X-linked gene product, this Dosage Compensation Mechanism is responsible for keeping levels of the X-linked gene product similar in both males and females. Therefore, such a mechanism only exists in females. Dosage compensation requires RNA polymerase, Tsix transcript and Xist transcript as well as the X chromosomes of developing embryonic cells. Note that each transcript is transcribed on one chromosome each. Xist acts as to ensure inactivation of X chromosome whereas Tsix ensures X chromosome remains active. Such activity is carried out from the X inactivation centre ., also known as XIC. The XIC is located at the centromere and is where decativation of the X Chromosome begins. This mechanism follows the process of X-inactivation, also termed Lyonization or single active X principle, in which regardless of the amount of X chromosomes are present, all but one are inactivated. However, although one of the X chromosomes is said to be inactivated, there is still around 15% of the genes on the inactivated X chromosome which aren't inactivated

In other organisms

Dosage compensation in other sexually-reproducing organisms is achieved through other mechanisms. *Drosophila* transcribe X chromosome genes in males at twice the level of the female X chromosomes. This increase in transcription is caused by changes in chromatin over the male X chromosome. A dosage-compensation complex forms and brings about this up-regulation. It is associated with histone-modifiying enzymes and two non-coding RNAs transcribed from the male X chromosome. The result is equal transcription of the X chromosome genes in males and females.

In nematode worms, there are two sexes - male and hermaphrodite. Males possess one X chromosome, whereas hermaphrodites contain two X chromosomes. Dosage compensation is brought about through the two-fold decrease in transcription in each of the X chromosomes in the hermaphrodite. A dosage compensation complex forms on each X chromosome, dissimilar to that in *Drosophila*, and results in chromosome changes and decreased X chromosome transcription.

Extra chromosomal inheritance

Extra-nuclear Inheritance by Cellular Organelles:

Extra-nuclear inheritance is also associated with certain cytoplasmic organelles (mitochondria, plastids) that contain naked circular DNA and protein synthesizing apparatus. These extra nuclear genetic materials present in the organelles axe autonomous and code only for limited number of enzymes and polypeptides. Certain enzymes required for cellular respiration are synthesized in the mitochondria.

Similarly, chlorophyll and other pigments axe synthesized in the plastid. Besides the involvement of such biosynthetic activities, these organelles DNAs axe directly associated with the inheritance of some phenotypes which are not controlled by the nuclear genes. The genetic material of chloroplasts and mitochondria are transmitted almost exclusively via the egg.

The inheritance pattern is well-illustrated by the following examples:

Plastid Inheritance in Mirabilis:

Plastid inheritance means the inheritance of plastid characteristics due to plasma genes located in plastids. Plastid inheritance was first described by C. Corens (1908) in the four o'clock plant, *Mirabilis jalapa*.

Leaves of *Mirabilis jalapa* may be green, white or variegated and some branches may have only green, only white or only variegated leaves. Variegation means the presence of white or yellow spots of variable size on the green background of leaves.

Thus it forms the mosaic pattern of coloration on a leaf. Due to certain inheritable defects chloroplast of all cells or some cells of leaf often are unable to synthesize the chlorophyll pigments. Such cells remain non- green and form white or yellow coloured leaf, or white or yellow patches, interspersed with areas containing normal green cells with healthy chloroplasts.

Variegation may be produced by:

(a) Some environmental factors,

(b) Some nuclear genes,

(c) Plasma-genes in some cases.

Since the first and second causes of leaf variegation do not concern cytoplasmic inheritance, the inheritance of variegation due to plasma-genes will be discussed in this article.

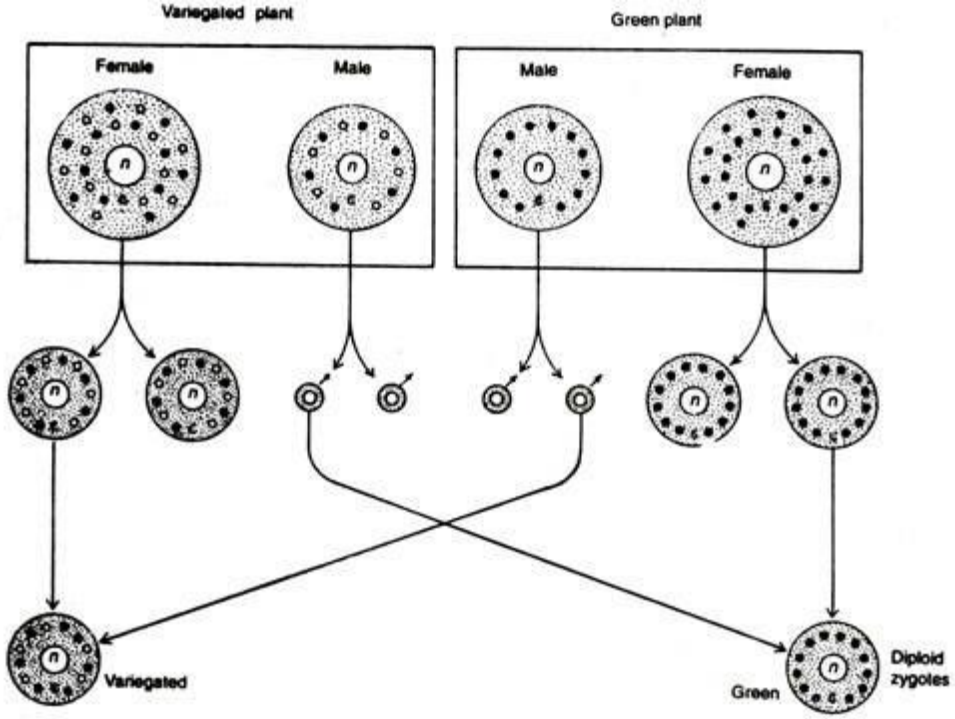


Fig. 22.3: Uniparental (*maternal*) inheritance in *Mirabilis jalapa* which has little or no cytoplasm in pollen gametes.

Correns made reciprocal crosses (Fig. 22.3) in all combinations among the flowers produced on these three types of branches.

These results are summarized in Table 22.1.:

Table 22.1: Plastid Inheritance in Variegated four O'clock Plant

Leaf phenotype of branch used as male plant	Leaf phenotype of branch used as female plant	Leaf phenotype of the progeny (F_1)
Green	Green	Green
	White or yellow	White or Colourless
	Variegated	Green, White or Colourless, Variegated
White or Colourless	Green	Green
	White or Colourless	White or Colourless
	Variegated	Green, White or Colourless, Variegated
Variegated	Green	Green
	White or Colourless	White or Colourless
	Variegated	Green, White or Colourless, Variegated

The results obtained from various crosses of leaf phenotypes of *Mirabilis jalapa*, as shown in Table 22.1, clearly indicates that leaf phenotype of the progeny is the same as that of the female parent (Fig. 22.3). The phenotype of male parent did not contribute anything to the progeny.

This phenomenon is referred to as uniparental transmission. Again, the results of the crosses of *Mirabilis jalapa* cannot be explained by sex-linkage.

The inheritance of different leaf colours in *Mirabilis jalapa* might be explained if the plastids are somehow autonomous and are never transmitted through male parent. For an organelle to be genetically autonomous, it must be provided with its own genetic determinants that are responsible for its phenotype.

Since the bulk amount of cytoplasm containing many plastids is contributed by the egg and the male gametes contribute negligible amount of cytoplasm, therefore plastids present in the cytoplasm of egg is responsible for the appearance of maternal colour in the offspring and the failure of male plant to transmit its colour to offspring is reasonable.

In the offspring from variegated female parents, green, white and variegated progeny are recovered in variable proportions. The variegated parent produces three kinds of egg- some with colourless plastids, some contains only green plastids, and some are with both chloroplasts and leucoplasts.

As a result, zygotes derived from these three types of egg cells will develop into green, white and variegated offspring's, respectively.

Inheritance of Lojap Trait in Maize:

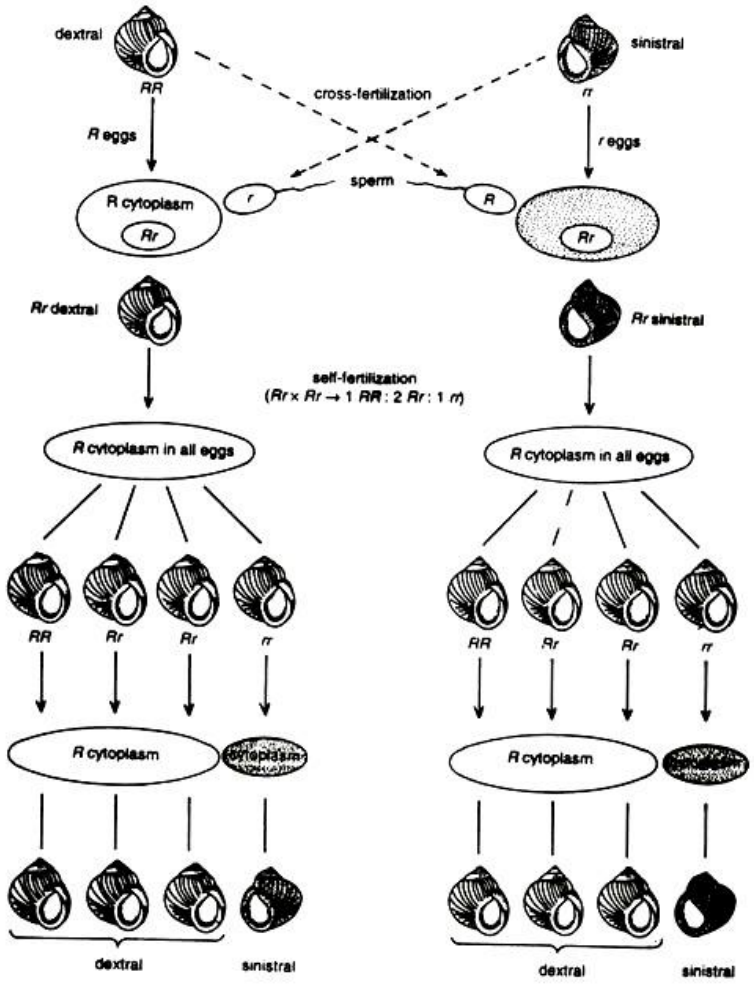


Fig. 22.2: Maternal effect in direction of coiling of the shell in *Limnaea peregra*.

In maize plant, *iojap* is a trait which produces green and white stripped leaves. This trait is controlled by a recessive chromosomal gene (ij) when present in homozygous state. The name iojap was derived from 'Iowa' state (USA), the source of maize strain and japonica, the name of a stripped variety.

When a normal plant with green leaves used as a female parent is crossed (Fig. 22.4) with iojap parent, the offspring will be green leaved:

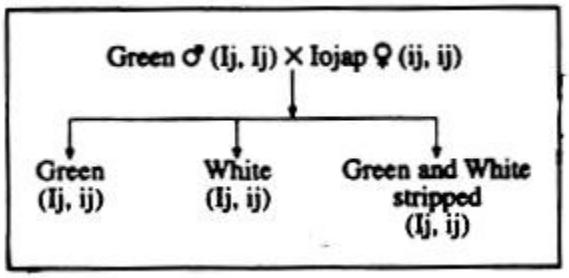
Green ♀ (Ij, Ij) × Iojap ♂ (ij, ij)

↓

Green leaved (Ij, ij)

Again, when a reciprocal cross is made between a normal green plant (used as male) and iojap plant (used as female).

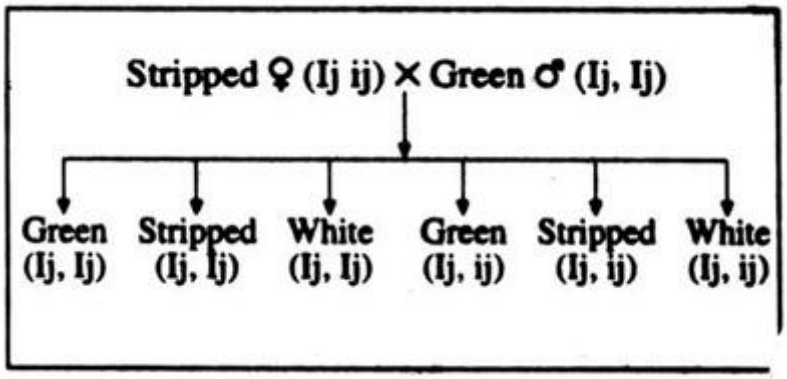
The offspring will be of three different types:



In iojap plants, green and white stripped trait of leaf is inherited from the female parent due to maternal inheritance. It seems that iojap plants contain two types of plastids— normal green, and abnormal iojap plastids.

During the formation of egg cells plastids are randomly distributed in the egg cells. If the egg cell receives normal green plastids it will produce green leaved plants irrespective of which plant acted as pollen parent. If the egg cell receives abnormal colourless plastids, it will give rise to white leaved plants. If the egg cell receives both green and abnormal plastids it will give rise to plants with green and white stripped leaves.

If stripped leaved F_x iojap (I_j,i_j) as female parent is crossed with normal green leaved (Ij Ij) as male parent the following types of offspring are obtained:



This backcross experiment shows that green males have no effect upon progeny. The appearance of iojap trait has been explained by two hypotheses. One hypothesis states that frequent mutation in the chloroplast genome produces the abnormal plastids.

Another hypothesis suggests that certain cytoplasmic elements other than chloroplast mutation bring about the bleaching of chloroplasts. It is also suggested that a nuclear gene controls the development of abnormal plastids in the cytoplasm. So this type of inheritance is a case of inaction between nuclear and cytoplasmic inheritance.

Extra-Nuclear Inheritance by Mitochondria of Yeast:

Yeast, *Saccharomyces cerevisiae*, are unicellular ascomycetes fungi. In this fungi, sexual reproduction takes place by the fusion of two somatic cells to form a diploid zygote nucleus. Next follows two

successive nuclear divisions forming four haploid daughter nuclei, all of which take part in ascospore formation. Now the mother cell, i.e., zygote cell, is called ascus.

The diploid zygote can also be grown vegetatively as a diploid strain that will later sporulate. Respiration of yeast cell takes place both aerobically and anaerobically (fermentation). Certain mutant yeast cells are unable to utilise oxygen and are comparatively small- sized and slow growing producing small colonies on agar medium. These small colonies forming mutant strains of yeast are known as petites.

In petite strains, the necessary components (cytochrome b, c_1) and some enzymes (cytochrome oxidase a, a_3) for aerobic terminal respiration activity are absent. But these components are present in the cell of normal strain where they are associated with the inner membrane of mitochondria.

Petite strain can be maintained indefinitely in the vegetative state and can be mated with normal yeast cells. When such mating are carried out, three petite varieties can be classified:

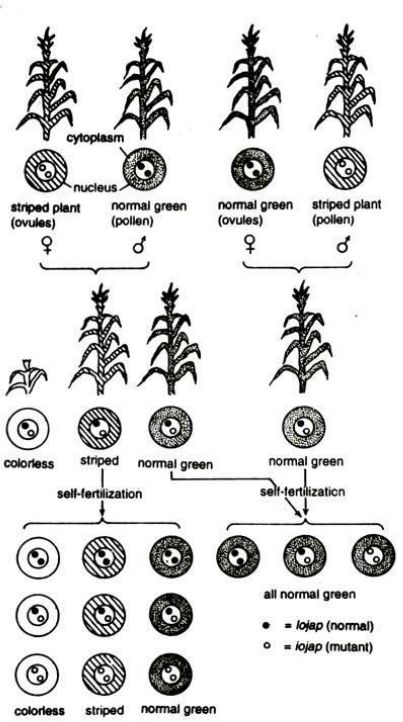


Fig. 22.4: Inheritance of lojap trait in maize.

Nuclear (segregational) Petites:

When a normal haploid strain of yeast is crossed with a haploid petite strain, a normal diploid zygote is produced. The haploid ascospores produced from zygote by sporulation are segregated in the ratio 1 : 1 (petite : normal). Hence the result of such cross follows ordinary nuclear mendelian inheritance (Fig. 22.5).

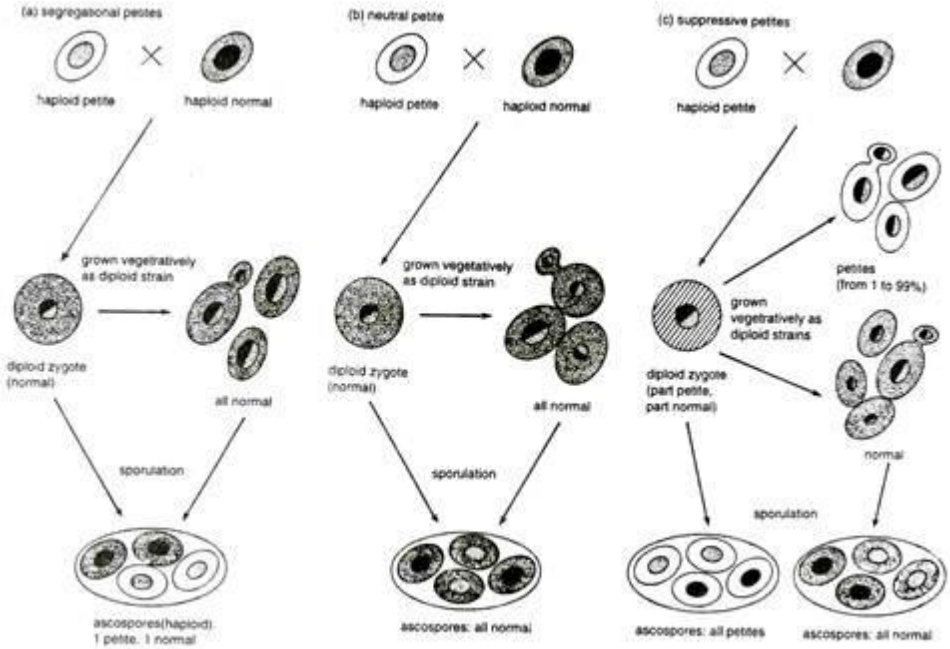


Fig. 22.5: Results of crosses between different kinds of *petites* and normal strain of yeast.

Neutral Petites:

In this type, only normal wild ascospores are produced from matting between petite and normal strain of yeast. The petite characteristics is absent in the product of segregation. So it shows the non-Mendelian inheritance. This non-Mendelian behaviour is very difficult to explain on the basis of nuclear genes and indicates that such petite characteristics are caused by extra-nuclear inheritance.

Suppressive Petites:

In this type, all ascospores produced from mating between normal and petite strain are petite type. Such petites seem to suppress normal respiratory behaviour and the suppressive petite factor acts as a dominant. Fig. 22.5 shows diagrammatic scheme for explaining some differences between neutral and suppressive petite in terms of DNA.

Therefore, there are two different genetic causes for respiratory deficiency in yeast. One is nuclear and the other is extra nuclear. On this basis a neutral petite having the nuclear gene for normally functioning mitochondria is crossed with a segregational petite (Fig. 22,6). The diploid zygote produced from such cross can use the normal nuclear genes from neutral petite and respire normally.

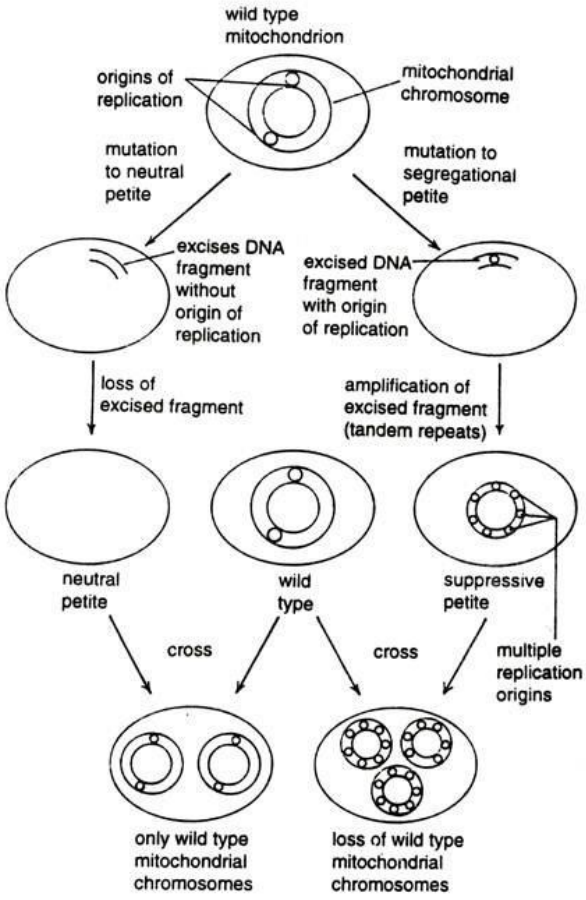


Fig. 22.6: Differences between neutral and suppressive petite in terms of DNA.

When such diploid zygotes are grown vegetatively as a diploid strain, they produce diploid colonies that are of normal size and respire normally. But when such diploid zygotes are allowed to sporulate, they undergo meiosis and produce four haploid ascospores of which two are petites and other two are normal. It indicates that normal and petite characteristics segregate in the 1 : 1 ratios expected from mendelian segregation.

It is noted that the neutral petite contains the normal nuclear gene for the respiratory enzymes but the segregational petite does not contain respiratory enzymes, so it is obvious that the cytoplasmic factor of the neutral petite appears in the cytoplasm of diploid zygotes where the factor is possibly independent of nuclear control.

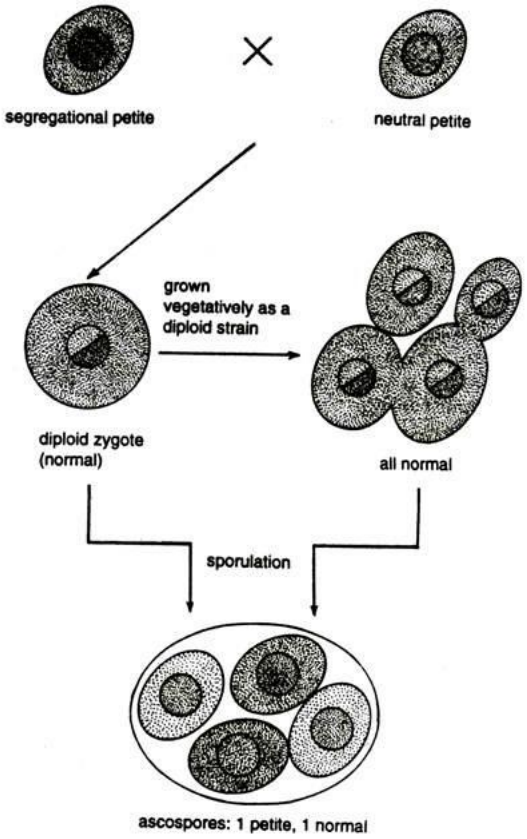


Fig. 22.7: Results of a cross between a segregational petite and a neutral peptite.

It is also noted that neutral petite strains are readily produced by subjecting normal strain in low doses of acriflavines dyes as well as ethidium bromide. But the treatment of such doses of dye does not induce any nuclear changes. Thus it strongly indicates the involvement of extra-nuclear change of gene controlling petite characteristics. Such changes ultimately shows the extra-nuclear inheritance of petite characteristics.

(viii) Extra-Nuclear Inheritance by Mitochondria of Porky Strain of *Neurospora*:

There are many examples for mitochondrial enzyme deficiency which are cases of extra chromosomal inheritance—petite yeast came from the studies of *Neurospora crassa*, a member of ascomycetes group of fungi. In this fungus, there is a slow-growing mutant strain called porky.

Such strain exhibits poorly differentiated mitochondria which are deficient in the membrane-bound cytochromes b, a_1 and a_3, essential proteins of the respiratory electron transport chain and also possess greatly reduced numbers of the small ribosomal units. As in yeast, this trait—inherited via the female parent In some strains in non-Mendelian fashion, indicate its extra chromosomal nature.

When porky as female parent (proto perithecial parent) is crossed with a normal strain as a male parent (mitochondrial parent), the progeny axe found to be porky. In reciprocal cross, the progeny are normal.

This non-Mendelian uniparental inheritance suggests that the cytoplasm of female parent is important because the only difference between reciprocal crosses is the contribution of cytoplasm.
Thus nuclear genotype has no effect on this particular phenotype.

(ix) Extra-Nuclear Inheritance by Symbionts:

There are many cases of cytoplasmic inheritance which are actually due to the presence of certain intra-cellular parasites like bacteria, virus particles etc. that make a symbiotic relationship with the host cell. They are able to reproduce within the host cell and look like the cytoplasmic inclusions.
These cytoplasmic symbionts provide some evidences regarding the cytoplasmic inheritance of the host cell. These symbionts are variously designated by Greek alphabets as a (sigma), k (kappa), etc. The various types of extra-nuclear inheritances due to parasites or symbionts are discussed next.

Kappa Particles in Paramoecium:

One of the most stricking and spectacular example of cytoplasmic inheritance due to symbiont bacteria is noted in the most common ciblate protozoam *Paramoecium aurelia*. In 1943, T. M. Sonneborn reported that some strains of P. aurelia contain kappa particles and are known as killer strain.
Kappa particles are the symbiont bacteria called *Caedobacter taeniospiralis*. The diameter of kappa particles are about 0.2µ. They are bounded by a membrane and contain a little bit of cytoplasm with DNA. The strain of Parameocium in which the kappa particles are absent are called sensitive strain. The sensitive strains are killed by the killer strain.
The destruction of sensitive strain occurs through secretion of a toxic substance called paramecin. This toxic substance is believed to breakdown the food vacuole membrane of the sensitive strain. Paramecin is diffusible in the liquid medium (Fig. 22.8).
When killers are allowed to remain in a medium for a time, they are not killed. It means that paramecin has no effect on killers. Paramecin is associated with a particular kind of kappa that occurs in about 20 percent of a kappa population.

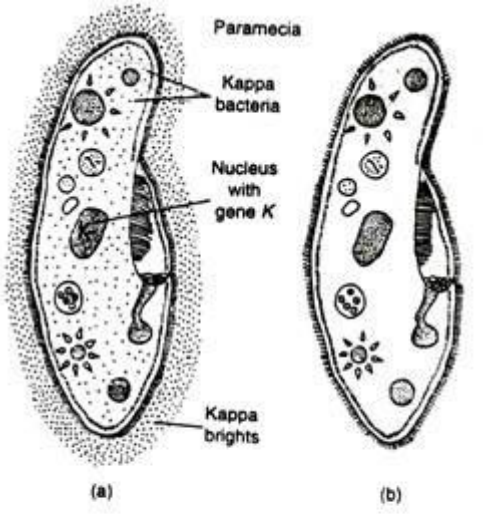


Fig. 22.8: (a) Killer strain Paramoecium with Kappa particles and nucleus with gene K; (b) Sensitive Paramoecium with no Kappa particles and nucleus with gene k.

These kappa bacteria possess a refractile protein containing 'R' body and are called brights because they are infected with a virus that controls the synthesis of a viral protein as well as R protein body in kappa bacterium.

The virus may act as the toxin in the killing response and R body facilitates the penetration of the toxin. The non-bright kappa bacteria may also contain virus but the virus may be in provirus state in them.

The killer character of Paramoecium has a nuclear as well as cytoplasmic basis. The existence of kappa particles is determined by presence of a nuclear dominant gene K. Kappa particles, like other bacteria, multiply through fission.

But their multiplication in the cytoplasm of Paramoecium depends on the presence of a dominant nuclear gene K which helps to make an environment necessary for the bacteria to reproduce.

When killer strain of *Paramoecium* conjugates with sensitive strain under appropriate condition for brief period and no cytoplasm exchange occurs, two kinds of clones result- one from the original killer cell which contains allele K (Kk) and kappa particles and the other from the original sensitive cell which carries the allele K (Kk) and lacks kappa particles.

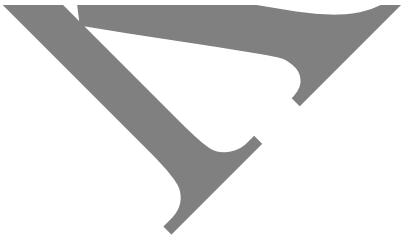
It indicates that homozygous (either KK or kk) strains become heterozygous following an exchange of K and k genes without cytoplasmic exchange.

Following autogamy (a process of self- fertilization within one undivided cell resulting in homozygosity), half the progency (50%) are sensitive Paramecia. But all progenies of sensitives following autogamy will be sensitive's.

In this conjugation, following autogamy of killers, 50% progeny will receive Kk genotype with cytoplasmic kappa particles other 50% progeny will receive kk genotype with cytoplasmic kappa particles. But it will be sensitive, because kappa cannot reproduce in the cells unless a K allele is present in the nucleus and, as a consequence the kappa are eliminated.

On the other hand, in this conjugation the product of autogamy of sensitive strain obtained after conjugation are all sensitive. All through, 50% progeny of autogamy have KK genotype without cytoplasmic kappa particles because no cytoplasm has been transferred in this conjugation. Remaining 50% progeny of autogamy of sensitive's have kk genotype and no cytoplasmic kappa particles.

Under some conditions of conjugation persists much longer; a long connection is established between conjugants (killer and sensitive). In this conjugation, cytoplasm as well as nuclear genes are exchanged (Fig. 22.9). As a consequence both ex-conjugants will receive the genotype Kk and the cytoplasm with kappa particles.



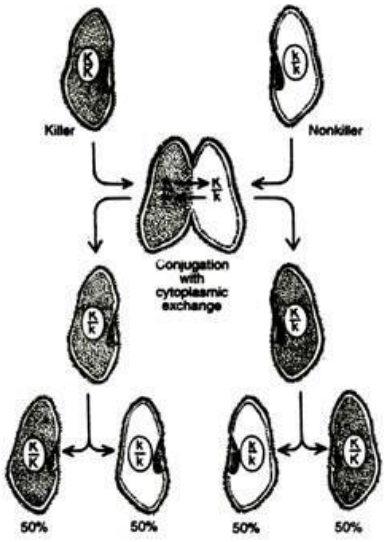


Fig. 22.9: Effect of conjugation for longer period with cytoplasmic exchange followed by autogamy.

Therefore, conjugation for longer period with cytoplasmic exchange will produce all killer strains. Autogamy of both ex-conjugants produces homozygotes KK (killer) and kk (sensitive) cell in the 1 : 1 ratios, respectively, as expected from Mendelian segregation.

Therefore, conjugation for shorter period without cytoplasmic exchange does not follow the Mendelian pattern of inheritance. Hence it confirms the cytoplasmic basis of inheritance of killer trait.

Mu Particles in Paramoecium:

There is another type of killer trait found in certain strain of *Paramoecium* due to presence of 'mu' particles in the cytoplasm. A *Paramoecium* with a 'mu' particle is called mate killer. On the other hand, a *Paramoecium* having no 'mu' particles is called mate sensitive.

It is so named because when a *Paramoecium* with 'mu' particle conjugates with a partner *Paramoecium* without 'mu' particle then the former kills the latter. The 'mu' particles exist only in those cells whose micronucleus contains at least one dominant of either of the two pairs of unlinked chromosomal genes such as M_1 and M_2. The 'mu' particles are symbionts which are made of DNA, RNA and other substances.

The maintenance of the 'mu' symbiont in a *Paramoecium* is dependent upon the genotype of the *Paramoecium*. In fact, the mate-killers of few genotypes maintain their normal number of particles for about seven generations. From the eighth generation, the particles suddenly and completely disappear from the a small fraction of the cell.

Gibson and Beale (1962) suggested that the maintenance of 'mu' particle in *Paramoecium* was due to the presence of another cytoplasmic particle called metagon. It is possibly a long-lived messenger RNA or informosome and may be a product of M_1 and M_2gene. One metagon may be necessary for the maintenance of hundred 'mu' particles.

(x) Sigma Virus in Drosophila:

Some strains of Drosophila melanogaster are sensitive to $C0_2$ as they die when briefly exposed to $C0_2$, while normal flies can be exposed for long periods to pure $C0_2$ without permanent damage. The high

degree of $C0_2$– sensitivity is associated with the presence of a DNA virus called sigma factor found in the cytoplasm of $C0_2$ sensitive Drosophila.

Sigma factor is transmitted through the egg cytoplasm. When a cross is made between $C0_2$– sensitive female with normal male, all offspring's are $C0_2$ sensitive. Again, in reciprocal cross, i.e., a cross between normal female and $C0_2$ sensitive male, most of the offspring's are normal except for a small proportion of progeny which are $C0_2$sensitive. Therefore, the inheritance pattern of $C0_2$ sensitivity is non-Mendelian and confirms the cytoplasmic basis of inheritance.

(xi) Spirochaetes and Maternal Sex Ratios in Drosophila:

Spirochaetes sometimes enter into the female body cell of Drosophila and live there as endoparasites. When spirochaetes enter the egg cell and these infected egg cells are fertilised, the zygotes having XY sex. chromosome are killed early in embryonic development and XX zygotes survive.

Therefore, the presence of spirochaete in the female body gives rise to exclusively female progeny—this condition is known as maternal sex ratio. It is evident that XY embryos are killed by a toxic substance which may derive from a DNA virus present within spirochetes that live as endoparasite in the female body of Drosophila. Maternal sex ratios in Drosophila is also considered as an example of extra nuclear inheritance.

Milk Factor in Mice:

This is an interesting example of extra nuclear inheritance. It is found that certain types of mice are very susceptible to mammary cancer and this characteristic is found to be transmitted maternally.The results of reciprocal cross between susceptible mice and low-incidence mice depend on the trait of female parent.

When the young mice of low cancer incidence parent are allowed to feed milk by a susceptible foster mother, it produce, a high rate of cancer in them. Hence this is a case of infective agent transmitted in the milk. The milk factor responsible for causing cancer is possibly a virus. The presence of milk factor depends on nuclear gene.

RNA Viruses in Fungi:

Like *Paramoecium*, there are two strains of yeast (*Saccharomyces cerevisiae*). One strain is killer and other one is sensitive. The killer strain secretes a proteineceous toxic substance that kills the sensitive strain of yeast cell.

When a cross is made between killer and sensitive strain of yeast, only killer offspring's are produced—indicating uniparental inheritance. There are some other strains of yeast which are called neutral strains.

Neutral strains are neither killed by killer nor do they kill the sensitive strain. But the cytoplasm of both killer and neutral strains contain two types of double-stranded RNA in the form of isometric virus-like particles (about 39 nm in diameter).

The existence and maintenance of virus particles in the yeast cytoplasm are controlled by some dominant nuclear genes called MAK genes (maintenance of killer). Some other nuclear genes—e.g., KEXx (killer expression) and KEX_2—convert killers into neutrals.

A similar situation is noted is case of *Ustilago maydis*, a maize smut fungus. Here the cytoplasm of killer strain also contains maycovirus like particle containing double-stranded RNA. Killer strain secretes a toxin which kills sensitive strains but it has no lethal effect on resistant strains. Resistant strains are particularly resistant to one of the killer strains designated as p_1, p_4 and p_6. Some nuclear genes denoted as P^r_1,P^r_4 and p^r_6 convert sensitive strain into resistant ones.

In all such cases mentioned above, the virus like particles are not the integral part of the normal cellular organization but their existence and transmission indirectly provides some evidences in favor of cytoplasmic inheritance.

Uniparental Inheritance in Chlamydomonas Reinhardi:

R. Sager (1970) and N. Gilham (1968) have reported some cases of extra-chromosomal inheritance in green alga *Chlamydomonas reinhardi*. The alga reproduces by asexual as well as sexual means. The sexual reproduction takes place by fusion between two morphologically similar but physiologically dissimilar haploid gametes coming from different haploid parents designated as '+' and '-'.

The gametic fusion produces the zygote. The sex is determined by a single chromosomal gene. When meiosis occurs in the zygote, four haploid daughter protoplasts are formed which give rise to a new plant body. Although both the sexes contribute equally to the zygote, there is maternal transmission of certain cytoplasmic traits.

Chlamydomonas is a haploid unicellulate green alga. It has two mating types—'+' and '-'.The two mating types are governed by two alleles of a nuclear single gene. The alleles axe named as mt^+ and mt^-. The + mating type is considered as female, while the – mating type is regarded as male. During sexual reproduction one mt^+ and one mt^-cell pair and fuse together to form a zygote where there is mixture of cytoplasm coming from both mt+ and mt^- gametes.

The zygote undergoes meiosis to produce 4 haploid meiozospores of which two zoospores contain '+' alleles and other two contain '-' alleles, i.e., it shows typical 1 : 1 segregation for nuclear genes. But for their plasma genes all zoospores are identical and contain only mt^+type plasma genes by mt^+ plasma genes. The inactivation is not clear but it may involve an enzymatic process.

R. Sager isolated two strains of *Chlamydomonas:* one strain was resistant (S_r) to 500/xg of streptomycin per ml of culture solution and the other one is sensitive. The trait of streptomycin resistance is believed to be located in its cp-DNA (chloroplast DNA).

Mating between mt+ streptomycin resistant (S_r) and mt sensitive (S_s) cells produce only resistant progeny but the nuclear genes for mating type segregate as expected (Fig. 22.10). But the reciprocal cross between mt+ susceptible and mt^- resistant shows again the expected segregation for mating type but all progenies are sensitive type. Therefore, it clearly provides an example for extra-nuclear inheritance. It is also observed that in less than 0.1% of zygotes plasma genes from mt" parent are not inactivated and produce cytohets, i.e., heterozygotes for cytoplasmic genes.

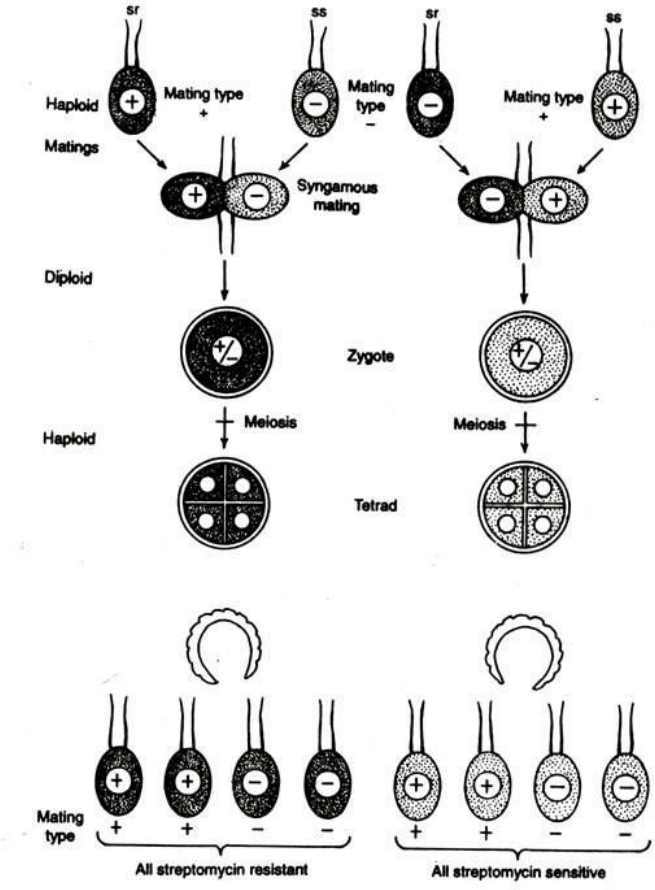


Fig. 22.10: Uniparental inheritance in *Chlamydomonas reinhardi*.

Inheritance of Preformed Structures:

In some lower group of animals like ciliated protozoa there are some characteristics which show cytoplasmic inheritance. These traits are not controlled by any plasma gene. In *Paramoecium*, the cytoplasm is differentiated into cortex or ectoplasm and medulla or endoplasm.

The mouth and the contractile vacuole are the prominent preformed traits that are present in the cortical region of *Paramoecium*. These preformed structures can be transmitted independent of the transmission of nuclear genes and plasma genes.

For normal sexual reproduction two individuals of *Paramoecium* called conjugants come close together, exchange their nuclear material, and then separate as ex-conjugants with zygote nucleus. In some rare cases, conjugants do not separate and remain as doublet animal with two sets of mouth and contractile vacuole structures.

When this doublet animal reproduces asexually by binary fission, they also give rise to doublet ex-conjugants. When doublets are mated with normal singlet's, the progeny of doublets ex-conjugants are doublets and the progeny of singlet's are singlet's. This type of inheritance is also found when the animals reproduces through autogamy.

In these crosses it is noted that the nuclear genes of doublets and singlet's are inherited in normal Mendelian fashion. But the mode of duplicated mouth and contractile vacuole is independent of the mode of inheritance of nuclear genes as well as cytoplasmic hereditary factors.

Furthermore, the transplantation experiments reveal that such cortical structure (mouth, contractile vacuole) are autonomous and are maintained by cell division.

Some born has suggested that different parts of the cortex might serve as sites for the specific absorption and orientation of molecules derived from the milieu and genetic action. Besides this, preformed cortical structures could act by determining where , some gene products go in the cell, how these combine and orient and what they do.

Extra-Nuclear Inheritance in Bacteria:

In many cases, extra-nuclear inheritance has also been reported in bacteria. In addition to main chromosome, bacterial cell has got a unique extra-nuclear genetic system in their cytoplasm. Such extra-nuclear genetic system plays an important role for cytoplasmic basis of inheritance.

In bacteria, the cytoplasmic inheritance is performed by:

(i) plasmids, and

(ii) episome.

In addition to main chromosome, subsidiary DNA is also present in the bacterial cell in the form of plasmid. Plasmids are mini-circular DNA duplex and are capable of independent replication and transmission. By definition, a plasmid is a relicon that is stably inherited in an extra-chromosomal state.

Plasmids are not essential for the survival of bacterial cells, i.e., disposable-except under certain environmental conditions. Plasmids vary in size and contain three to several hundred genes. A bacterial cell may contain more than one plasmid.

There are several types of bacterial plasmids of which three general types have been studied extensively such as:

(a) F plasmid;

(b) R plasmids, and

(c) Col plasmids.

F plasmids carry genes for the development of F pili and are responsible for conjugation.

R plasmids carry genes which are responsible for resistance to antibiotics or other antibacterial drugs.

Col plasmids carry genes which code for colicins. Colicins are proteins that kill sensitive *E.coli* cells.

Plasmids may again be divided into two types on the basis of whether or not they mediate conjugative self-transfer.

They are:

(a) Conjugative and

(b) Non-conjugative.

Conjugative plasmids are also known as transmissible plasmids that mediate the transfer of DNA through conjugation. All F plasmids, R plasmids and some col plasmids are the examples of conjugative plasmids.

These plasmids spread rapidly among the bacterial cells of a population. Transmission of R plasmids is responsible for many pathogenic bacteria to become resistant to many of the widely used antibiotics such as penicillin, tetracycline, streptomycin, kanamycin, chloramphenicol etc.

The transmission of these plasmids are not only restricted among the population of the same species but are also known to transfer to others like Proteus, Salmonella, Hemophilus, Pasteurella, Shigella etc.

Non-conjugative or non-transmissible plasmids do not mediate DNA transfer through conjugation. Some R and Col plasmids are of this type.

Some plasmids are capable of becoming attached and integrated into the bacterial chromosome. Then they are named episomes.

Plasmid and episomes contain insertion sequences which are also present in bacterial chromosomes. Insertion sequences are transposable in that they can move about within and between chromosomes and mediate genetic recombination between otherwise non-homologous genetic elements within which they are located.

Insertion sequence are also responsible for the transfer of genetic controlling resistance to antibiotics from one genetic element to the other.

Considering the mode of transmission, location and the presence of genes controlling certain characteristics, it is clear that the phenomenon of extra-nuclear inheritance still exists even in most simple, prokaryotic unicellular organism like bacteria.

Considering the discussion of this article we can summarize the characteristic features of cytoplasmic inheritance as:

i. In case of cytoplasmic inheritance, reciprocal crosses show marked difference for characteristics governed by plasma genes.

ii. In most cases female parent contributes the plasma genes, i.e., uniparental inheritance or maternal inheritance.

iii. In general, F_2, F_3 and so on generations do not show segregation for cytoplasmically inherited characteristics. It is a non-Mendelian inheritance.

iv. In case of bi-parental inheritance, irregular segregation takes place in F_1.

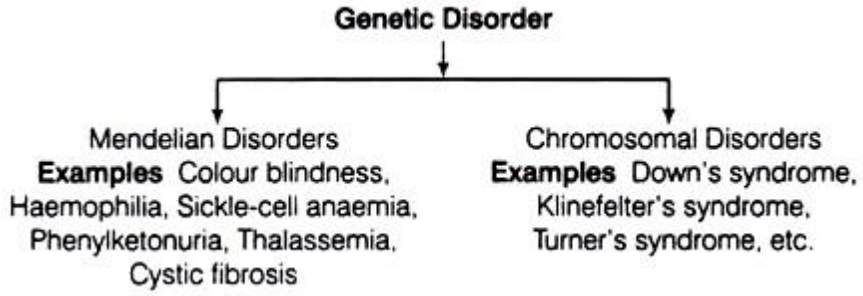
v. Several plasma genes are associated with cp-DNA or mt DNA in higher eukaryotic organisms and with plasmids or eRisomes in prokaryotes.

vi. In many cases, a cytoplasmically inherited characteristic is associated with an endosymbiont or parasite or virus present in the cytoplasm of the organism.

In some specific cases the cytoplasmic inheritance of some preformed characteristics is not affected by exchange of cytoplasm and is not controlled by nuclear genes. They are autonomous and are maintained by cell division.

Genetic abnormalities

The genetic disorders can be divided as:



1. Mendelian Disorders:

These are mainly determined by alteration or mutation in the single gene. These disorders are transmitted in next generation according to the principle of inheritance and can be studied by pedigree analysis. These can be dominant or recessive.

For example, Autosomal dominant disorder are Osteogenesis imperfecta, polycystic kidney disease, Huntington's Disease, Fatal familial Insomnia, etc. Autosomal recessive disorder are Sickle cell anaemia, cystic fibrosis, xeroderma pigmentosum, Albinism etc.

Some of Mendelian disorders are discussed below

Haemophilia:

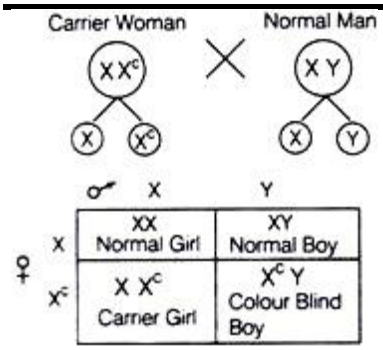
It is a sex-linked recessive disease, which is transmitted from an unaffected carrier female to some of the male offsprings. Due to this, patient continues bleeding even on a minor injury because of defective blood coagulation. The gene for haemophilia is located on X-chromosome. The defective alleles produce non-functional proteins, which later form a non-functional cascade of proteins involved in blood clotting.

The possibility of a female becoming a haemophilic is extremely rare because mother of such a female has to be at-least carrier and father should be haemophilic. For example, females suffer from this disease only in homozygous condition, i.e., X^CX^C. Queen Victoria was a carrier of haemophilia and produced haemophilic individuals.

Colour Blindness:

It is a sex-linked recessive disorder, which results in defect in either red or green cone of eye. It does not mean not seeing any colour at all, in-fact it leads to the failure in discrimination between red and green colour. The gene for colour blindness is present on X-chromosome.

It is more present in males (X^CY) because of the presence of only one X-chromosome as compared to two chromosomes in females. A heterozygous female has normal vision, but is a carrier and passes on the disorder to some of her sons. Colour blindness like any other inheritance show crisscross inheritance.

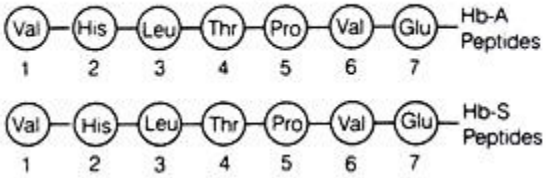


Sickle-cell Anaemia:

It is an autosome-linked recessive trait that can be transmitted from parents to the offsprings, when both the partners are carrier for the gene (heterozygous).

The disease is controlled by a single pair of allele Hb and Hb . Only the homozygous individuals for Hb^S, i.e., Hb^S Hb^S show the diseased phenotype. The heterozygous individuals are carriers (Hb^A Hb^S). It is caused by the substitution of glutamic acid (Glu) by valine (Val) at the sixth position of the beta globin chain of the haemoglobin molecule.

The normal (Hb-A) and the defective (Hb-S) peptides are as follows



The mutant haemoglobin molecule undergoes polymerisation under low oxygen tension causing the change in the shape of the RBC from biconcave disc to the elongated sickle like structure.

Thalassemia:

It is an autosome-linked recessive disease, which occurs due to either mutation or deletion, resulting in reduced rate of synthesis of one of the globin chains of haemoglobin. Anaemia is the main feature of this disease.

Thalassemia is classified into three types on the basis of globin chain affected:

(i) Alpha (α) Thalassemia:

It is controlled by the closely linked genes HBA1 and HBA2 on chromosome 16. In this, the production of globin gene is affected due to the mutation or deletion of one or more of the four alleles.

(ii) Beta (β) Thalassemia:

It is controlled by a single gene HBB on chromosome 11. It occurs due to the mutation in one or both the alleles of the gene. Hence, there is a reduced synthesis of beta globin of haemoglobin.

(iii) Delta (δ) Thalassemia:

As well as alpha and beta chains present in haemoglobin about 3% of adult haemoglobin is made up of alpha and delta chains. Just like beta thalassemia mutations can occur which affect the ability of this gene to produce delta chains.

Phenylketonuria (PKU):

It is an inborn error of metabolism, which is inherited as an autosomal recessive trait. It is a rare disease in which baby is born without the ability to properly breakdown an amino acid called phenylalanine. Babies with this disease have a missing enzyme called phenylalanine hydroxylase, which is needed to break down an essential amino acid phenylalanine into tyrosine in liver. This phenylalanine is accumulated and gets converted into phenyl pyruvic acid and other derivatives leading to mental retardation.

2. Chromosomal Disorders:

These are caused by the absence or excess or abnormal arrangement of one more chromosomes.

The examples are given below

Down's Syndrome:

It was described by J Langdon Down in 1866. It occurs due to the additional copy of chromosome number 21 or trisomy of chromosome 21 in humans and also seen in chimpanzees and other related primates.

Symptoms:

i. Individuals are short statured with small, round head and furrowed tongue.
ii. Partially open mouth, palm is broad with characteristic palm crease.
iii. Slow mental development.

Klinefelter's Syndrome:

It occurs due to the presence of an additional copy of X-chromosome resulting in the karyotype 45 + XXY which results into 47 chromosomes.

Symptoms:

i. Individuals have masculine development but feminine characters like development of breasts, (gynaecomastia) etc.
ii. Poor bread growth and often sterile and feminine pitched voice.

Turner's Syndrome:

i. It is a disorder caused due to the absence of one of the X-chromosome, i.e., 45 with XO.

Symptoms:

i. Affected females are sterile as ovaries are rudimentary.
ii. Lack of secondary sexual characters and poor breasts development. Short stature, small uterus, puffy fingers and webbed neck.
iii. The chromosomal disorders can be studied by the analysis of karyotypes.

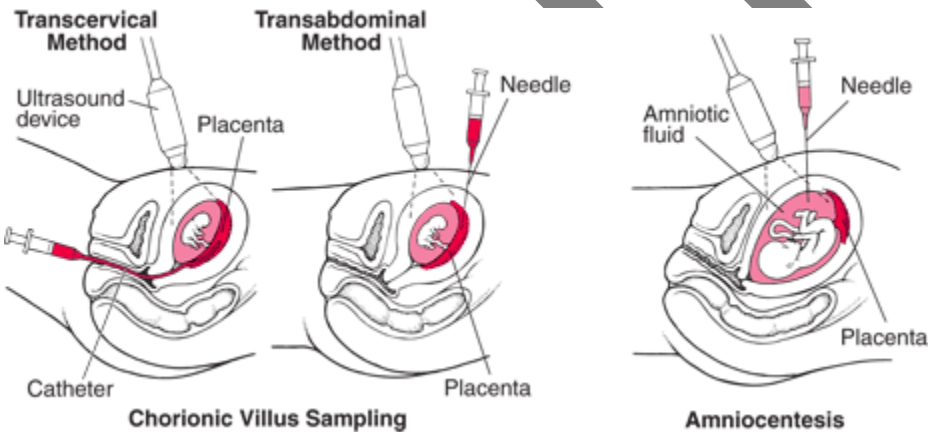
Possible questions

1. Differentiate incomplete dominance and codominance with suitable examples.
2. What are the methods employed for sex determination? Write a brief note on it.
3. Discuss about the penetrance and expressivity.
4. Write a brief note on epistasis.
5. Discuss on Mendelian principles and his experiments.
6. Write a short note on multiple allele with examples.
7. Write a note on complementary gene and duplicate gene.
8. Comment a brief note on environmental effects and twin studies.
9. Comment on Mendelian inheritance and his experiments. Explain with monohybrid, dihybrid and trihybrid crosses.

UNIT-II
SYLLABUS

Mendelian Prenatal Screening: Amniocentesis; Prenatal diagnosis of genetic diseases, XX and XY karyotyping, DNA/RNA probes. DNA probes in the diagnosis of infectious diseases; Mycobacterial, plasmodial, HIV and HPV infections during development. Molecular probes in diagnosis of genetic diseases: Down syndrome, Cystic fibrosis, Sickle cell anemia, Alkaptonuria, Phenylketonuria, Kleinfelter syndrome and Cancer (breast cancer,Leukaemia, Burkets lymphoma).

Amniocentesis



Amniocentesis is a diagnostic test that may be recommended by your health care provider following an abnormal triple test result. Inherited or genetic concerns lead some parents to choose amniocentesis to determine if specific genetic disorders may be present in their baby.

Amniocentesis is a procedure wherein a sample of fluid is removed from the amniotic sac for analysis. The amniotic sac is found within the uterine cavity. The amniotic sac is the fluid-filled structure inside a pregnant woman's uterus within which the baby lives. Fetal cells, proteins, and fetal urine freely move within this sac.

During amniocentesis, fluid is removed by placing a long needle through the abdominal wall into amniotic sac. Sometimes, the woman's skin is injected first with a local anesthetic, but this is not usually necessary. The amniocentesis needle is typically guided into the sac with the help of ultrasound imaging performed either prior to or during the procedure. Once the needle is in the sac, a syringe is used to withdraw the clear amber-colored amniotic fluid, that resembles urine. The volume of fluid withdrawn depends upon the age of the fetus and the reason for the procedure.

The fluid can then be sent for evaluation of fetal lung maturity, genetic evaluation, evidence of spina bifida (a birth defect in spinal cord development) or other neural-tube defects, the presence of infection, or fetal chromosomal analysis. Chromosomes are structures which contain all of the genetic information within our cells. Amniotic fluid contains numerous free-floating fetal cells that can be grown in the laboratory. When these cells multiply and reach a certain number, their chromosomes are extracted and analyzed. It takes approximately two weeks to perform chromosomal analysis. The fluid also contains proteins, minerals and other compounds that can be tested. These additional studies may require 1 to 7 days to perform. Data obtained from amniotic fluid can help women make informed decisions regarding their pregnancies.

For most patients, amniocentesis is a fairly rapid and comfortable procedure. Some women experience uterine cramping after the procedure. They may also feel faint during or following the test.

Women over the age of 35 have an increased chance of having a baby with chromosomal abnormalities. Therefore, the current recommendation is that women who will be age 35 or older at the time of delivery be offered a genetic amniocentesis for the testing of fetal chromosomes. Chromosomal abnormalities in the fetus can lead to intellectual disabilities or other birth defects, as seen with Down's syndrome. The possibility of having a baby with a chromosomal defect increases with the age of the mother.

- At age 35, the chance is about 1 in 178.
- By age 40, the rate increases to 1 in 63.
- By age 48, the ratio is 1 in 8.
- For women younger than 35, the risk of complications as a result of amniocentesis outweighs the possibility that the baby has a chromosomal abnormality
- After 35, the chance of delivering a baby with a chromosomal abnormality may be more common than experiencing the complications associated with this procedure.

If there is a family history of chromosomal problems or history of prior births in which a chromosomal defect was identified, amniocentesis may be recommended. However, the exact benefit of amniocentesis in these situations is uncertain. Many chromosomal defects may not reappear in subsequent pregnancies, and genetic counseling can help a woman decide whether amniocentesis would be of value in these situations.

Amniocentesis is very accurate in detecting chromosomal abnormalities and the gender of the fetus. Occasionally, the fetal cells harvested by amniocentesis do not grow in the culture, and no chromosomal data can be obtained. Additionally, physician may be unable to extract fluid from the uterus during amniocentesis due to a variety of technical reasons. In both instances, a subsequent extraction of fluid may be attempted.

Amniocentesis testing for lung maturity can help the doctor make decisions regarding timing of delivery for women in premature labor, those with diabetes, or other medical conditions requiring possible early delivery. Lung maturity testing is not perfect. In rare instances, a baby with lungs that are predicted to perform normally can still experience difficulty breathing.

The AFP (alpha fetoprotein) blood test is currently available and can be used to screen for neural tube defects such as spina bifida, as well as Down's syndrome. An elevated AFP blood level may indicate the possibility that the fetus has a defect in the brain and/or spinal cord development. A

low AFP level may indicate the potential for Down's syndrome. When abnormal blood levels of AFP are found, further evaluation with ultrasound and amniocentesis can be done to look for birth defects and chromosomal abnormalities.

Amniocentesis can also help determine if there are specific genetic problems present in the fetus. Testing for Tay-Sachs disease, cystic fibrosis, sickle cell anemia, and other inherited disorders can be performed on samples of amniotic fluid. Checking the cells for specific genes can now be done on the fetal cell cultures. It is also possible to look for a specific protein in the amniotic fluid that can signal a genetic abnormality. Testing for genetic (inherited) diseases is not routinely performed, but is available when necessary.

Another common reason for performing amniocentesis is to determine if the fetal lungs are mature enough so that the baby can be safely delivered before the due date. If the fetal lungs are not mature, an attempt can be made to stop premature labor with medications. If the lung tissues are mature, it may be safe for the premature baby to be born. This test can also help in the timing of delivery when the due date is uncertain.

Occasionally, a pregnant woman may have a fever without an obvious site of infection. In these instances, an amniocentesis may be performed to determine if the cause of the mother's fever is from an intrauterine infection.

The overall possibility of birth defects in any pregnancy is approximately 3%. For defects such as cleft lip, hernia, and extra fingers or toes, amniocentesis will not be diagnostic. Ultrasound may be helpful in detecting these defects in the fetus.

Summary

- Amniocentesis may be performed for chromosome analysis or the detection of genetic defects and conditions in the fetus.
- Pregnant women over the age of 35 years are candidates for amniocentesis to detect chromosomal abnormalities in the fetus.
- Amniocentesis can also be used to determine the maturity of the fetal lungs or the cause of a fever in the mother.
- For genetic testing, chromosome analysis, and the evaluation of an abnormal alpha feto-protein test, amniocentesis is usually performed between the 16th and 21st weeks of pregnancy.
- The risk of fetal loss from the amniocentesis procedure is less than 1%.

Prenatal diagnosis of genetic diseases

Prenatal diagnosis employs a variety of techniques to determine the health and condition of an unborn fetus. Without knowledge gained by prenatal diagnosis, there could be an untoward outcome for the fetus or the mother or both. Congenital anomalies account for 20 to 25% of perinatal deaths. Specifically, prenatal diagnosis is helpful for:

- Managing the remaining weeks of the pregnancy
- Determining the outcome of the pregnancy
- Planning for possible complications with the birth process
- Planning for problems that may occur in the newborn infant
- Deciding whether to continue the pregnancy

- Finding conditions that may affect future pregnancies

There are a variety of non-invasive and invasive techniques available for prenatal diagnosis. Each of them can be applied only during specific time periods during the pregnancy for greatest utility. The techniques employed for prenatal diagnosis include:

- Ultrasonography
- Amniocentesis
- Chorionic villus sampling
- Fetal blood cells in maternal blood
- Maternal serum alpha-fetoprotein
- Maternal serum beta-HCG
- Maternal serum estriol

Ultrasonography

This is a non-invasive procedure that is harmless to both the fetus and the mother. High frequency sound waves are utilized to produce visible images from the pattern of the echos made by different tissues and organs, including the baby in the amniotic cavity. The developing embryo can first be visualized at about 6 weeks gestation. Recognition of the major internal organs and extremities to determine if any are abnormal can best be accomplished between 16 to 20 weeks gestation.

Although an ultrasound examination can be quite useful to determine the size and position of the fetus, the size and position of the placenta, the amount of amniotic fluid, and the appearance of fetal anatomy, there are limitations to this procedure. Subtle abnormalities may not be detected until later in pregnancy, or may not be detected at all. A good example of this is Down syndrome (trisomy 21) where the morphologic abnormalities are often not marked, but only subtle, such as nuchal thickening.

Amniocentesis

This is an invasive procedure in which a needle is passed through the mother's lower abdomen into the amniotic cavity inside the uterus. Enough amniotic fluid is present for this to be accomplished starting about 14 weeks gestation. For prenatal diagnosis, most amniocenteses are performed between 14 and 20 weeks gestation. However, an ultrasound examination always proceeds amniocentesis in order to determine gestational age, the position of the fetus and placenta, and determine if enough amniotic fluid is present. Within the amniotic fluid are fetal cells (mostly derived from fetal skin) which can be grown in culture for chromosome analysis, biochemical analysis, and molecular biologic analysis.

In the third trimester of pregnancy, the amniotic fluid can be analyzed for determination of fetal lung maturity. This is important when the fetus is below 35 to 36 weeks gestation, because the lungs may not be mature enough to sustain life. This is because the lungs are not producing enough surfactant. After birth, the infant will develop respiratory distress syndrome from hyaline membrane disease. The amniotic fluid can be analyzed by fluorescence polarization (fpol), for lecithin:sphingomyelin (LS) ration, and/or for phosphatidyl glycerol (PG).

Risks with amniocentesis are uncommon, but include fetal loss and maternal Rh sensitization. The increased risk for fetal mortality following amniocentesis is about 0.5% above what would normally be expected. Rh negative mothers can be treated with RhoGam. Contamination of fluid

from amniocentesis by maternal cells is highly unlikely. If oligohydramnios is present, then amniotic fluid cannot be obtained. It is sometimes possible to instill saline into the amniotic cavity and then remove fluid for analysis.

Chorionic Villus Sampling (CVS)

In this procedure, a catheter is passed via the vagina through the cervix and into the uterus to the developing placenta under ultrasound guidance. Alternative approaches are transvaginal and transabdominal. The introduction of the catheter allows sampling of cells from the placental chorionic villi. These cells can then be analyzed by a variety of techniques. The most common test employed on cells obtained by CVS is chromosome analysis to determine the karyotype of the fetus. The cells can also be grown in culture for biochemical or molecular biologic analysis. CVS can be safely performed between 9.5 and 12.5 weeks gestation.

CVS has the disadvantage of being an invasive procedure, and it has a small but significant rate of morbidity for the fetus; this loss rate is about 0.5 to 1% higher than for women undergoing amniocentesis. Rarely, CVS can be associated with limb defects in the fetus. The possibility of maternal Rh sensitization is present. There is also the possibility that maternal blood cells in the developing placenta will be sampled instead of fetal cells and confound chromosome analysis.

Maternal blood sampling for fetal DNA

This technique makes use of the phenomenon of fetal blood cells gaining access to maternal circulation through the placental villi. Ordinarily, only a very small number of fetal cells or cell free DNA enter the maternal circulation in this fashion (not enough to produce a positive Kleihauer-Betke test for fetal-maternal hemorrhage). The sequencing of maternal plasma cell-free DNA (cfDNA testing) can detect fetal autosomal aneuploidy, but without the risks that invasive procedures inherently have. Fluorescence in-situ hybridization (FISH) is another technique that can be applied to identify particular chromosomes of the fetal cells recovered from maternal blood and diagnose aneuploid conditions such as the trisomies and monosomy X.

The problem with this technique is that it is difficult to get large amounts of fetal DNA. There may not be enough to reliably determine anomalies of the fetal karyotype or assay for other abnormalities.

Maternal serum alpha-fetoprotein (MSAFP)

The developing fetus has two major blood proteins--albumin and alpha-fetoprotein (AFP). Since adults typically have only albumin in their blood, the MSAFP test can be utilized to determine the levels of AFP from the fetus. Ordinarily, only a small amount of AFP gains access to the amniotic fluid and crosses the placenta to mother's blood. However, when there is a neural tube defect in the fetus, from failure of part of the embryologic neural tube to close, then there is a means for escape of more AFP into the amniotic fluid. Neural tube defects include anencephaly (failure of closure at the cranial end of the neural tube) and spina bifida (failure of closure at the caudal end of the neural tube). The incidence of such defects is abbout 1 to 2 births per 1000 in the United States. Also, if there is an omphalocele or gastroschisis (both are defects in the fetal abdominal wall), the AFP from the fetus will end up in maternal blood in higher amounts.

In order for the MSAFP test to have the greates utility, the gestational age must be known with certainty. This is because the amount of MSAFP increasses with gestational age (as the fetus and the amount of AFP produced increase in size). Also, the race of the mother and presence of

gestational diabetes are important to know, because the MSAFP can be affected by these factors. The MSAFP is typically reported as multiples of the mean (MoM). The greater the MoM, the more likely a defect is present. The MSAFP has the greatest sensitivity between 16 and 18 weeks gestation, but can still be useful between 15 and 22 weeks gestation.

However, the MSAFP can be elevated for a variety of reasons which are not related to fetal neural tube or abdominal wall defects, so this test is not 100% specific. The most common cause for an elevated MSAFP is a wrong estimation of the gestational age of the fetus.

Using a combination of MSAFP screening and ultrasonography, almost all cases of anencephaly can be found and most cases of spina bifida. Neural tube defects can be distinguished from other fetal defects (such as abdominal wall defects) by use of the acetylcholinesterase test performed on amniotic fluid obtained by amniocentesis--if the acetylcholinesterase is elevated along with MSAFP then a neural tube defect is likely. If the acetylcholinesterase is not detectable, then some other fetal defect is suggested.

NOTE: The genetic polymorphisms due to mutations in the methylene tetrahydrofolate reductase gene may increase the risk for NTDs. Folate is a cofactor for this enzyme, which is part of the pathway of homocysteine metabolism in cells. The C677T and the A1298C mutations are associated with elevated maternal homocysteine concentrations and an increased risk for NTDs in fetuses. Prevention of many neural tube defects can be accomplished by supplementation of the maternal diet with only 4 mg of folic acid per day, but this vitamin supplement must be taken a month before conception and through the first trimester.

The MSAFP can also be useful in screening for Down syndrome and other trisomies. The MSAFP tends to be lower when Down syndrome or other chromosomal abnormalities is present.

Maternal serum beta-HCG

This test is most commonly used as a test for pregnancy. Beginning at about a week following conception and implantation of the developing embryo into the uterus, the trophoblast will produce enough detectable beta-HCG (the beta subunit of human chorionic gonadotropin) to diagnose pregnancy. Thus, by the time the first menstrual period is missed, the beta-HCG will virtually always be elevated enough to provide a positive pregnancy test. The beta-HCG can also be quantified in serum from maternal blood, and this can be useful early in pregnancy when threatened abortion or ectopic pregnancy is suspected, because the amount of beta-HCG will be lower than expected.

Later in pregnancy, in the middle to late second trimester, the beta-HCG can be used in conjunction with the MSAFP to screen for chromosomal abnormalities, and Down syndrome in particular. An elevated beta-HCG coupled with a decreased MSAFP suggests Down syndrome.

Very high levels of HCG suggest trophoblastic disease (molar pregnancy). The absence of a fetus on ultrasonography along with an elevated HCG suggests a hydatidiform mole. The HCG level can be used to follow up treatment for molar pregnancy to make sure that no trophoblastic disease, such as a choriocarcinoma, persists.

Maternal serum estriol

The amount of estriol in maternal serum is dependent upon a viable fetus, a properly functioning placenta, and maternal well-being. The substrate for estriol begins as dehydroepiandrosterone (DHEA) made by the fetal adrenal glands. This is further metabolized in the placenta to estriol.

The estriol crosses to the maternal circulation and is excreted by the maternal kidney in urine or by the maternal liver in the bile. The measurement of serial estriol levels in the third trimester will give an indication of general well-being of the fetus. If the estriol level drops, then the fetus is threatened and delivery may be necessary emergently. Estriol tends to be lower when Down syndrome is present and when there is adrenal hypoplasia with anencephaly.

Inhibin-A

Inhibin is secreted by the placenta and the corpus luteum. Inhibin-A can be measured in maternal serum. An increased level of inhibin-A is associated with an increased risk for trisomy 21. A high inhibin-A may be associated with a risk for preterm delivery.

Pregnancy-associated plasma protein A (PAPP-A)

Low levels of PAPP-A as measured in maternal serum during the first trimester may be associated with fetal chromosomal anomalies including trisomies 13, 18, and 21. In addition, low PAPP-A levels in the first trimester may predict an adverse pregnancy outcome, including a small for gestational age (SGA) baby or stillbirth. A high PAPP-A level may predict a large for gestational age (LGA) baby.

"Triple" or "Quadruple" screen

Combining the maternal serum assays may aid in increasing the sensitivity and specificity of detection for fetal abnormalities. The classic test is the "triple screen" for alpha-fetoprotein (MSAFP), beta-HCG, and estriol (uE3). The "quadruple screen" adds inhibin-A.

Condition	MSAFP	uE3	HCG
Neural tube defect	Increased	Normal	Normal
Trisomy 21	Low	Low	Increased
Trisomy 18	Low	Low	Low
Molar pregnancy	Low	Low	Very High
Multiple gestation	Increased	Normal	Increased
Fetal death (stillbirth)	Increased	Low	Low

Note: the levels of these analytes change markedly during pregnancy, so interpretation of the measurements depends greatly upon knowing the proper gestational age. Otherwise, results can be misinterpreted.

Techniques for Pathologic Examination

A variety of methods can be employed for analysis of fetal and placental tissues:

Gross Examination

The most important procedure to perform is simply to look at the fetus or fetal parts. Obviously, examination of an intact fetus is most useful, though information can still be gained from examination of fetal parts.

The pattern of gross abnormalities can often suggest a possible chromosomal abnormality or a syndrome. Abnormalities can often be quite subtle, particularly the earlier the gestational age.

Consultations are obtained with clinical geneticists to review the findings. A description of the findings is put into a report (surgical pathology or autopsy).

Examination of the placenta is very important, because the reason for the fetal loss may be a placental problem.

Microscopic Examination

Microscopic findings are generally less useful than gross examination for the fetus, but microscopic examination of the placenta is important. Microscopy can aid in determination of gestational age (lung, kidney maturity), presence of infection, presence of neoplasia, or presence of "dysplasia" (abnormal organogenesis).

Radiography

Standard anterior-posterior and lateral radiographic views are essential for analysis of the fetal skeleton. Radiographs are useful for comparison with prenatal ultrasound, and help define anomalies when autopsy consent is limited, or can help to determine sites to be examined microscopically. Conditions diagnosed by postmortem radiography may include:

- Skeletal anomalies (dwarfism, dysplasia, sirenomelia, etc.)
- Neural tube defects (anencephaly, iniencephaly, spina bifida, etc.)
- Osteogenesis imperfecta (osteopenia, fractures)
- Soft tissue changes (hydrops, hygroma, etc.)
- Teratomas or other neoplasms
- Growth retardation
- Orientation and audit of fetal parts (with D&E specimens)
- Assessment of catheter or therapeutic device placement

Microbiologic Culture

Culture can aid in diagnosis or confirmation of congenital infections. Examples of congenital infection include:

T - toxoplasmosis
O - other, such as Listeria monocytogenes, group B streptococcus, syphilis
R - rubella
C - cytomegalovirus
H - herpes simplex or human immunodeficiency virus (HIV)

Cultures have to be appropriately obtained with the proper media and sent with the proper requisitions ("routine" includes aerobic and anaerobic bacteria; fungal and viral cultures must be separately ordered).

Viral cultures are difficult and expensive. Separate media and collection procedures may be necessary depending upon what virus is being sought.

Bacterial contamination can be a problem.

Karyotyping

Tissues must be obtained as fresh as possible for culture and without contamination.

A useful procedure is to wash the tissue samples in sterile saline prior to placing them into cell culture media.

Tissues with the best chance for growth are those with the least maceration: placenta, lung, diaphragm.

Obtaining tissue from more than one site can increase the yield by avoiding contamination or by detection of mosaicism.

FISH (performed on fresh tissue or paraffin blocks)

In addition to karyotyping, fluorescence in situ hybridization (FISH) can be useful. A wide variety of probes are available. It is useful for detecting aneuploid conditions (trisomies, monosomies).

Fresh cells are desirable, but the method can be applied even to fixed tissues stored in paraffin blocks, though working with paraffin blocks is much more time consuming and interpretation can be difficult. The ability to use FISH on paraffin blocks means that archival tissues can be examined in cases where karyotyping was not performed, or cells didn't grow in culture.

DNA Probes

Fetal cells obtained via amniocentesis or CVS can be analyzed by probes specific for DNA sequences. One method employs restriction fragment length polymorphism (RFLP) analysis. This method is useful for detection of mutations involving genes that are closely linked to the DNA restriction fragments generated by the action of an endonuclease. The DNA of family members is analyzed to determine differences by RFLP analysis.

In some cases, if the DNA sequence of a gene is known, a probe to a DNA sequence specific for a genetic marker is available, and the polymerase chain reaction (PCR) technique can be applied for diagnosis.

There are many genetic diseases, but only in a minority have particular genes been identified, and tests to detect them have been developed in some of these. Thus, it is not possible to detect all genetic diseases. Moreover, testing is confounded by the presence of different mutations in the same gene, making testing more complex.

Biochemical Analysis

Tissues can be obtained for cell culture or for extraction of compounds that can aid in identification of inborn errors of metabolism. Examples include:

- long-chain fatty acids (adrenoleukodystrophy)
- amino acids (aminoacidurias)

Flow Cytometry

Flow cytometry is useful only for determination of the amount of DNA and can yield no information about individual chromosomes with aneuploidy. Thus, the condition that flow cytometry can routinely detect is triploidy.

Very little sample (0.1 gm) is required. The technique can also be applied to fixed tissues in paraffin blocks.

Electron Microscopy

Rarely used and requires prompt fixation with no maceration. Examples of conditions to be diagnosed with EM include:

- mitochondrial myopathies
- viral infections

XX and XY karyotyping

Chromosome analysis or karyotyping is a test that evaluates the number and structure of a person's chromosomes in order to detect abnormalities. Chromosomes are thread-like structures within each cell nucleus and contain the body's genetic blueprint. Each chromosome contains thousands of genes in specific locations. These genes are responsible for a person's inherited physical characteristics and they have a profound impact on growth, development, and function.

Humans have 46 chromosomes, present as 23 pairs. Twenty-two pairs are found in both sexes (autosomes) and one pair (sex chromosomes) is present as either XY (in males) or XX (in females). Normally, all cells in the body that have a nucleus will contain a complete set of the same 46 chromosomes, except for the reproductive cells (eggs and sperm), which contain a half set of 23. This half set is the genetic contribution that will be passed on to a child. At conception, half sets from each parent combine to form a new set of 46 chromosomes in the developing fetus. Chromosomal abnormalities include both numerical and structural changes. For numerical changes, anything other than a complete set of 46 chromosomes represents a change in the amount of genetic material present and can cause health and development problems. For structural changes, the significance of the problems and their severity depends upon the chromosome that is altered. The type and degree of the problem may vary from person to person, even when the same chromosome abnormality is present.

A chromosomal karyotyping examines a person's chromosomes to determine if the right number is present and to determine if each chromosome appears normal. It requires experience and expertise to perform properly and to interpret the results. While theoretically almost any cells could be used to perform testing, in practice it is usually performed on amniotic fluid to evaluate a fetus and on lymphocytes (a white blood cell) from a blood sample to test all other ages. Alternately, white blood cells may be obtained from bone marrow aspirations to look for changes in individuals suspected of having hematologic or lymphoid diseases (e.g., leukemia, lymphoma, myeloma, refractory anemia).

The test is performed by:

- Taking a sample of a person's cells, culturing them in nutrient-enriched media to promote cell division *in vitro*. This is done in order to select a specific time during the cells' growth phase when the chromosomes are easiest to distinguish.

- Isolating the chromosomes from the nucleus of the cells, placing them on a slide, and treating them with a special stain.
- Taking microphotographs of the chromosomes.
- In jigsaw puzzle fashion, rearranging the pictures of the chromosomes to match up pairs and arrange them by size, from largest to smallest, numbers 1 to 22, followed by the sex chromosomes as the 23rd pair.
- The pictures also allow the chromosomes to be vertically oriented. Each chromosome looks like a striped straw. It has two arms that differ in length (a short arm (p) and a long arm (q)), a pinched-in area between the arms called a centromere, and a series of light and dark horizontal bands. The length of the arms and the location of the bands help determine top from bottom.
- Once the chromosome photo arrangement is completed, a laboratory specialist evaluates the chromosome pairs and identifies any abnormalities that may be present.

Some chromosomal disorders that may be detected include:

- Down syndrome (Trisomy 21), caused by an extra chromosome 21; this may occur in all or most cells of the body.
- Edwards syndrome (Trisomy 18), a condition associated with severe mental retardation; caused by an extra chromosome 18.
- Patau syndrome (Trisomy 13), caused by an extra chromosome 13.
- Klinefelter syndrome, the most common sex chromosome abnormality in males; caused by an extra X chromosome.
- Turner syndrome, caused by missing one X chromosome in females.
- Chronic myelogenous leukemia, a classic 9;22 translocation that is diagnostic of the disease.

DNA / RNA Probes

DNA probes are small segments of DNA which help to detect the presence of a gene of a long DNA sequence, in a biological systems. These DNA probes are are the most sophisticated and sensitive means to identify genes or specific DNA sequences.DNA probes provide commercial avenues for diagnosis of infection diseases, identification of food contaminants for isolation of genes and in other microbiological tests.

The production of DNA probes can be done by any of the following methods. Such as (a) using a template DNA with the help of purified biological enzymes (b) DNA probe of specific sequence can also be obtained by using automated DNA synthesizers (c) DNA probe can also be included in viral DNA and may even multiply in bacteria, thus by this way many copies of DNA probe can be obtained.

However, the DNA probe assay consists of the following steps. Sample to be tested is treated with detergents and enzymes to remove non DNA components. Then DNA is denatured by low PH. Single stranded DNA binds on filters and is exposed to excess of DNA probes but only one of which will hybridize. At the same time unbound DNA is detected by a variety of available methods using florescence and dye etc.

DNA indirect methods are based on an examination of probands (person being examined) family. The main instrument here is a **pedigree**. All we need is a sample of blood from a close family – proband, his parents, siblings and so on.

The sample of *10 ml* of blood is enough. We extract the white blood cells – leucocytes. DNA is extracted from them. The next step is the use of Southern blotting. It is a method which allows us to divide the DNA due to restriction endonucleasis. They should then be lined according to their size.

DNA Probes

In indirect methods we used two types of **probes** – *intragenetic* or *extragenetic*. The probe is a segment of the DNA or RNA which binds specifically to some part of the DNA strand. The probes can be synthesized in the laboratory. It depends just on the targets DNA nucleotide sequence. It is an easy way how to find some specific part of the DNA, which can be associated with some disease under investigation.

The first type of probes – **intragenetic** – is located right on the spot of the examined gene. The second type – **extragenetic** – lies just close to it. In that case we can use our knowledge about a **gene linkage**. It can highly influence the results of the test.

Test Results

After the DNA is synthesized and the probes bind, we can discuss the results. The most important is to identify which segment of DNA was inherited from which parent. We can put the results of the electrophoresis into the scheme of the family tree. If we are able to determine an origin of inherited DNA segments – the family is called to be **informative**. In case of intragenetic probe we can be sure about the result (positive or negative). On the other hand the extragenetic probe´s results are just highly probable.

Another situation is the case, when we can´t say from which parent the segment was inherited. The family is then called to be **uninformative**. This method is based on the differences of RFLP in a population. This time we have to use another type of restriction endonucleasis. The result can then became informative.

The Use of DNA Indirect Methods

It is used for a **prenatal diagnosis** of inherited diseases. It is necessary to know, which segment of DNA is connected with it. According to its location we then synthesize a suitable probe - with suitable nucleotide sequence. Within the finding of affected fetuses we are also able to determine the **carriers** of the disease.

Molecular Basis of Disease Diagnosis and Treatment

A disease, in molecular sense, can be defined as any abnormality in the living system. The abnormality can be caused due to infection by virus, bacteria, fungi, parasites, proteins or small molecules in/from humans, animals, plants, water and soil. The abnormality can also arise due to changes in the molecular structure within the cells. As an example, a change in the DNA sequence known as mutation can cause various disorders/diseases.

The prevention and treatment of these diseases is possible only if the causative agent of the disease can be diagnosed at the appropriate time. Hitherto many costly and laborious clinical procedures were used in the diagnosis and treatment of these diseases. With the advancement of

Molecular Biotechnology, various molecular diagnostic methods are now applied in the diagnosis and also treatment of these diseases.

Molecular Diagnosis:

A diagnostic test can be effective only if it is:

(a) Specific for the target molecule
(b) Sensitive to detect even minute levels of the target and
(c) Technically simple.

There are two classes of molecular diagnostic techniques:

(1) DNA detection methods—which uses nucleic acid hybridization or the polymerase chain reaction to detect a specific nucleic acid sequence.
(2) Immunological methods—are based upon the specificity of an antibody for a particular antigen.

1. DNA Detection Methods:

Various methods have been devised for the detection of various diseases, based on the sequence of DNA, built in a specific manner.

Some methods discussed here are:

(a) Detection of a pathogenic organism by nucleic acid hybridization
(b) Diagnosis of genetic disease using restriction endonuclease
(c) Diagnosis of genetic disease by P.C.R./oligonucleotide ligation assay (PCR/OLA) and
(d) detection of mutants at different sites within one gene.

(a) Detection of a pathogenic organism by nucleic acid hybridization:

The disease causing (pathogenic) organism can be detected very specifically in biological samples by nucleic acid hybridization i.e. if the nucleic acid sequence of a disease causing organism is present in the blood, urine, faeces, etc., then it can be hybridized with a nucleic acid probe complementary to the sequence of this target nucleic acid. If the pathogenic organism is present in the biological sample, hybridization occurs and if not, there is no hybridization.

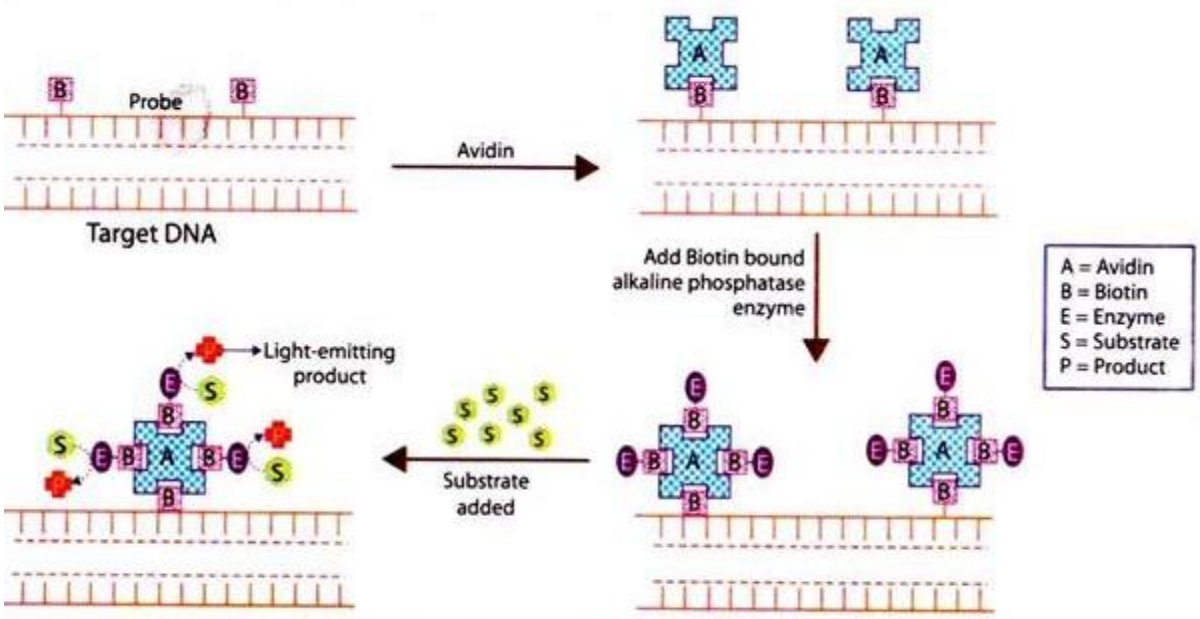
The parasite Plasmodium falciparum causes malaria in man. A specific gene (thereby its product) is the causative agent in this parasite. A complementary DNA probe to this gene is synthesized chemically with radiolabelled ^{32}P. This probe is bonded to a membrane support. Then the biological sample to be analysed is added, under appropriate conditions of temperature and ionic strength to promote base pairing between the probe and the target DNA in the sample.

It is then washed to remove the excess of the sample and then the hybridized double stranded DNA is extracted and the hybrid sequences are detected by autoradiography. The specific DNA probe chosen will hybridize only with P. falciparum but not with P. vivax, P. cyanomolgi or human DNA. This probe can detect as little as 10 picogram of purified P. falciparum DNA or 1 Nano gram of P. falciparum DNA in blood samples. If hybridization has occurred then the pathogenic organism is present and if no hybridization occurs (i.e. no radiations) then the pathogenic organism is absent.

The above procedure is adopted for detection of all pathogenic organisms in any biological sample. Here the disadvantage of using the radioactive phosphorus is that it is hazardous, hence now-a-days nonradioactive hybridization procedures are used. In this method all the DNA from

the sample is extracted and is bonded to a support, then the biotin-labelled DNA probe complementary to the pathogen DNA is hybridized to the target DNA.

Then, either avidin or streptavidin is added, which will bind to the biotin on the hybridized probe-target DNA. Then a biotin labelled enzyme like alkaline phosphatase is added which binds to the avidin bonded on the probe. Then the substrate specific for this enzyme is added, which will convert the colourless substrate into a coloured product. Appearance of the colour indicates the presence of the pathogenic DNA and non-development of the colour is an indication of the absence of the organism.



Detection of pathogenic organism by DNA hybridization

(b) Diagnosis of genetic disease using restriction endonuclease:

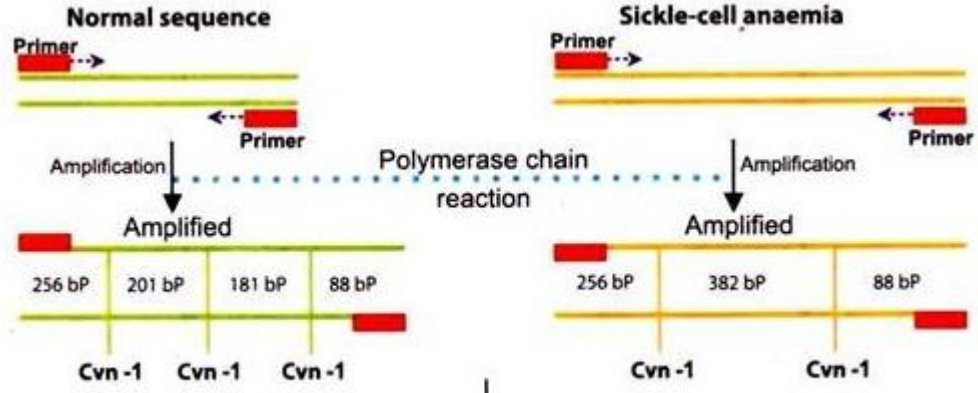
Sickle-cell anemia is a genetic disease due to the change in a single nucleotide in the codon for the sixth amino acid of the beta-chain of the hemoglobin molecule. The gene for beta-globin in normal persons is designated as A/A, in heterozygous individuals as A/a, and in homozygous individuals as S/s. Individuals containing the sickle gene are screened before the expression of the symptoms and for screening the carrier, who are at risk of transmitting this gene to their offspring.

The principle for the detection is that, within the beta-globin gene of a normal individual, there are three sites for the restriction endonuclease Cvn-1, but in sickle-cell gene one of these sites is lost due to replacement of the single nucleotide.

In the normal gene, the DNA sequence is CCTGAGG whereas in the sickle-cell anemia gene, the sequence is CCTGTGG. Further the recognition sequence and site of cleavage by Cvn-1 is CCTNAGG. Thus, the difference in sequence of normal and sickle-cell gene in the recognition site of Cvn—1 forms the basis of this DNA diagnosis.

Two primers with sequences that can pair within the Cvn-1 sites in the beta-globin gene are added and this part of DNA is amplified using P.C.R. and then digested with Cvn-1. Finally the cleavage products are separated by gel electrophoresis and stained by ethidium bromide.

The results indicate that in the normal gene, four DNA fragments are obtained with 88, 181, 201 and 256 base pairs. But in heterozygous individual five DNA fragments are obtained containing 88, 181, 201, 256 and 382 base pairs and in homozygous individuals only three fragments are obtained with 88, 256 and 382 nucleotide base pairs, indicating the loss of one of the recognition site in the sickle cell gene.



Electrophoresis after Cleavage and Staining

Fragment Size – bP	Genotype		
	AA Normal	AS Heterozygous	SS Homozygous
382		▬	▬
256	▬	▬	▬
201	▬	▬	
181	▬	▬	
88	▬	▬	▬

Detection of genetic disease by restriction endonuclease

(c) Diagnosis of genetic disease by PCR/OLA procedure:

This procedure is applied for those disorders, due to genetic mutations, which does not affect the restriction endonuclease sites. Let us take an example of a gene, which has undergone mutation at position 98. At this specific site the base pair in the normal gene be C=G and in the mutant gene let it be A=T.

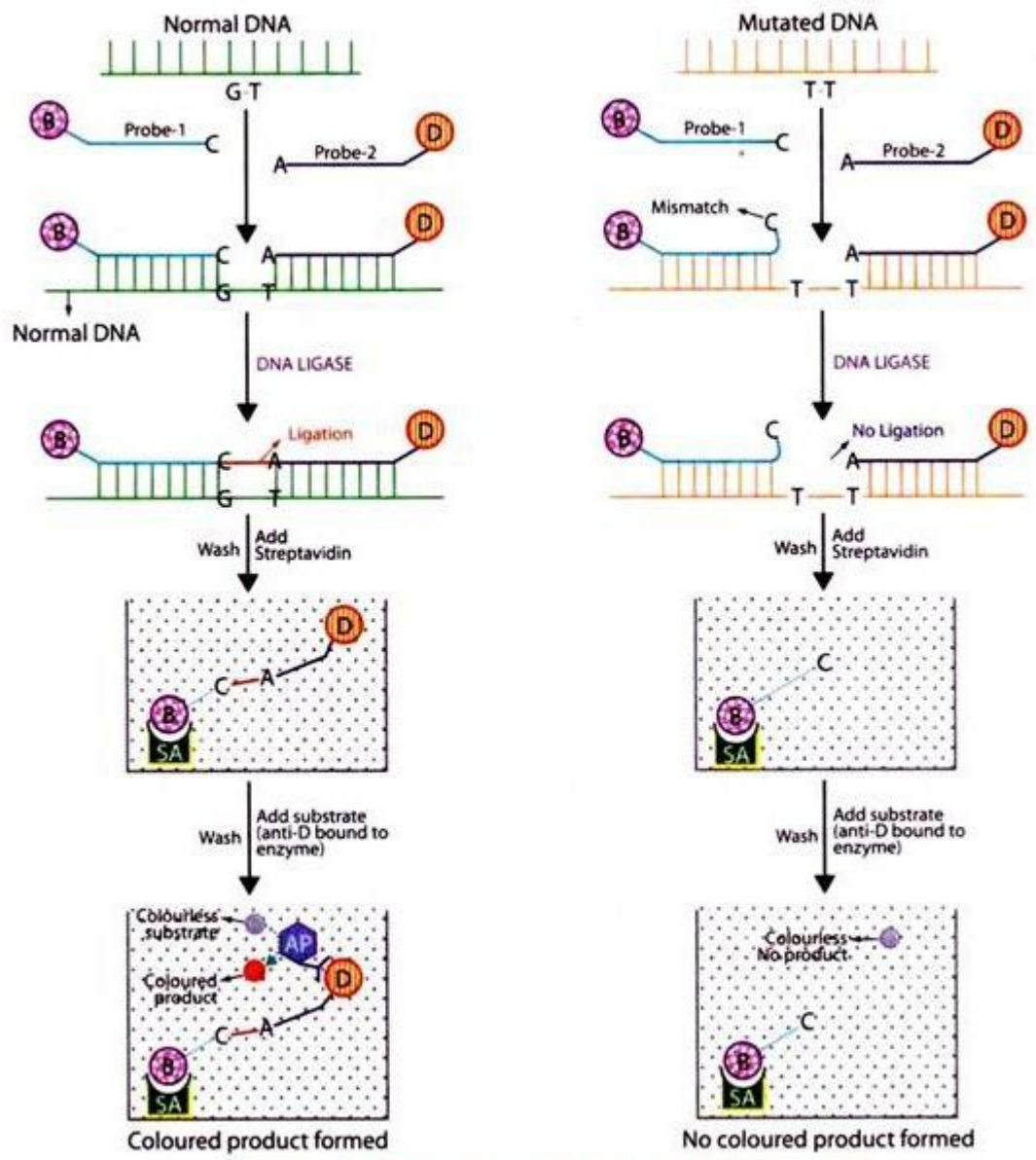
Two oligonucleotides of about 20 nucleotide length each are synthesized with sequence complementary to one of the strands of this gene on either side of position 98. One of these oligonucleotides has biotin at its 5′ end and ‘C’ as the terminal nucleotide at the 3′ end. The other

oligonucleotide probe has 'A' base nucleotide at the 5′ end and digoxigenin (compound 'D') at its 3′ end.

The target DNA is amplified by PCR and then is hybridized with the synthesized probes. The two probes base pair such that the 5′ end of the 2nd probe lies next to the 3′ end of the 1st probe. Then DNA ligase is added, which will ligate only the normal DNA fragment but not the mutated fragment hybridized with the probes.

This is because of the mismatch between the 2nd probe and the mutated gene, which cannot base pair. Further, in order to determine whether ligation has occurred or not, the hybrid probes are taken into a well containing avidin which binds to biotin. Then it is washed, which removes the un-ligated probe.

Then anti-digoxigenin ('D' compound) antibody- alkaline phophatase conjugate is added and washed in both the wells (the normal and mutated hybridized probes). It is expected that the antibody enzyme will bind only to the ligated probe well. When substrate is added, the coloured product is produced only in the well where ligation has occurred, whereas no colour is formed where no ligation has taken place.



Diagnosis of genetic disease by PCR/OLA procedure

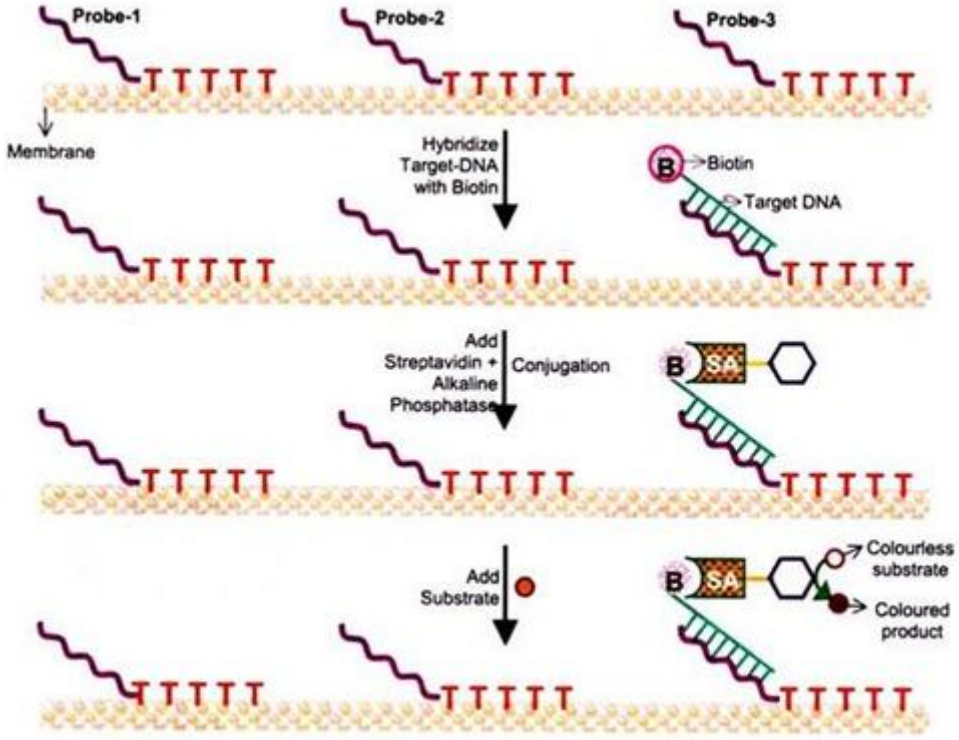
(d) Detection of mutations at different sites within one gene:

Beta-thalassemia is a genetic disease that is caused due to mutation in beta-globulin at eight or more sites, thus results in low rate of its synthesis. Hence instead of detecting each mutation separately all the eight sites are scanned at the same time.

DNA probes are synthesized to all these eight sites of beta-globin gene where mutations are expected. Each probe is 20 nucleotide in length with a poly T' tail at the 3′ end. This is used to attach the probe to a membrane. Segments of the sample DNA (beta-gene) that includes each of the possible mutant sites are amplified by PCR, using primers labeled with biotin at the 5′ end.

The amplified target DNA is then hybridized to the membrane bound probes under conditions that allow only perfect matches to hybridize. Then streptavidin with attached alkaline phosphatase is added, the membrane washed and a colourless substrate is added.

A coloured spot on the membrane appears wherever there is a perfect nucleotide match between the amplified target DNA segment and one of the specific oligoneucleotide probes. Where there is no hybridization (mutant DNA segments) no colour appears. In the illustration given below, gene 1 and 2 are mutated but gene 3 is normal. (Represented as probe 1, 2 and 3 respectively in the figure).



Detection of mutations at different sites within one gene

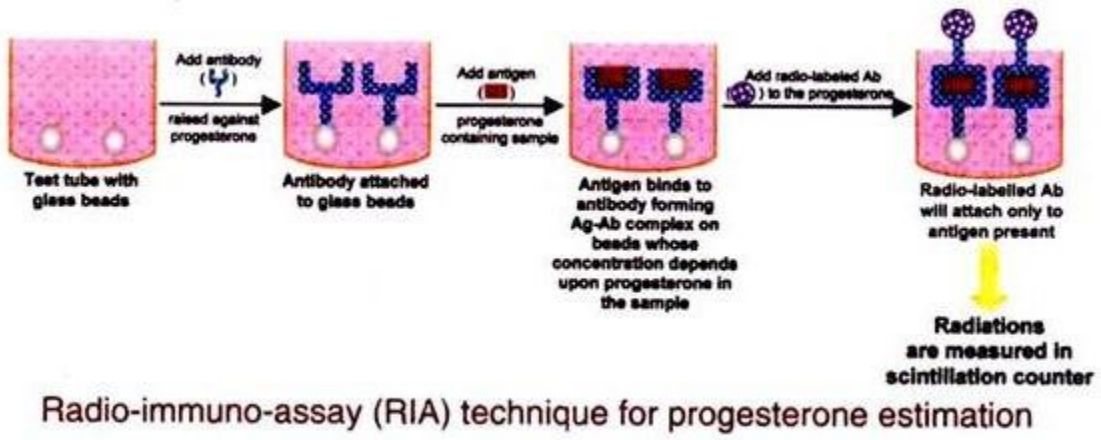
2. Immunological Methods:

Antibody molecules consist of four chains, two identical light chains and two identical heavy chains. The fv (fragment variable) region of each antibody molecule binds tightly to a specific site (epitope) on an antigen. This specificity is used to identify the presence of a particular epitope of a disease causing molecule or organism in a biological sample. There are two methods by which the antigen-antibody reaction or binding is detected.

(a) Radio-immuno assay (RIA):

The concentration of progesterone in blood (for example) is to be determined by RIA. First of all antibodies to progesterone are raised and taken in a test tube containing glass beads. The antibodies get readily attached to the glass beads. Then progesterone containing sample is added to this test tube which binds the antibodies forming antigen-antibody complex, whose concentration depends upon the amount of progesterone in the blood sample.

Another test tube is taken and the antibodies are labelled with radioactive compounds like ^{123}I or ^{3}H or ^{14}C. This radio labelled antibody is then added to the first test tube containing progesterone attached to un-labelled antibodies. Radio labelled antibodies will now attach to the progesterone and form labelled antigen-antibody complex which is measured using a scintillation counter.



Radio-immuno-assay (RIA) technique for progesterone estimation

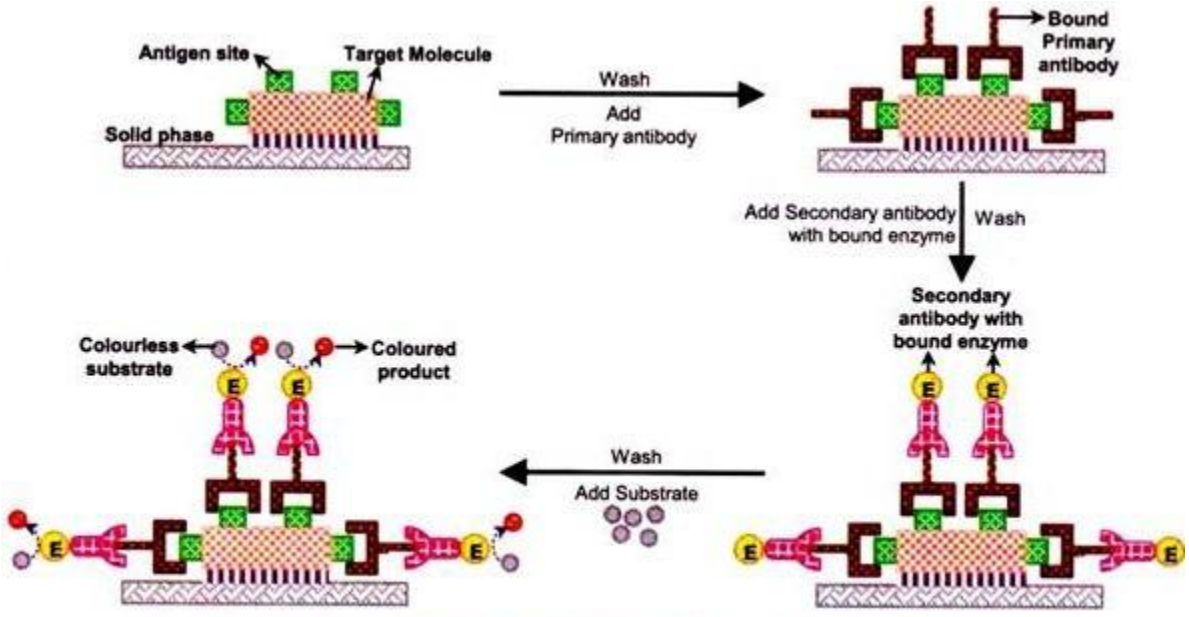
(b) Enzyme linked immunosorbent assay (ELISA):

The sample which is to be tested for the presence of a specific molecule or organism is bound to a solid support such as a plastic plate. Then a marker- specific antibody (primary antibody) is added to the bound material and then the support is washed to remove unbound primary antibody.

Then a second antibody (secondary antibody) is added, which binds specifically to the primary antibody and not to the target molecule. The secondary antibody contains bound enzyme like alkaline phosphatase which catalyses the conversion of a colorless substrate into a colored product. The system is washed again to remove any unbound secondary antibody-enzyme conjugate.

Then a colorless substrate is added which is converted to a colored product only if the specific antigen is present, if not there is no colour. If there is no antigen (or the causative agent) then the primary antibody will not bind to the target site in the sample, hence the first washing step removes it. Consequently, the secondary antibody—enzymes conjugate will have nothing to bind to and is removed during the second washing step, and the final mixture remains colorless.

Conversely, if the antigen (or the causative agent) or the target site is present in the sample, then the primary antibody binds to it, the secondary antibody binds to the primary antibody and the attached enzyme will catalyze the reaction to from a colored product which can be detected colorimetrically.



ELISA tests for detection of antigen-antibody reaction

Molecular Treatment or Gene Therapy:

In order to treat a genetic disease, the normal gene for that disease has to be sequenced and cloned. This cloned normal gene can be used to correct the defect in individuals who have a mutant form of that gene. Here, the objective is to add a normal functioning gene to defective cells, thereby providing the required protein and correcting the genetic disease. In addition, it will be necessary to prevent the over expression of a deregulated normal gene, in some diseases.

There are three methods for the therapy of genetic diseases:

(1) Ex vivo gene therapy
(2) In vivo gene therapy and
(3) Antisense therapy.

(a) Ex vivo Gene Therapy:

Somatic cells from an affected individual are collected. The isolated cells are grown in culture. These cells are then transfected by retroviral cloning vectors containing the remedial gene construct. The cells are further grown and those cells which contain the gene of interest are selected and finally transplanted or transfused back into the patient. These transplanted transfected cells will synthesize the gene product i.e. the protein. Examples for this type of treatment include gauche disease, sickle cell anaemia, thalassemia etc.

(b) In vivo Gene Therapy:

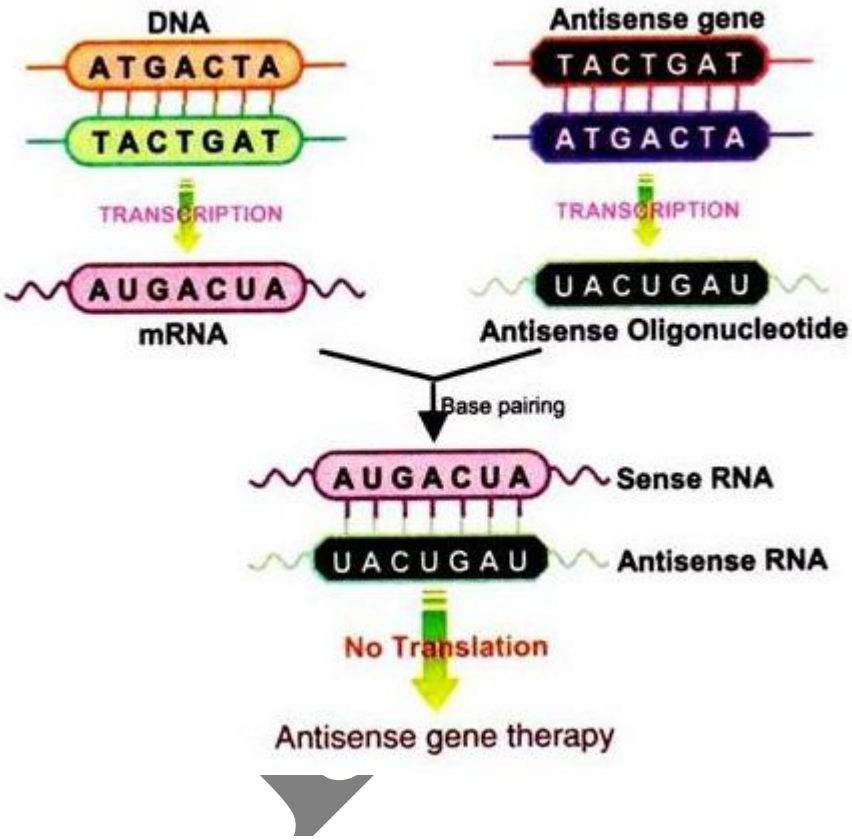
In this type of treatment there is the direct delivery of the remedial gene into the cells of a particular tissue of the patient, using retroviral vectors. Even plasmid DNA constructs are used. This type of treatment is used in case of muscular dystrophy, neuronal degeneration and brain cancer patients.

(c) Antisense Therapy:

Antisense therapy is designed to prevent or lower the expression of a specific gene. In some type of genetic diseases and cancers, the genes are deregulated or over expressed resulting in the production of excess of the gene product or its continuous presence in the cell will disrupt the normal functioning of the cell.

In such type of diseases the addition of normal gene will not solve the problem; instead blocking the synthesis of the gene product (protein) will be helpful. Thus in anti-sense therapy a nucleic acid sequence is introduced into the target cell which is complementary to complete or a part of that specific mRNA.

Hence the mRNA produced by the normal transcription of the gene will hybridize with the antisense oligonucleotide by base pairing, thereby preventing the translation of this mRNA, resulting in reduced amount of target protein. The antisense therapy is used in treatment of various cancers, AIDS, atherosclerosis, leukemia and sickle cell anaemia.



DNA probes in the diagnosis of infectious diseases

The use of DNA analysis (by employing DNA probes) is a novel and revolutionary approach for specifically identifying the disease-causing pathogenic organisms. This is in contrast to the traditional methods of disease diagnosis by detection of enzymes, antibodies etc., besides the microscopic examination of pathogens.

Although at present not in widespread use, DNA analysis may soon take over the traditional diagnostic tests in the years to come. Diagnosis of selected diseases by genetically engineered techniques or DNA probes or direct DNA analysis is briefly described.

Mycobacterial

Tuberculosis:

Tuberculosis is caused by the bacterium Mycobacterium tuberculosis. The commonly used diagnostic tests for this disease are very slow and sometimes may take several weeks. This is because M. tuberculosis multiplies very slowly (takes about 24 hrs. to double; E. coli takes just 20 minutes to double).

A novel diagnostic test for tuberculosis was developed by genetic engineering, and is illustrated in Fig. 14.3. A gene from firefly, encoding the enzyme luciferase is introduced into the bacteriophage specific for M. tuberculosis. The bacteriophage is a bacterial virus, frequently referred to as luciferase reporter phage or mycophage.

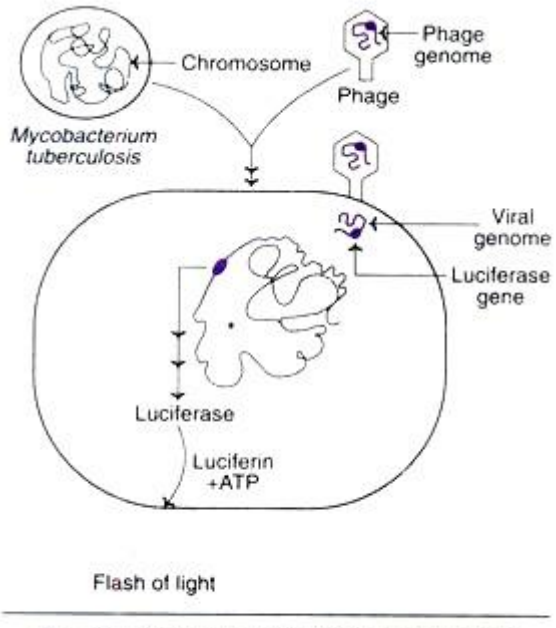


***Fig. 14.3** : Diagnosis of tuberculosis by using a genetically engineered bacteriophage (phage).*

The genetically engineered phage is added to the culture of M. tuberculosis. The phage attaches to the bacterial cell wall, penetrates inside, and inserts its gene (along with luciferase gene) into the M. tuberculosis chromosome. The enzyme luciferase is produced by the bacterium.

When luciferin and ATP are added to the culture medium, luciferase cleaves luciferin. This reaction is accompanied by a flash of light which can be detected by a luminometer. This diagnostic test is quite sensitive for the confirmation of tuberculosis. The flash of light is specific for the identification of M. tuberculosis in the culture. For other bacteria, the genetically engineered phage cannot attach and enter in, hence no flash of light would be detected.

Plasmodial

Malaria:

Malaria, mainly caused by Plasmodium falciparum and P. vivax, affects about one-third of the world's population. The commonly used laboratory tests for the diagnosis of malaria include microscopic examination of blood smears, and detection of antibodies in the circulation. While the former is time consuming and frequently gives false-negative tests, the latter cannot distinguish between the past and present infections.

A specific DNA diagnostic test for identification of the current infection of P. falciparum has been developed. This is carried out by using a DNA probe that can bind and hybridize with a DNA fragment of P.falciparum genome and not with other species of Plasmodium. It is reported that this DNA probe can detect as little as 1mg of P. falciparum in blood or 10 pg of its purified DNA.

HIV and HPV infections during development

HIV Acquired Immunodeficiency Syndrome (AIDS):

AIDS is caused by the virus, human immunodeficiency virus (HIV). The commonly used laboratory test for detection of AIDS is the detection of HIV antibodies. However, it might take several weeks for the body to respond and produce sufficient HIV antibodies. Consequently, the antibodies test may be negative (i.e., false-negative), although HIV is present in the body. During this period, being a carrier, he/she can transmit HIV to others.

DNA probes, with radioisotope label, for HIV DNA are now available. By using PCR and DNA probes, AIDS can be specifically diagnosed in the laboratory. During the course of infection cycle, HIV exists as a segment of DNA integrated into the T-lymphocytes of the host. The T-lymphocytes of a suspected AIDS patient are isolated and disrupted to release DNA.

The so obtained DNA is amplified by PCR, and to this DNA probes are added. If the HIV DNA is present, it hybridizes with the complementary sequence of the labeled DNA probe which can be detected by its radioactivity. The advantage of DNA probe is that it can detect the virus when there are no detectable antibodies in the circulation.

HIV diagnosis in the newborn:

Detection of antibodies is of no use in the newborn to ascertain whether AIDS has been transmitted from the mother. This is because the antibodies might have come from the mother but not from the virus. This problem can be solved by using DNA probes to detect HIV DNA in the newborn.

Human Papilloma Virus:

Human papilloma virus (HPV) causes genital warts. HPV is also associated with the cervical cancer in women. The DNA probe (trade name Virapap detection kit) that specifically detects HPV has been developed. The tissue samples obtained from woman's cervix are used. HPV DNA, when present hybridizes with DNA probe by complementary base pairing, and this is the positive test.

Chagas' Disease:

The protozoan parasite Trypanosoma cruzi causes Chagas' disease. This disease is characterized by destruction of several tissues (liver, spleen, brain, lymph nodes) by the invading parasite. Chagas' disease is diagnosed by the microscopic examination of the fresh blood samples. Immunological tests, although available, are not commonly used, since they frequently give false-positive results.

Scientists have identified a DNA fragment with 188-base pair length present in T. cruzi genome. This is however, not found in any other related parasite. A PCR technique is employed to amplify the 188 bp DNA fragment. This can be detected by using polyacrylamide gel electrophoresis. Thus, PCR-based amplification can be effectively used for the diagnosis of Chagas' disease.

DNA Probes for Other Diseases:

Lyme Disease:

Lyme disease is caused by the bacterium, Borrelia burgdorferi. This disease is characterized by fever, skin rash, arthritis and neurological manifestations. The diagnosis of Lyme disease is rather difficult, since it is not possible to see B. burgdorferi under microscope and the antibody detection tests are not very reliable. Some workers have used PCR to amplify the DNA of B. burgdorferi. By using appropriate DNA probes, the bacterium causing Lyme disease can be specifically detected.

Periodontal Disease:

Periodontal disease is characterized by the degenerative infection of gums that may ultimately lead to tooth decay and loss. This disease is caused by certain bacteria. At least three distinct species of bacteria have been identified and DNA probes developed for their detection. Early diagnosis of periodontal disease will help the treatment modalities to prevent the tooth decay.

In principle, almost all the pathogenic organisms can be detected by DNA probes. Several DNA probes (more than 100) have been developed and many more are in the experimental stages. The ultimate aim of the researchers is to have a stock of probes for the detection of various pathogenic organisms—bacteria, viruses, parasites. The other important DNA probes in recent years include for the detection of bacterial infections caused by E. coli (gastroenteritis) Salmonella typhi (food poisoning), Campylobacter hyoitestinalis (gastritis).

Diagnosis of tropical diseases:

Malaria, filariasis, tuberculosis, leprosy, schistosomiasis, leishmaniasis and trypanosomiasis are the tropical diseases affecting millions of people throughout the world. As already described for the diagnosis of malaria caused by P. falciparum, a DNA probe has been developed. A novel diagnostic test, by genetic manipulations, has been devised for the diagnosis of tuberculosis. Scientists are continuously working to develop better diagnostic techniques for other tropical diseases.

Molecular probes in diagnosis of genetic diseases

Traditional laboratory tests for the diagnosis of genetic diseases are mostly based on the estimation of metabolites and/or enzymes. This is usually done after the onset of symptoms. The laboratory tests based on DNA analysis can specifically diagnose the inherited diseases at the genetic level.

DNA-based tests are useful to discover, well in advance, whether the individuals or their offspring's are at risk for any genetic disease; Further, such tests can also be employed for the prenatal diagnosis of hereditary disorders, besides identifying the carriers of genetic diseases.

By knowing the genetic basis of the diseases, the individuals can be advised on how to limit the transmission of the disease to their offspring's. It may also be possible, in due course of time, to treat genetic diseases by appropriate gene therapies. Theoretically, it is possible to develop screening tests for all single-gene diseases. Some of the important genetic diseases for which DNA analysis is used for diagnosis are briefly described.

Cystic Fibrosis:

Cystic fibrosis (CF) is a common and fatal hereditary disease. The patients produce thick and sticky mucus that clogs lungs and respiratory tract. Cystic fibrosis is due to a defect in cftr gene that encodes cystic fibrosis trans membrane regulator protein, cftr gene is located on chromosome 7 in humans, and a DNA probe has been developed to identify this gene.

The genetic disease cystic fibrosis is inherited by a recessive pattern, i.e., the disease develops when two recessive genes are present. It is now possible to detect CF genes in duplicate in the fetal cells obtained from samples of amniotic fluid. As the test can be done months before birth, it is possible to know whether the offspring will be a victim of CF. One group of researchers have reported that CF gene can be detected in the eight-celled embryo obtained through in vitro fertilization.

Sickle-Cell Anemia:

Sickle-cell anemia is a genetic disease characterized by the irregular sickle (crescent like) shape of the erythrocytes. Biochemically, this disease results in severe anemia and progressive damage to major organs in the body (heart, brain, lungs, and joints).

This disease is caused by a recessive gene Hb^s. The normal gene Hb^Apresent on chromosome 11 has undergone mutation to produce the recessive Hb^s gene which cause sickle-cell anaemia in homozygous condition ($Hb^s Hb^s$) and the patient dies. In heterozygous condition ($Hb^A Hb^s$) the patient survives, only few R.B.Cs are affected. The R.B.Cs in this disease become sickle-shaped in venous blood owing to the lower concentration of oxygen. This causes rupture of cells and severe haemolytic anaemia.

Haemoglobin is a conjugate protein of heme and globulin. It is formed of about 600 amino acids, two identical a chains and two identical β chains. The sixth amino acid in chain of normal haemoglobin is glutamic acid. In sickle-cell haemoglobin glutamic acid is replaced by valine. The children homozygous for sickle-cell gene (Hb^sHb^s) produce rigid chains. When oxygen level of blood drops below certain level, R.B.Cs undergo sickling.

Such cells do not transport oxygen efficiently they are removed by spleen causing severe anaemia. Individuals with Hb^AHb^A genotype are normal, those with Hb^sHb^s genotype have sickle-cell disease and those with Hb^AHB^s geno-type have the sickle-cell trait. Two individual

with sickle-cell trait can produce children with all three phenotypes. Individuals of sickle-cell trait are immune to malaria.

	β chain of Haemoglobin-A	β chain of Haemoglobin-S	
1.	Valine	Valine	1.
2.	Histidine	Histidine	2.
3.	Leucin	Leucin	3.
4.	Threonine	Threonine	4.
5.	Proline	Proline	5.
6.	glutamic acid	Valine	6.
7.	Glutamic acid	Glutamic acid	7.
8.	Lysine	Lysine	8.

Fig. 5.30. β chain of normal haemoglobin and sickle shaped haemoglobin showing difference in the arrangement of amino acids.

Sickle-cell anemia occurs due to a single amino acid change in the β-chain of hemoglobin. Specifically, the amino acid glutamate at the 6th position of β-chain is replaced by valine. At the molecular level, sickle-cell anemia is due to a single-nucleotide change (A → T) in the β-globin gene of coding (or antisense) strand.

In the normal β-globin gene the DNA sequence is CCTGAGGAG, while in sickle-cell anemia, the sequence is CCTGTGGAG. This single-base mutation can be detected by using restriction enzyme Mstll to cut DNA fragments in and around β-globin gene, followed by the electrophoretic pattern of the DNA fragments formed.

The change in the base from A to T in the β-globin gene destroys the recognition site (CCTGAGG) for Mstll (Fig. 14.4). Consequently, the DNA fragments formed from a sickle-cell anemia patient for β-globin gene differ from that of a normal person. Thus, sickle-cell anemia can be detected by digesting mutant and normal β-globin gene by restriction enzyme and performing a hybridization with a cloned β-globin DNA probe.

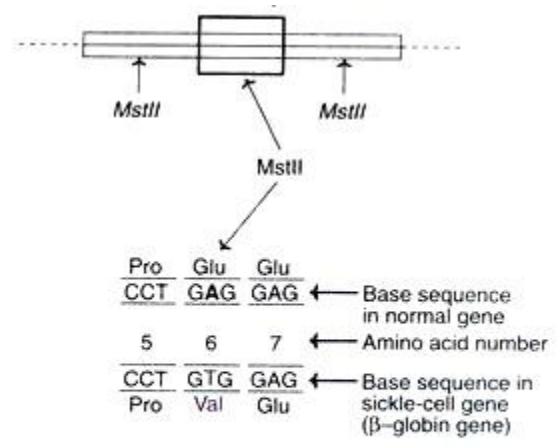


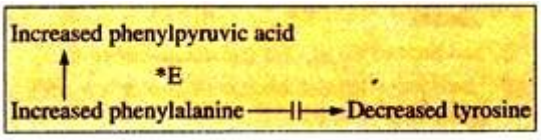
*Fig. 14.4 : Single-base change resulting in sickle-cell anemia. (**Note** : A small and relevant DNA fragment of β-globin gene magnified and shown with encoded amino acids).*

Single-nucleotide polymorphisms:

The single base changes that occur in some of the genetic diseases (e.g., sickle-cell anemia) are collectively referred to as single-nucleotide polymorphisms (SNPs, pronounced snips). It is estimated that the frequency of SNPs is about one in every 1000 bases. Sometimes SNPs are associated with amino acid change in the protein that is encoded. A point mutation in α_1-antitrypsin gene is also a good example of SNPs, besides sickle-cell anemia.

Phenylketonuria (PKU):

This genetic disease was discovered by the Norwegian physician A. Foiling in 1934. It is a single gene disorder caused by the mutation (= change) of a gene on chromosome 12. PKU results when there is deficiency of liver enzyme phenylalanine hydroxylase which converts phenylalanine (an amino acid) into tyrosine (amino acid). Thus, there is high level of phenyl alanine in the blood and tissue fluid of the patient causing disease.



Lack of enzyme phenyl alanine hydroxylase (an inborn metabolic disorder) is due to the homozygous recessive gene. Affected babies are normal at birth but within a few weeks there is rise in plasma phenylalanine level which damages the development of brain. Generally by six months severe mental retardation is observed. If these affected children are not treated properly one third of them are unable to walk and two-thirds cannot talk.

Besides mental retardation other symptoms of the disease include decreased pigmentation of hair and skin and eczema. Large amount of phenylalanine and its metabolites although excreted through urine and sweat, their excess accumulation damages the brain. The heterozygous individuals are normal but carriers.

Early Diagnosis of Certain Inborn Errors to Avoid Permanent Damage:

Treatment must be commenced immediately in certain inborn errors, otherwise the victimized will be permanently damaged (e.g., PKU and galactosemia).

A number of useful clues are given below:

Table 44.3 : Useful clues for the diagnosis of an inborn error of metabolism
(a) Family history of a genetic disease.
(b) Positive screening test (eg, for PKU).
(c) Sick newborn with other conditions (infections, cardiovascular, etc.) excluded.
(d) Physical findings, such as enlarged organs (eg, hepatomegaly in Gaucher's and Niemann-Pick disease).
(e) Unusual odour from breath or urine (eg, maple syrup urine disease).
(f) Low blood sugar (eg, Glycogen storage disease).
(g) Low blood pH (eg, due to accumulation of organic acids in maple syrup urine disease).

The sources of material that can be analysed and the major tests used in investigating patients or fetuses suspected of having genetic diseases are listed below. Chorionic villus sampling and amniocentesis apply only to investigation of the fetus.

Material	:	Plasma red cells, white cells, fibroblasts, urine, organ biopsy, chorionic villus sample, amniotic cells.
General tests	:	Blood and urine glucose, blood pH, blood ammonia, amino acids in plasma and urine, detection of organic acids in urine.
Specific tests	:	Measurement of low amount of product or increased amount of precursor (eg, phenylalanine in PKU).

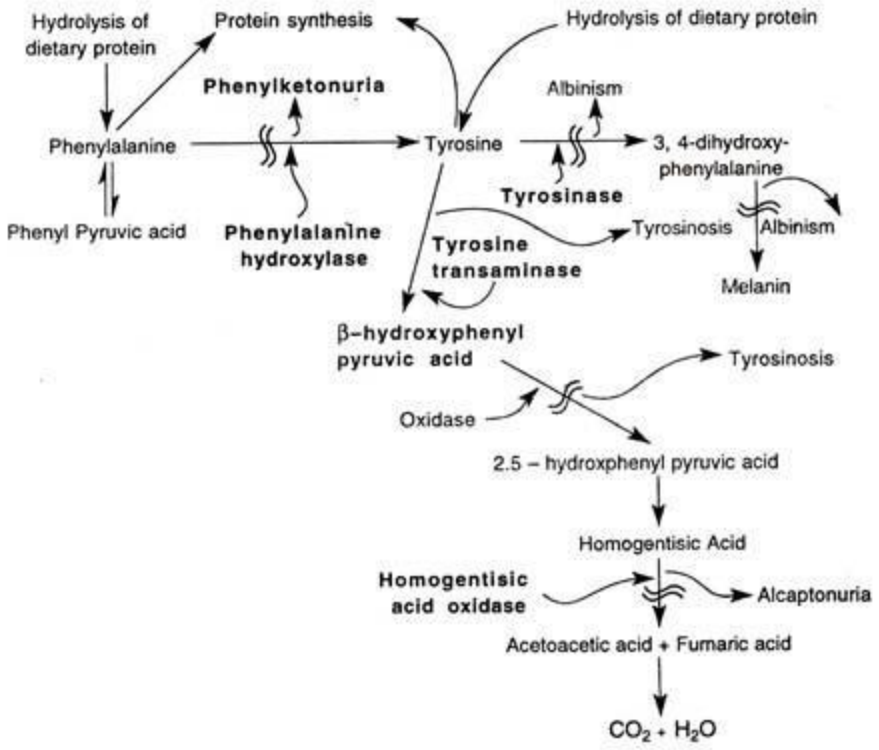
Measurement of activity of enzyme in red cells, white cells, or tissue biopsy.
Electrophoresis (e.g., for Hbs).
Analyses by Southern blotting of restriction fragment length polymorphisms (RFLPs) and other features of DNA structure linked to or causing specific diseases (e.g., Hbs, Huntington's chorea, DNA, etc.)
Identification of novel metabolite in urine of plasma by GLC-Ms.

Alkaptonuria (Black Urine Disease):

Alkaptonuria is a useful model for discussion of inborn errors of metabolism and has historical precedence, since it was the condition that was the basis for Garrod's concept.
One of the end points of phenylalanine and tyrosine metabolisnn is the breakdown of homogentisic acid to CO_2 and H_2O. This reaction is accomplished under the influence of an

enzyme that is present in the liver, named homogentisic acid oxidase. When the enzyme is defective, large amount of homogentisic acid are excreted in the urine, which turns black upon exposure to the air.In addition, quantities of homogentisic acid accumulate in the body and become attached to the collagen of cartilage and other connective tissues.

Table 11.3: Metabolic pathways showing with defects for producing differen' inherited human diseases



Clinical Symptoms:

The cartilage of the ears and the sclera, which is collagenous in nature are stained black. These manifestations are called ochronosis. In the joints such as those of the spine, the accumulation leads to arthritis. When alkaptonurics are fed increased quantities of phenylalanine or tyrosine, there is corresponding increased amount of homogentisic acid excreted in their urine.

Garrod's Interpretation:

Garrod interpreted alkaptonuria as being caused by the congenital deficiency of a particular enzyme due to the presence in double dose of an abnormal Mendelian factor or gene. An important implication of the idea was that the normal allele of this gene must in some way be necessary for the formation of the enzyme in the normal organism.

This was the first clue to the now well-established generalisation that genes exert their effects in the organism by directing the synthesis of enzymes and other proteins.

Frequency of Individual with Alcaptonuria:

Approximately one person in every million is homozygous for the mutant gene and suffers from disorder.

Detection:

The condition is present from birth and can be recognized because of the dark color appearing on wet diapers.

Clinical Advice:

An increase of phenylalanine or tyrosine in the diet of normal persons is not followed by the appearance of homogentisic acid in their urine.

Inheritance Pattern:

Alkaptonuria is inherited as an autosomal recessive trait and is an example of a genetic enzyme block in which the phenotypic features are due to accumulation of a substance just proximal to the block. The pedigrees were quite characteristic, and Garrod had little hesitation in concluding that they implied an hereditary or genetical basis for the condition.

He consulted Bateson, one of the earliest geneticists, who pointed out that the situation could be readily explained in terms of laws of Mendel. The pedigrees were exactly those to be expected if alkaptonuria was determined by a rare recessive Mendelian factor, or as we should now say, gene.

The affected individuals could be presumed to be homozygous for the abnormal gene. According to Stern, most pedigrees fit recessive inheritance but at least one in which affected individuals descended from affected parents for three generations, has been frequently claimed to be evidence for dominant inheritance.

However, recent genealogical studies show connections between members of this kindred with others in which the trait is undoubtedly recessive. This suggests that the same recessive allele is responsible for alkaptonuria in all these kindreds.

Conclusion:

Garrod inferred that homogentisic acid, although it had never been detected in tissues, was itself a normal intermediate in the catabolism of Phenylalanine and tyrosine, and that in alkaptonuric subjects the essential defect was a failure in this degradation, due to the lack of a necessary enzyme.

In a alkaptonuric homogentisic acid cannot be broken down further, so it tends to accumulate in the cells of the liver where this metabolic process mainly occurs, leaks into the circulation and is excreted into the urine in large quantities. Alkaptonuria is relatively a benign disease.

The amino acids phenylalanine and tyrosine are normal dietary constituents. However, the diet supplies much more than the body can use as such and the excess is metabolized via homogentisic acid. No essential metabolites are formed subsequent to homogentisic acid.

The fact that the kidney cannot retain excess homogentisic acid prevents major deviation from normal metabolism. The only pathological consequence of alkaptonuria is a slow deposition of pigment in some of the joints, leading ultimately to a form of arthritis.

Down's syndrome (=Monogolian Idiocy, Monogolism, 21-trisomy):

Down's syndrome is one of the most common chromosome abnormality of man (fig. 5.31). It was first reported by a British physician Langdon Down in 1866. It is caused by the presence of an extra chromosome 21 (Fig. 5.31). During normal oogenesis a chromosome of the pair 2] enters into an ovum.

But due to non-disjunction (= non-separation) of chromosomes of pair 21 during meiosis both the chromosomes of pair 21 pass into a single ovum. Thus the ovum possesses 24 chromosomes in stead of 23 and offspring has 47 chromosomes (45 + XY in male, 45 + XX in female) in stead of 46. One in every 600 children is a victim of this genetic disorder. Persons suffering from Down syndrome resemble Mongolians.

They have broad fore head, short and broad neck, short and stubby fingers, permanently open mouth, protruding tongue, projecting lower lip, fiat hands. The victim suffers from severe mental retardation (IQ generally below 40) because of malformation of central nervous system, heart and other organs may be defective.

Gonads and genitalia are underdeveloped. Women around 45 years of age are more likely to produce children having Down's syndrome. Translocation of a portion of chromosome 21 on autosome 14 also results in Down's syndrome. In translocation Down's syndrome chromosome number is 2n – 46.

Klinefelters Syndrome (2n = 47 or 44 + XXY):

H.F. Klinefelter first described this genetic disorder in 1942. The chromosome number of the patient is 2n = 47 and the chromosome formula is 44A + XXY. Thus, there is an extra chromosome in male. This syndrome originates when ovum with XX chromosomes (ab normal egg) unites with a normal sperm with an Y chromosome or a normal ovum (with an X chromosome) unites with an abnormal sperm carrying XY chromosomes. The individual has 47 chromosomes (44 + XXY).

Such persons are sterile males with undeveloped testes, mental retardation, thinly scattered body hair, and long limbs and with some female characteristics such as enlarged breasts (gynaecomastia) and feminine pitched voice. It is reported that the more the X chromosomes, the greater is the mental defect klinefelter's syndrome is generally seen in one out of every 500 male births. It arises by the nondisjunction of sex chromosomes during meiosis.

Klinefelter syndrome may be diagnosed prenatally from fetal cytogenetic analyses performed on chorionic villi or amniocytes. If Klinefelter syndrome is not diagnosed prenatally, a patient with 47,XXY karyotype may demonstrate various subtle, age-related clinical signs that would prompt diagnostic testing. Karyotype analysis on peripheral blood lymphocytes, the XCAT-KS buccal swab test, fluorescence in-situ hybridization (FISH), and microarrays are options for postnatal diagnostic testing.

Early identification and anticipatory guidance are extremely helpful in Klinefelter syndrome. Management and treatment should focus on 3 major facets of the syndrome: hypogonadism, gynecomastia, and psychosocial problems. Androgen (testosterone) replacement therapy is an important aspect of treatment.

Cancers

It is now agreed that there is some degree of genetic predisposition for the occurrence of cancers, although the influence of environmental factors cannot be underestimated. In fact, cancer susceptible genes have been identified in some families e.g., genes for melanoma susceptibility in humans are located on chromosomes 1 and 9.

p^{53} Gene:

The gene p^{53} encodes for a protein with a molecular weight 53 kilo Daltons (hence the name). It is believed that the protein produced by this gene helps DNA repair and suppresses cancer development. Certain damages that occur in DNA may lead to unlimited replication and uncontrolled multiplication of cells.

In such a situation, the protein encoded by p^{53} gene binds to DNA and blocks replication. Further, it facilitates the faulty DNA to get repaired. The result is that the cancerous cells are not allowed to establish and multiply. Thus, p^{53} is a cancer-suppressor gene and acts as a guardian of cellular DNA.

Any mutation in the gene p^{53} is likely to alter its tumor suppressor function that lead to cancer development. And in fact, the altered forms of p^{53} recovered from the various tumor cells (breast, bone, brain, colon, bladder, skin, lung) confirm the protective function of p^{53} gene against cancers.

It is believed that the environmental factors may cause mutations in p^{53} gene which may ultimately lead to cancer. Some of the mutations of p^{53} gene may be inherited, which probably explains the occurrence of certain cancers in some families.

Genes of breast cancer:

Two genes, namely BRCAI and BRCAII, implicated in certain hereditary forms of breast cancer in women, have been identified. It is estimated that about 80% of inherited breast cancers are due to mutations in either one of these two genes — BRCAI or BRCAII. In addition, there is a high risk for ovarian cancer due to mutations in BRCAI.

It is suggested that the normal genes BRCAI and BRCAII encode proteins (with 1863 and 3418 amino acids respectively) that function in a manner comparable to gene p^{53} protein (as described above). As such, BRCAI and BRCAII are DNA- repair and tumor-suppressor genes. Some researchers believe that these two proteins act as gene regulators. Diagnostic tests for the analysis of the genes BRCAI and BRCAII were developed. Unfortunately, their utility is very limited, since there could be hundreds of variations in the base sequence of these genes.

Genes of colon cancer:

The occurrence of colon cancer appears to be genetically linked since it runs in some families. Some researchers have identified a gene linked with hereditary non-polyposis colon cancer or HNPCC (sometimes called Lynch syndrome). This gene encoded a protein that acts as a guardian and brings about DNA repair whenever there is a damage to it.

However, as and when there is a mutation to this protective gene, an altered protein is produced which cannot undo the damage done to DNA. This leads to HNPCC. It is estimated that the occurrence of this altered gene is one in every 200 people in general population.

Microsatellite marker genes:

Microsatellites refer to the short repetitive sequences of DNA that can be employed as markers for the identification of certain genes. For colon cancer, microsatellite marker genes have been identified on chromosome 2 in humans. There is a lot of variability in the sequence of microsatellites.

Early detection of the risk for colon cancer by DNA analysis is a boon for the would be victims of this disease. The suspected individuals can be periodically monitored for the signs and treated

appropriately. Unlike many other cancers, the chances of cure for colon cancer are reasonably good.

Gene of retinoblastoma:

Retinoblastoma is a rare cancer of the eye. If detected early, it can be cured by radiation therapy and laser surgery or else the eyeball has to be removed. Scientists have identified a missing or a defective (mutated) gene on chromosome number 13, being responsible for retinoblastoma. The normal gene when present on chromosome 13 is anticancer and does not allow retinoblastoma to develop.

Possible questions

1. Give a short note on amniocentesis.
2. Comment on molecular probes diagnosis in genetic diseases (Down syndrome and sickle cell anemia).
3. What is karyotyping? Explain the method in detail.
4. Comment on HIV and HPV infections during development.
5. Write in brief about prenatal diagnosis of genetic diseases.
6. Discuss about DNA and RNA probes.
7. Comment about mycobacterial and plasmodial infections during development.
8. Explain in detail how the molecular probes are used in diagnosis of genetic diseases.

UNIT-III
SYLLABUS

Developmental Stage I: Gametogenesis – Origin of germ cells – Significance of different stages of gametogenesis Oogenesis – Types of eggs–growth, development and maturation of oocyte, Egg envelopes, Polarity and symmetry, Spermatogenesis–Sperm Structure, Types of sperm, Fertilization – Approach of spermatozoon–Reaction of egg, essence of activation – Changes in egg cytoplasm during fertilization.

Developmental Stage I:

Gametogenesis

At the end of meiosis, four haploid cells have been produced, but the cells are not yet gametes. The cells need to develop before they become mature gametes capable of fertilization. The development of haploid cells into gametes is called gametogenesis.

Gametogenesis is arbitrarily designated as the first stage of embryonic development.

In embryology, the gametes are usually discussed first, as they provide both the blue print and the raw material from which the embryo is formed.

Gametogenesis i.e. formation of gametes in the sexes is tailored to their future roles in reproduction. The female gamete is usually non-mobile, larger and nutrient filled cell, the ovum or egg.

The female gamete must be competent to be fertilized, which means that it must develop a number of specialized properties to enable it to interact with the sperm. The male gamete is usually small and mobile sex cell, the spermatozoon or sperm. The formation of female gamete, ova or egg is known as oogenesis, whereas formation of male gamete, sperm is termed as spermatogenesis.

In some animals, the spermatozoa and ova are produced by a single individual which is called hermaphrodite or monoecious or bisexual, whereas in others the two types of gametes are produced by different individuals, and such individuals are called Dioecious or unisexual. This type of seperation of individuals is called gonochorism.

Both classes of gametes, spermatozoon and ova make an equal contribution to the nucleus of the zygote. It is said that egg and sperm possess the ‘information’ that is needed to build a new organism in the encoded form. During the development of egg, the encoded information is decoded. The decoding or reading of the information is equivalent to the process of ontogenetic development (i.e. transformation of zygote into new adult individual).

Another aspect of gametogenesis requires that chromosome number be reduced from the diploid number to the haploid condition. Sexual reproduction involves union of gametes from two different individuals, so either gamete possesses one half the number of chromosomes of the parents.

The reduction of chromosome number is accomplished by meiotic (Z-44/D.B.) division. In both the sexes of initial cells (germinal cells) giving rise to the gametes are very similar, and the steps in the production of gametes include (i) proliferation of cells by mitosis (ii) growth and (iii) maturation.

Origin of Germ Cells

The initial cells of the early embryo which migrate to a new site to produce sperm or ova are called primordial germ cells. The primordial germ cells of both sexes are indistinguishable from one another. The primordial germ cells may arise at some distance from the presumptive gonads. After the gonads are formed, the germ cells migrate into them, become established and increase in number by mitosis. Regarding the place of origin of primordial germ cells, a great costroversy exists among the embryologists.

(1) In Lepidosteus and Amia the germ cells arise from the endoderm, whereas in Petromyzon the germ cells derive from the coelomic epithelium.

(2) In urodeles, the germ cells originate in the postero-ventral part of the lateral mesoderm (Nienwkoop, 1946). In anurans, the germplasm of primordial germ cells originates before cleavage, within the subtropical area of the vegetal pole of the egg. In amphibians, the primordial germ cells migrate to the gonads by amoeboid movements.

(3) In reptiles and birds, the germ cells have been assigned an extragonadal origin. In chick, the primordial germ cells are said to originate from the endodermal layer of the extra-embryonic part of the blastoderm just anterior to the head region of the embryo.

The primordial germ cells have been observed to leave the endoderm epithelium to move slowly into the space between endoderm and mesoderm, and then to penetrate into the blood vessels of the area vasculosa, via the blood vessel, they migrate into the developing gonad.

Origin of primordial germ cells in mammals:

In mammals the primordial germ cells have extragonadal origin. Earlier it was believed that in mouse the germ cells originated from the germinal epithelium of the gonad, but recent studies suggest that in mouse like the vertebrates, germ cells originate into the endoderm before migrating into the gonad. By utilizing the marking capacity of alkaline phosphatase enzyme of the germ cells, it was possible to determine the location of these cells within the caudal endoderm and their subsequent migration into the developing gonad.

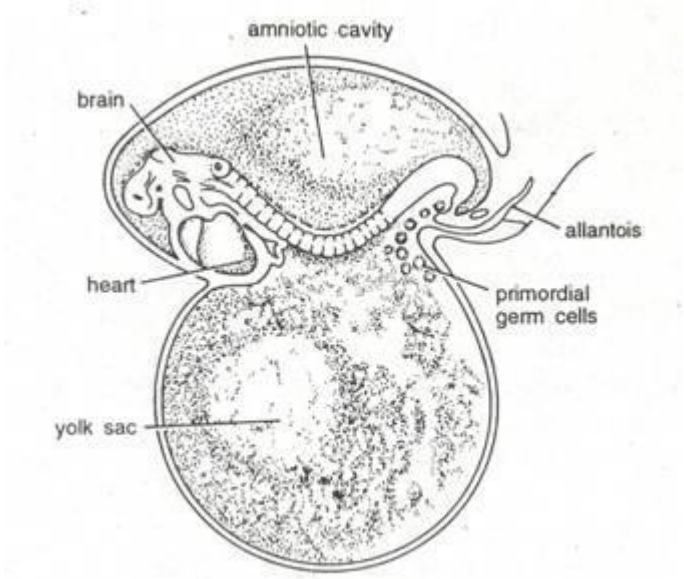


Fig. 1. Origin of primordial germ cells.

Similar histochemical observation on the primordial germ cells of the human embryo have revealed that the primordial germ cells originate in the endodermal epithelium of the yolk sac in the vicinity of the allantoic stalk and from there these germ cells migrate into the adjoining mesenchyme and eventually take up their position in the germinal ridges (Witschi, 1948).

Thus, it becomes evident that the primordial germ cells have an extragonadal origin in most invertebrates and vertebrates. Then they migrate by their own or float passively through the blood vessels to reach their final abode in the gonads.

According to some workers, these primordial germ cells later give rise to the functional gametes of adult animals. But some other workers have found that these primordial germ cells degenerate before they are converted into functional gametes, and certain other cells of the gonads later on give origin to functional germ cells.

Significance of different stages of gametogenesis

Gametogenesis is the process by which diploid precursor cells undergo meiotic division to become haploid gametes (sex cells)

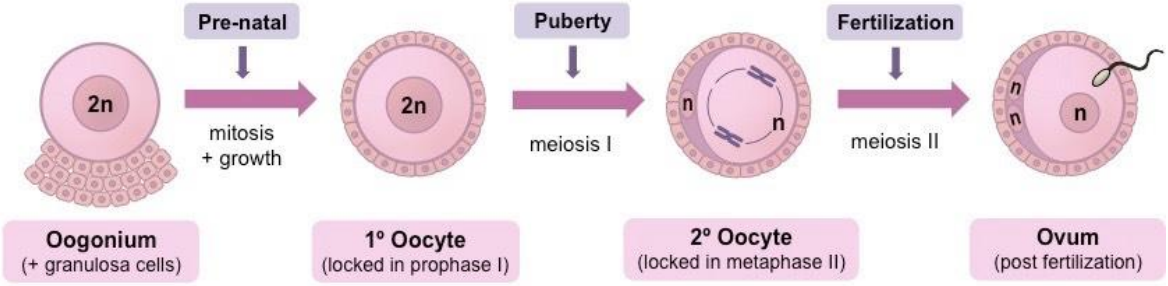
- In males, this process is called *spermatogenesis* and produce spermatozoa (sperm)
- In females, this process is called *oogenesis* and produce ova (eggs)

The process of gametogenesis occurs in the gonads and involves the following steps:

- Multiple *mitotic divisions* and *cell growth* of precursor germ cells
- Two *meiotic divisions* (meiosis I and II) to produce haploid daughter cells
- Differentiation of the haploid daughter cells to produce functional gametes

Oogenesis

- Oogenesis describes the production of female gametes (ova) within the **ovaries** (and, to a lesser extent, the oviduct)
- The process begins during foetal development, when a large number of primordial cells are formed by mitosis (~40,000)
- These cells (*oogonia*) undergo cell growth until they are large enough to undergo meiosis (becoming *primary oocytes*)
- The primary oocytes begin meiosis but are arrested in prophase I when granulosa cells surround them to form follicles
- The primary oocytes remain arrested in prophase I until puberty, when a girl begins her menstrual cycle
- Each month, hormones (FSH) will trigger the continued division of some of the primary oocytes
- These cells will complete the first meiotic division to form two cells of unequal size
- One cell retains the entirety of the cytoplasm to form a *secondary oocyte*, while the other cell forms a polar body
- The polar body remains trapped within the follicle until it eventually degenerates
- The secondary oocyte begins the second meiotic division but is arrested in metaphase II
- The secondary oocyte is released from the ovary (ovulation) and enters into the oviduct (or fallopian tube)
- The follicular cells surrounding the oocyte form a corona radiata and function to nourish the secondary oocyte
- If the oocyte is fertilised by a sperm, chemical changes will trigger the completion of meiosis II and the formation of another polar body (the first polar body *may* also undergo a second division to form a third polar body)
- Once meiosis II is complete the mature egg forms a *ovum*, before fusing its nucleus with the sperm nucleus to form a zygote



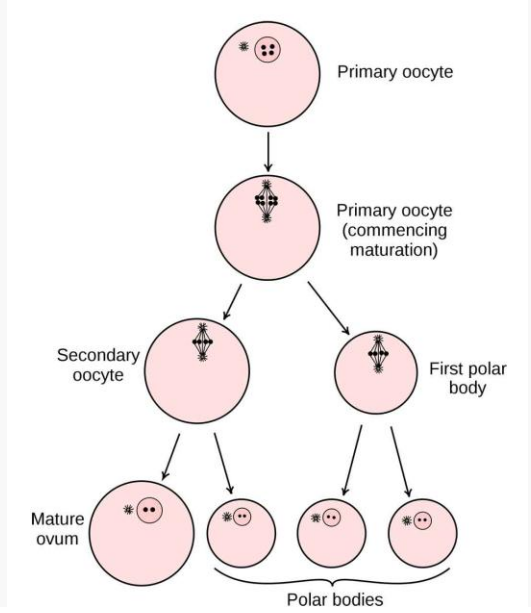
Types of eggs–growth, development and maturation of oocyte, Egg envelopes

The egg in all animals is a larger cell in comparison to other type of cells.

All contain reserve material of various kinds such as yolk, glycogen, nucleic acids (DNA, RNA, etc.) and various species of proteins.

Eggs are produced as large as possible, compatible with the number needed to ensure adequate variation, to cope of with the general enormous mortality rate of eggs, embryos and juveniles, and to distribute developing progeny beyond the terrestrial limits of the parental populations.

The number of eggs produced becomes greatly reduced and individual egg size correspondingly increased only in those cases, where special means are evolved to ensure survival whether by hiding, securing or otherwise protecting eggs, or above all by retaining developing eggs, within the maternal body as in mammals.



The egg cell or ovum has three basic functions: (i) to supply a nucleus containing half of the chromosomal component of the future embryo, (ii) to supply almost all the cytoplasm to the zygote, and (iii) to supply food reserves that will enable the embryo to develop up to a stage where it can begin to feed upon exogenous materials.

Therefore, for these future needs, an egg cell becomes immotile, large-sized, well-specialized, packaged and programmed during the process of oogenesis. Packaging i.e. the growth of the primary oocyte, and the accumulation of condensed food reserve, yolk, glycogen etc. within, it relates mainly to the number of cells that the developing egg can become, while, programming of egg relates to the determinative or directional information that egg may possess as a specialized type of reproductive cell.

The process of oogenesis is somewhat more complicated and different than spermatogenesis. Besides, the production of four unequal sized haploid cells, there is acquisition of food reserves in the egg cytoplasm for the development of embryo. Further, before the occurrence of meiosis,

enormous amount of growth and differentiation of egg-cytoplasm takes place. The oogenesis is more or less similar in all vertebrate groups.

During oogenesis, the cells of germinal epithelium detach from the surface epithelium and enter the cortex of the ovary. These germinal cells are diploid and are called primordial germ cells. They pass through the three stages to form a fully formed egg which are:

(1) Phase of multiplication
(2) Phase of growth
(3) Phase of maturation.

Oogenesis in insects:

In insects, eggs are produced in the ovarioles of an ovary. The thinner part of an ovariole is called germanum, which contains oogonia enveloped by mesodermal cells. The stouter part of the ovariole is called the vitellarium, which contains oocytes in a longitudinal row. Each oocyte is surronded by a single layer of cuboidal follicle cells.

In the germanum, oogonia produce oocytes by mitosis. One oogonium divides into two cells—one primary oogonium and a cytoblast. The blast divides by four consecutive mitosis into 16 cells (cytocysts). One of these becomes an oocyte and the other 15 becomes nurse cells (trophocytes). All the 16 cells are surrounded by a single layer of flattened follicle cells.

The oocyte and the nurse cells, with their follicular covering, come to the vitellarium, where the oocyte gets yolk granules from the haemolymph and the nurse cells. As deposition of yolk continues, the oocyte grows. Then by meiosis the oocyte forms two cells, one big cell—the ovum and the other very small cell—the first polar body. The latter degenerates after sometime. In this way, the ovum gets half the number of chromosomes of the species.

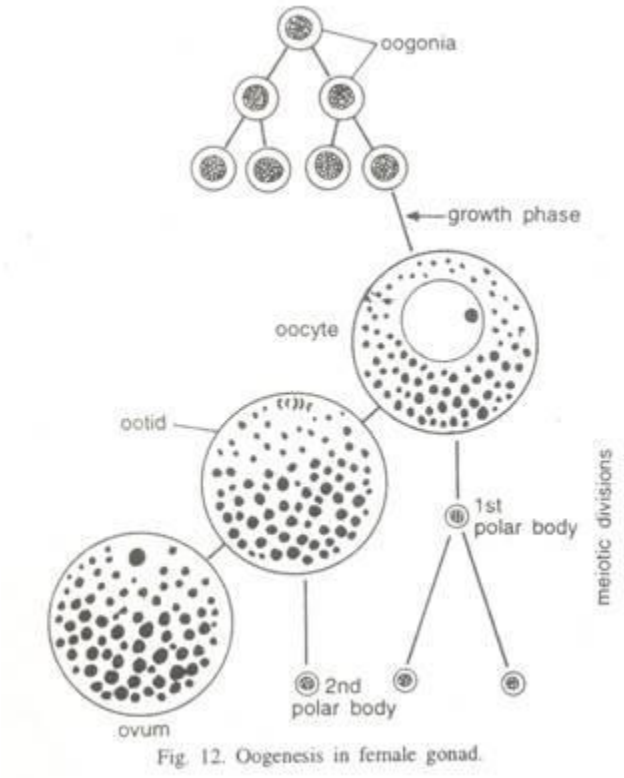


Fig. 12. Oogenesis in female gonad.

When the ovum is ready for the transfer to the oviduct, the follicle cells secrete the chorion or outer shell around it. In cockroach, the nurse cells are absent.

Oogenesis in mammals:

In mammals the process of oogenesis shows the following three phases:

1. Phase of multiplication:

The primordial germ cells become the oogonia—the egg mother cell. The oogonial cells eventually undergo proliferation by repeated mitotic divisions, giving rise to the eggs and become primary oocytes when cell division ceases. Now they enter into a period of growth.

2. Phase of growth:

Owing to the fact that the egg contributes the greater part of the substance used in the development, growth plays a much greater role in oogenesis than in spermatogenesis. The period of growth in the female gametes is very prolonged and tremendous growth of oocyte occurs during this phase.

Most of the primordial germ cells are approx., 10 µm (0.01 mm) in diameter. The young oocyte of amphibians may be about 50 µm (0.05 mm) and the mature amphibian egg is rather large about 1000 to 2000 µm (1.0 to 2.0 mm) in diameter. In birds the diameter of ovum is as large as 40,000 µm and in mammals it is only 200 µ.m.

The rate of growth of oocytes also varies; it may be slow or fast. The young oocyte start growing after the tadpole metamorphoses into the young frog and by the third year the eggs mature and the frog spawn for the first time.

In other animals, the growth of oocyte may proceed at a much higher rate and takes shorter time for completion. In hen, the last rapid growth of oocyte occurs in 10 to 14 days preceding ovulation, and during this time the volume of the oocyte increases 200 fold.

The progressive growth increase in nuclear as well as cytoplasmic substances) of oocytes may be divided into two stages—(a) previtellogenesis growth period and (b) vitellogenesis growth period.

(a) Previtellogenesis growth period:

During this phase, no synthesis and accumulation of food reserve material, the yolk, takes place, but tremendous increase in the volume of nucleus and cytoplasm of primary oocyte occurs. There is qualitative and quantitative increase in the amount of cytoplasm. The mitochondria increase in number, the network of endoplasmic reticulum with ribosomes becomes more complicated, the Golgi bodies manufacture cortical granules, besides performing their normal function.

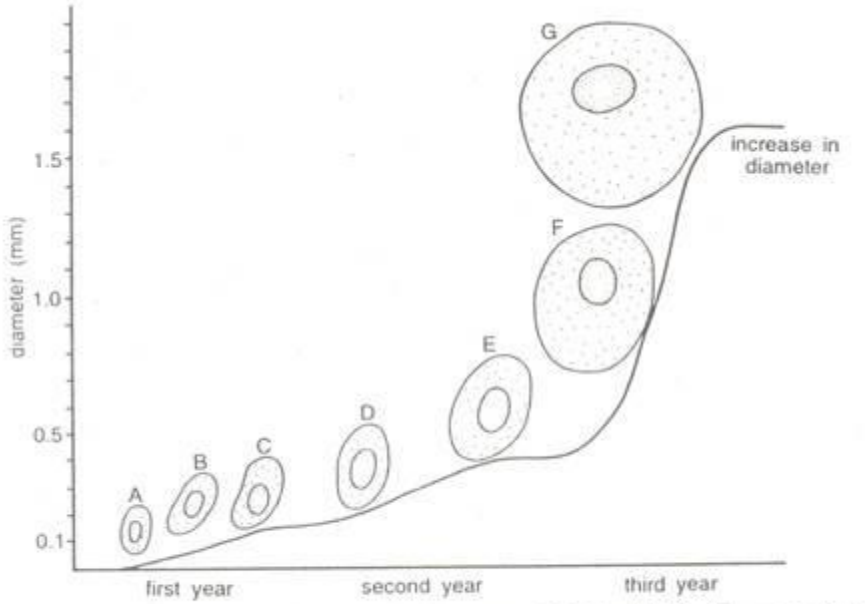


Fig. 13. Growth of frog oocytes during the first three years of the female's life. The curves show the increase in diameter of three generations of oocytes; the drawings represent the changes in size and structure of oocytes of the first generation.

(i) Growth of nuclear substances:

During this phase due to the production of the large amount of nuclear sap, the nucleus of the growing oocyte increase in size. A dark body appears at one place outside the nucleus, usually near the Golgi complex and is known as yolk nucleus of Balbiani. This large sized oocyte inflated with the fluid is now called germinal vesicle.

The nucleus of the occyte enters the prophase of meiotic division. Synapsis occurs between homologous chromosomes but the subsequent stages of meiosis are postponed and each chromosomes increases in its length, but the amount of DNA in each choromosomes does not increase in proportion to the enlargement of the nucleus. The increased chromosomes look like bottle brush; hence they are called the lamp-brush chromosomes.

It is believed that the loop of chromosomes represent actual site for the main activity of the genes, i.e. transcription of mRNA, which inturn controls translation process in which synthesis of proteins in the cell cytoplasm takes place.

During the growth period of ooctye, all mRNA molecules are not utilized during translation but some are inactivated by the wrapping of proteins around them and stored as informosomes to be used during early cleavage of egg, when chromosomal DNA remains more actively engaged in its own transcription of mRNA (messenger RNA) r- RNA (ribosomal RNA) or t-RNA (transfer RNA).

The RNAs are transcribed by r-DNA of 'nucleolar organizer region' of chromosomes. The nucleolus has a significant role in the storage and maturation of the ribosomal RNAs. It also synthesizes all the proteins required for the biogenesis of ribosomes.

Therefore, during growth period of primary oocytes, the nucleolus increases greatly in size and becomes very conspicuous. In many animals, particularly in amphibians, instead of one large nucleous, numerous small sized nucleoli are formed in their germinal vesicles. Most of them are localized on the periphery of the nucleus, immediately underneath the nuclear membrane.

The increased transcriptional activity (i.e. RNA synthesis) of chromosomal genes during growth period of oocytes, is called gene amplification (Ephel, 1973) or redundancy (De Robertis et al, 1982). When MRNA molecules are transcribed from DNA then it is known as transcriptional amplification. Each MRNA molecule in turn can be translocated several times into the corresponding proteins known as translational amplification.

This high rate of gene amplification or gene activity is correlated with the fact that gene reduction (meiosis) does not take place until after the growth of the oocytes has been completed. As a result the oocytes remain tetraploid for a long time.

(ii) Growth of cytoplasmic substances:

The amount of cytoplasm of oocyte increases both quantitatively and qualitatively during the Previtellogenesis growth period of oocyte. Young oocytes, in many animals, show a very simple organisation due to poor cytoplasmic inclusions and possess none of the specialized structures found in the adult oocyte and mature egg. The cytoplasm is finely granular having granules of ribonucleo-protein and DNA.

Mitochondria, the carriers of oxidative enzymes are fairly scarce in young oocytes but may increase in number very considerably during the growth of primary oocyte because overall oxygen consumption increases during this time. In amphibians and birds, the mitochondria become aggregated in the form of large 'Mitrochondrial clouds". Mitrochondria possess its own circular DNA. So in a growing oocyte, the amount of mitrochondrial DNA far exceeds the amount of nuclear DNA.

The young oocytes have the granular endoplasmic reticulum in the form of numerous, small vesicles. Annulated lamellae are also found in the cytoplasm of growing oocyte. These membranous structures appear in the form of stakes of cistemae, either in parallel or in spiral arrangement.

Sometimes, annulated lamellae are associated with ribosomes and RNA in high concentrations, and there is also an ATPase activity in the pore complexes of these lamellae. The lamella, thus serves as a storage site of RNA in cytoplasm and they are found to break down and disappear during late oogenesis.

In young oocyte the Golgi bodies are found around the Centrosome. In mature oocytes they form a large spherical mass in some mammals, or become located in the sub-cortical cytoplasm of frog and chick, or sometimes may disappear completely. The Golgi complex of oocyte is believed to synthesize cortical granules besides performing its normal function.

In the cortical region, cortical granules are present. These are membrane bound spherical bodies of diameter from 0.8 microns (sea urchin) to 2.0 microns (frog) and contain acid mucopolysaccharides. These mucopolysaccharides are used during fertilization, in the formation of fertilization membrane.

They are present in bivalve molluses, some annelids, fishes, frogs and some mammals (rabbit and man), but are absent in some insects, gastropodesurodeles, birds and some mammals (rat and guineapig). These granules are synthesized' by cisternae of Golgi complex in the interior of the oocyte and later they move to the periphery where they are arranged in a layer close to the plasma membrane of oocyte.

(b) Vitellogenesis growth period:

The process of formation and deposition of yolk is called vitellogenesis. In amphibians and fishes its synthesis takes place inside the modified mitochondria. In some insects yolk formation occurs in fat bodies whereas in most of the vertebrates yolk is formed in the liver of the mother. From the liver it is carried by blood to the ovary.

In the ovary, this material 'percolates' to the follicle cells and from there to the cytoplasm of the oocyte. However, in vertebrates, a very small quantity of the material for the formation of yolk (hardly 1%) is synthesised by the oocyte cytoplasm itself.

Thus a major part of the material forming the yolk is exogenous (formed outside the oocyte). Yolk is a general term that covers the major storage material such as glycogen, certain other carbohydrates, proteins and lipids. Hence, vitellogenesis is the period of rapid growth.

The primordial germinal cells divide repeatedly to form the oogonia (Gr., oon = egg). The oogonia multiply by the mitotic divisions and form the primary oocytes which pass through the growth phase.

Growth Phase:

The growth phase of the oogenesis is comparatively longer than the growth phase of the spermatogenesis. In the growth phase, the size of the primary oocyte increases enormously. For instance, the primary oocyte of the frog in the beginning has the diameter about 50µ but after growth phase the diameter of the mature egg reaches about 1000µ to 2000µ.

In the primary oocyte, large amount of fats and proteins becomes accumulated in the form of yolk and due to its heavy weight (or gravity), it is usually concentrated towards the lower portion of the egg forming the vegetative pole.

The portion of the cytoplasm containing the egg pro-nucleus remains often separated from the yolk and occurs towards the upper side of the egg forming the animal pole. The cytoplasm of the oocyte becomes rich in RNA, DNA, ATP and enzymes. Moreover, the mitochondria, Golgi apparatus, ribosomes, etc., become concentrated in the cytoplasm of the oocyte.

In certain oocytes (Amphibia and birds), the mitochondria become accumulated at some place in the occyte cytoplasm and form the mitochondrial clouds.

During the growth phase, tremendous changes also occur in the nucleus of the primary oocyte. The nucleus becomes large due to the increased amount of the nucleoplasm and is called germinal vesicle. The nucleolus becomes large or its number is multiplied due to excessive synthesis of ribosomal RNA (rRNA) by ribosomal DNA (rDNA) of nucleolar organizer region of chromosomes.

Thus, the nucleus or germinal vesicle of primary oocyte of Triturus has 600 nucleoli, of Siredon has 1000 nucleoli and of Xenopus has 600 to 1200 nucleoli due to synthesis of ribosomal RNA (rRNA).

The chromosomes change their shape and become giant lamp-brush chromosomes which are directly related with increased transcription of messenger RNA (mRNA) molecules and active protein synthesis in the cytoplasm. When the growth of the cytoplasm and nucleus of the primary oocyte is completed, it becomes ready for maturation phase.

Maturation Phase:

The maturation phase is accompanied by the maturation or meiotic division. The maturation division of the primary oocyte differs greatly from the maturation division of the spermatocyte. Here after the meiotic division of the nucleus, the cytoplasm of the oocyte divides unequally to form a single large-sized haploid egg and three small haploid polar bodies or polocytes at the end.

This type of unequal division has the great significance for the egg. If the equal divisions of the primary oocyte might have been resulted, the stored food amount would have been distributed equally to the four daughter cells and which might prove insufficient for the developing embryo.

Therefore, these unequal divisions allow one cell out of the four daughter cells to contain most of the cytoplasm and reserve food material which is sufficient for the developing embryo.

(i) First maturation division:

During the first maturation division or first meiosis, the homologous chromosomes of the primary oocyte nucleus pass through the pairing or synapsis, duplication, chiasma formation and crossing over. Soon after, the nuclear membrane breaks and the bivalent chromosomes move towards the opposite poles due to contraction of chromonemalfibres.

A new nuclear envelope is developed around the daughter chromosomes by the endoplasmic reticulum. After the karyokinesis, the unequal cytokinesis occurs and a small haploid polar body or polocyte and a large haploid secondary oocyte or ootid are formed.

(ii) Second meiotic division:

The haploid secondary oocyte and first polocyte pass through the second meiotic division. Due to the second meiotic division, the secondary oocyte forms amature egg and a second polocyte. By the second meiotic division, the first polocyte also divides into two secondary polocytes. These polocytes ooze out from the egg and degenerate while the haploid egg cell becomes ready for the fertilisation.

Polarity

The polarity of the embryo is seen in the forming of embryonic and abembryonalic poles. This is obvious when observing a blastocyst where an inner cell mass (ICM) has formed. This is concentrated at one pole in the interior of the hollow sphere and is made from blastomeres.

Blastocyst on the 5th day

1 Blastomeres of the trophoblast (abembryonic pole)
2 Inner cell mass (ICM = embryoblast) (embryonic pole)

This polarity was already set up in the fertilized oocyte. Its cytoskeleton is substantially involved with this phenomenon since the cytoskeleton is anchored at the oolemma. If the observation conditions are favorable, it is possible to distinguish between a somewhat smoother and a somewhat rougher surface on the fertilized oocyte. These two differing surfaces reflect the polarity of the future embryo.

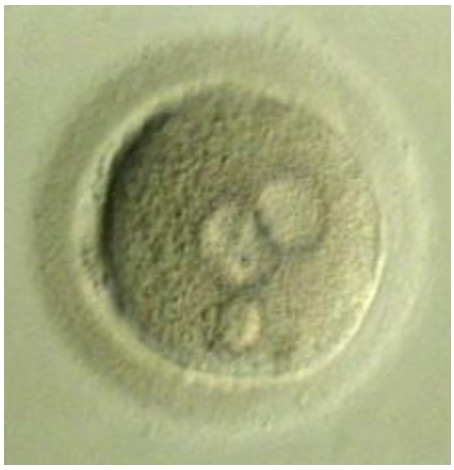
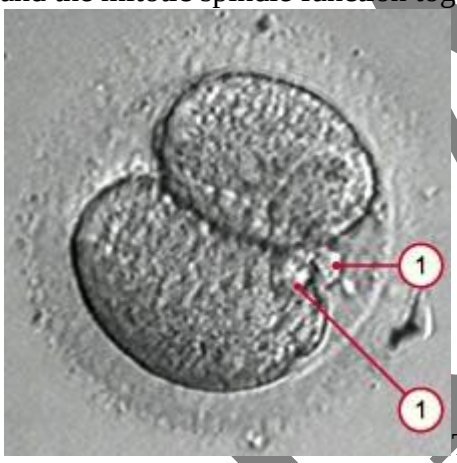


Fig.

In this picture, focused on the oocyte surface, one sees that the right hemisphere is smoother, while the left one exhibits a rougher surface structure. In the depth of the picture, two pronuclei in the typical vis-à-vis position and a polar body can be discerned.

The division of the zygote into the two-cell stage happens precisely so that one of the cells being formed is oriented to one pole and the other to the other one. The division always takes place so the polar bodies end up lying in the cleavage crease. This is not surprising since the cytoskeleton and the mitotic spindle function together to expel the polar bodies.



Two-cell embryo

1 Polar bodies

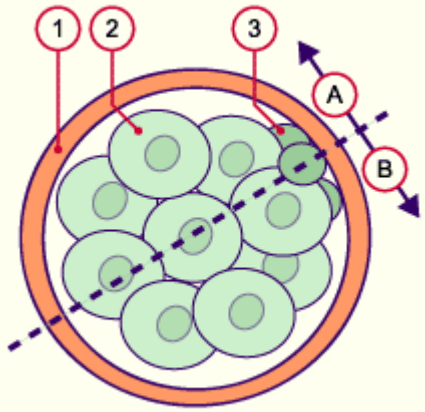
Fig.

The two recognizable polar bodies lie exactly in the cleavage crease between the two cells

Once the polarity, as reflected in the two cells, has been set up, it remains in existence so that one also finds it again in the morula stage. Here an imaginary equatorial plane, created by the polar

bodies, also separates the embryonic from the abembryonic poles.

Morula viewed schematically



1 Pellucid zone
2 Blastomere
3 Polar bodies

Symmetry

Symmetry, in biology, the repetition of the parts in an animal or plant in an orderly fashion. Specifically, symmetry refers to a correspondence of body parts, in size, shape, and relative position, on opposite sides of a dividing line or distributed around a central point or axis. With the exception of radial symmetry (*see* below), external form has little relation to internal anatomy, since animals of very different anatomical construction may have the same type of symmetry.

Certain animals, particularly most sponges and the ameboid protozoans, lack symmetry, having either an irregular shape different for each individual or else one undergoing constant changes of form. The vast majority of animals, however, exhibit a definite symmetrical form. Four such patterns of symmetry occur among animals: spherical, radial, biradial, and bilateral.

1. Asymmetrical Symmetry:

In some animals there are no body axis and no plane of symmetry, hence the animals are called asymmetrical. The amoeboid forms (e.g., Amoeba) and many sponges have irregular growth pattern of the body and cannot be divided into two equal halves (Fig. 9.1).

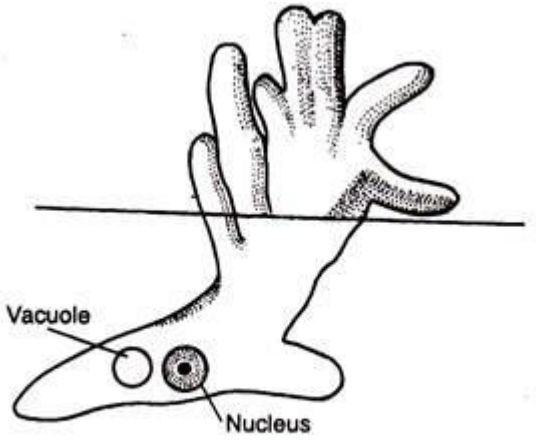


Fig. 9.1: Diagram of *Amoeba* showing the asymmetrical symmetry.

2. Spherical Symmetry:

In spherical symmetry the shape of the body is spherical and lack any axis. The body can be divided into two identical halves in any plane that runs through the organism's centre. In asymmetrical symmetry and spherical symmetry the polarity does not exist and spherical symmetry is seen in radiolarian protozoa (Fig. 9.2).

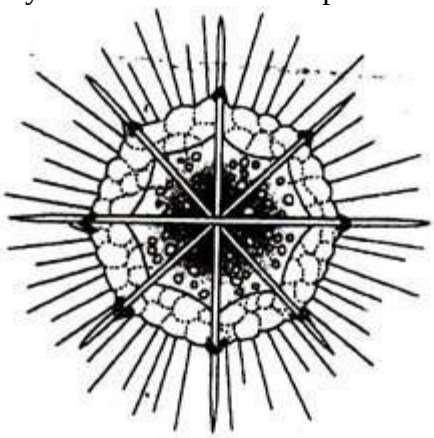


Fig. 9.2: Diagram of a radiolarian showing the spherical symmetry.

3. Radial Symmetry:

In radial symmetry the body can be divided into two roughly equal halves by any one of many vertical planes passing through the central axis (Fig. 9.3A-C) like the spokes of a wheel. The animals which exhibit primarily radial symmetry are cylinder in form and the similar parts of the body are arranged equally around the axis. The axis extends from the centre of the mouth to the centre of the aboral side.

The radial symmetry is seen among the sessile and sedentary animals such as in some sponges, hydroids, anthozoan polyps, medusae and sea stars.

Special forms of radial symmetry are observed in different groups of animals such as:

(i) Tetramerous symmetry:

Many jelly fishes possess 4 radial canals and the body can be divided into 4 equal parts. Hence the animals exhibit tetramerous raidal symmetry (Fig. 9.3B).

(ii) Pentamerous symmetry:

Most echinoderms possess pentamerous radial symmetry because the body can be divided into 5 roughly equal parts (Fig. 20.1). The body parts are arranged around the axis of the mouth at orientations of 72° apart. The larvae of echinoderms are bilaterally symmetrical but acquires radial symmetry in adult stage. The radial symmetry of echinoderms is regarded as a secondary acquisition.

(iii) Hexamerous symmetry:

The sea anemones and true coral polyps belong to the subclass Hexacorallia (class Anthozoa). The mesenteries and tentacles are arranged in the multiple of six. The mesenteries are usually paired and are arranged in the multiple of six. The body of hexacorallianpolyps exhibits hexameric plan and have sixfold internal symmetry.

(iv) Octomerous symmetry:

The body of octocorallian polyps (subclass Octocorallia) shows octomeric radial symmetry and contains 8 hollow marginal tentacles and 8 mesenteries and the body can be divided into 8 equal parts (Fig. 9.3C).

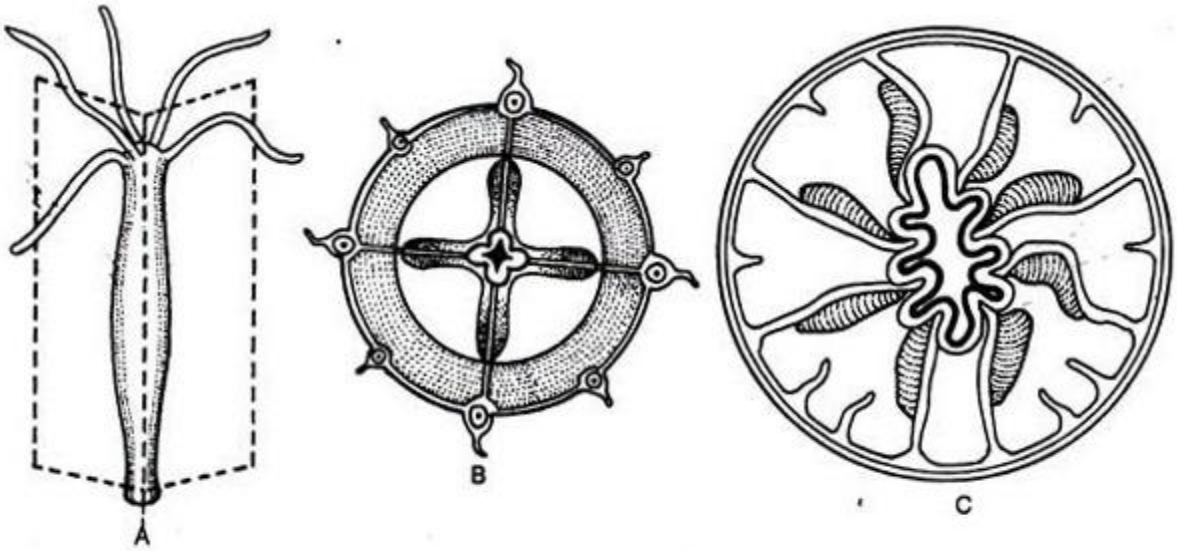


Fig. 9.3: Diagrams showing the different forms of radial symmetry. A. Radial symmetry (Hydra). B. Tetramerous radial symmetry (Jelly fish). C. Octomerous radial symmetry (a octocorallian polyp).

The animals with radial symmetry do not have anterior and posterior sides or dorsal and ventral surfaces. They have a mouth bearing oral side and the side away from the mouth called the aboral side.

4. Biradial Symmetry:

The body of animals which exhibits biradialsymmetry, represents a combination of both radial and bilateral symmetry. The organs are arranged radially and the body can be divided into two by a mid-longitudinal plane. Ctenophores exhibit biradial symmetry.

5. Bilateral Symmetry:

In bilateral symmetry the body parts are arranged in such a way that the animal is divisible into roughly mirror image halves through one plane (mid sagittal plane) only (Fig. 9.4A). This plane passes through the axis of the body to separate the two halves which are referred to as the right and left halves.

The animals which exhibit bilateral symmetry called bilateria. Bilaterally symmetrical animals include acoelomates, pseudo-coelomates and eucoelomates among invertebrates and both lower chordates and vertebrates.

The entire body of a bilateria can be divided into three planes such as— (i) frontal (ii) sagittal and (iii) transverse (Fig. 9.4). Any of the vertical planes perpendicular to the sagittal plane that passes through the body separating the upper and underside is called frontal plane.

The upper-side is also called dorsal which is usually away from the ground and near the back of the animal. The underside is also called ventral which is usually facing towards ground. A longitudinal plane that passes along the axis of the body of bilaterally symmetrical animal to separate right and left sides is called the mid- sagittal plane (Fig. 9.4B).

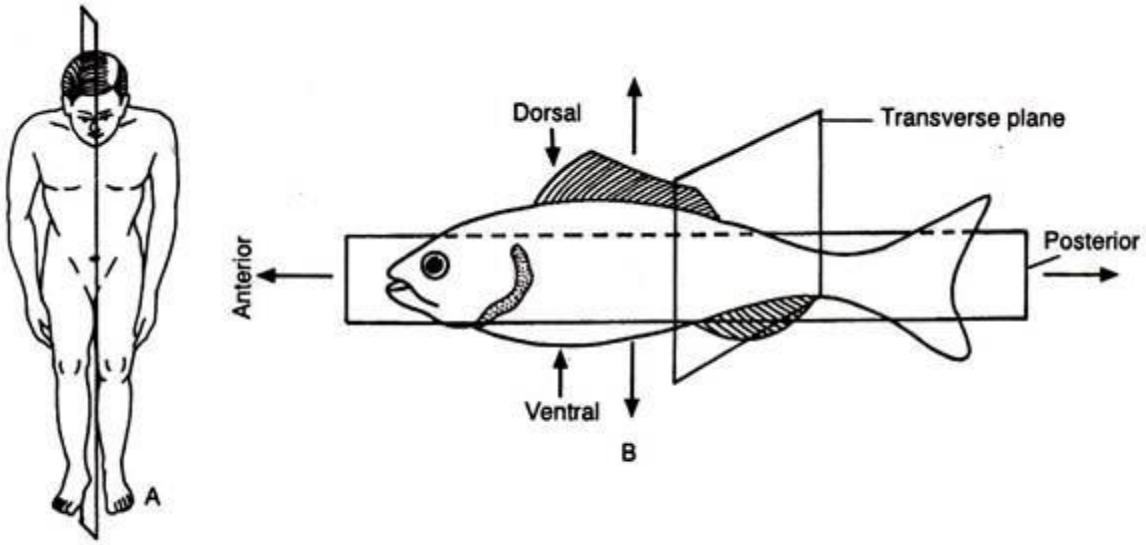


Fig. 9.4: A. Diagram showing the bilateral symmetry in man. B. A fish showing the different planes of bilateral symmetry.

An imaginary plane that crosses the body, perpendicular to the mid sagittal plane called transverse plane. The body of bilateria has the term lateral (two sides of the body), anterior (the

end which usually moves forward during movement and bears mouth) and posterior (Fig. 9.5) (the end opposite to anterior).

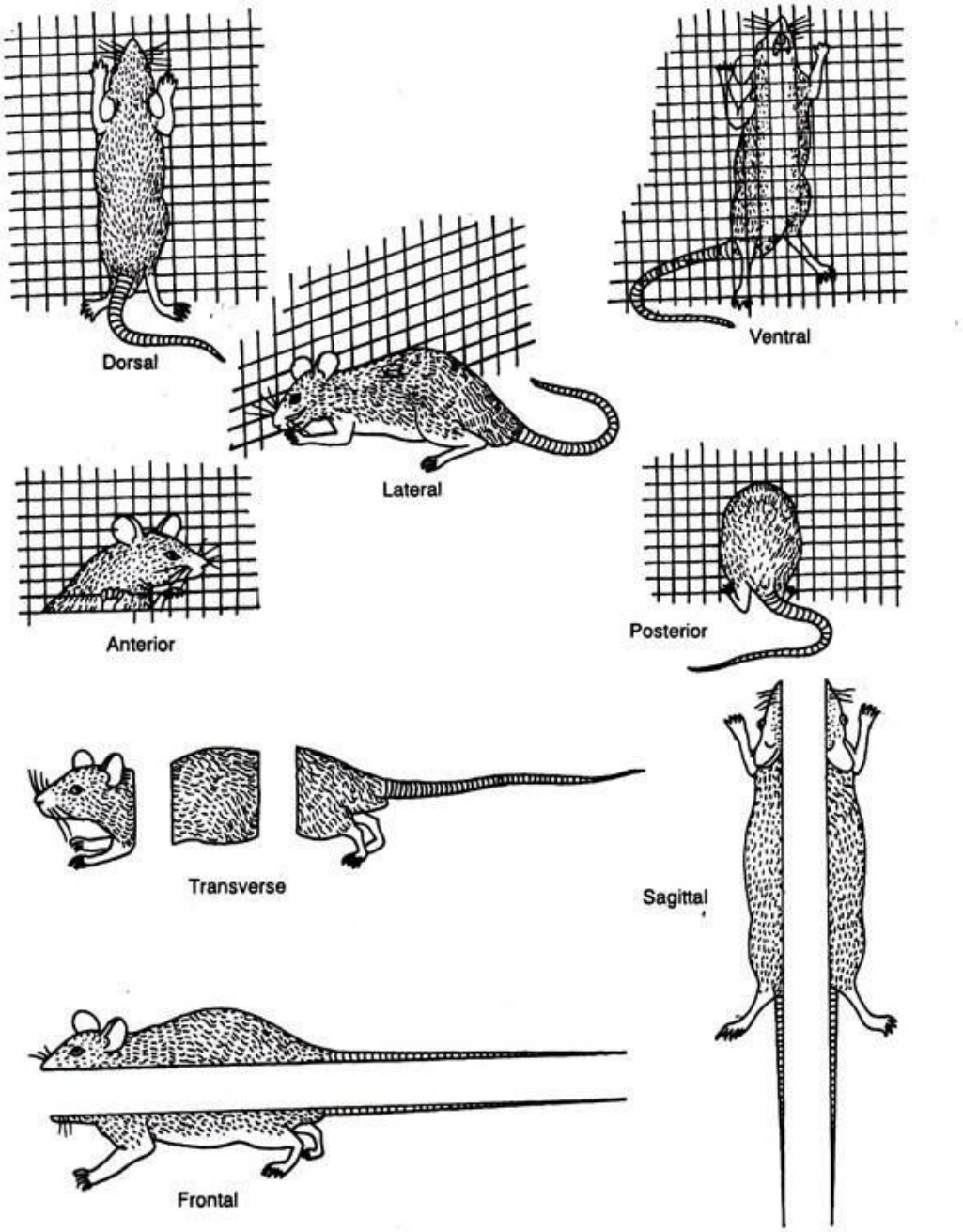


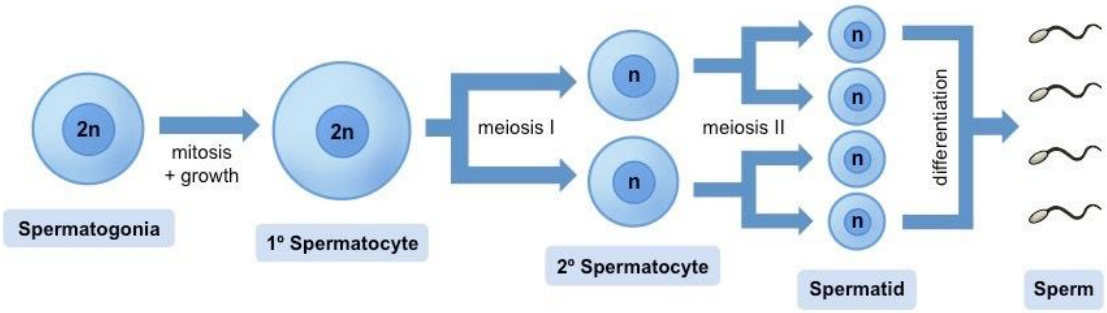
Fig. 9.5: For the convenience of study, the animal body is divided into a number of regions–dorsal, ventral, lateral anterior and posterior. The entire body may also be divided into three planes, transverse, frontal and sagittal.

Advantages of Symmetry:

1. Bilateral symmetry is associated with the term cephalization—meaning the specialization of the anterior end of the body to form the head where the nervous tissues, sense organs and feeding organs are concentrated.
2. Other advantages of this symmetry are the streamlining of the body, development of different organs in different body regions and more efficient unidirectional movement.
3. Radial symmetry helps the animals for collecting food and defence

Spermatogenesis

- Spermatogenesis describes the producton of spermatozoa (sperm) in the seminiferous tubules of the **testes**
- The process begins at puberty when the germline epithelium of the seminiferous tubules divides by mitosis
- These cells (*spermatogonia*) then undergo a period of cell growth, becoming *spermatocytes*
- The spermatocytes undergo two meiotic divisions to form four haploid daughter cells (*spermatids*)
- The spermatids then undertake a process of differentiation in order to become functional sperm cells (*spermatozoa*)



Structure of Sperm

The whole cell of sperm is streamlined and paired down for action of a special sort and of limited duration, namely, to swim and to meet an egg, to fuse with the cortex of an egg, and to introduce sperms nucleus and Centriole in the egg interior.

Structure of head of sperm:

The sperm head consists of mainly the nucleus and acrosome. Its shape, size and structure vary greatly in different groups of vertebrates (Fig. 7.) The head of sperm performs two functions—genetic and activation.

The genetic function is embodied in the sperm nucleus which consists almost entirely of DNA plus nuclear proteins and thus is responsible for the transmission of hereditary characters from the male. The nucleus of the sperm occupies major part of the head and its shape, ultimately,

determines the shape of the head of sperm. At the anterior end of the sperm nucleus occurs a cap-like structure called acrosome.

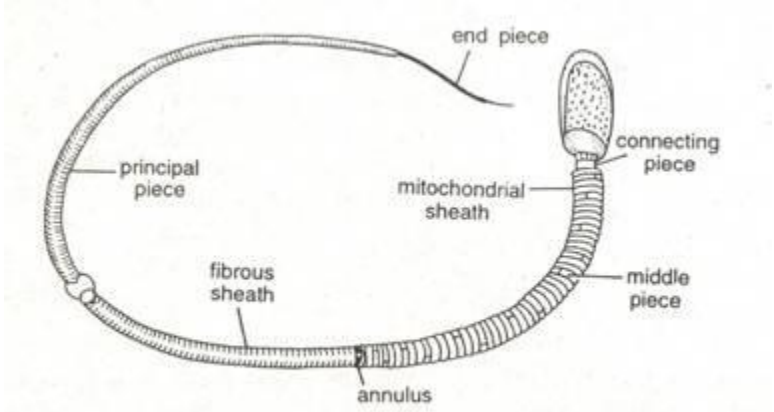


Fig. 7. Typical mammalian spermatozoon.

The shape and size of the acrosome vary among different species. The acrosome is bounded by an acrosomal membrane and it contains certain acrosomal polysaccharides like galactose, mannose, fructose, and hexosmine (Kopency, 1976). A large number of enzymes especially hydrolases are also present in the acrosome. It also contains two most important enzymes such as hyaluronidase and zonaIysin or acrosin which functions during sperm entry into the ovum (Nelson, 1985).

In the sperm of some animals such as fowl, there occurs a cone-shaped structure called axial body or acrosomal cone in between acrosome and nucleus (Fig. 8 A). The acrosomal cone develops into an acrosomal filament at the time of fertilization. Very little cytoplasm occurs in between nucleus and plasma membrane of sperm head. The cytoplasm in between the membrane of acrosomal vesicle and sperm plasma membrane is called periacrosomal cytoplasm.

Structure of Tail of Spermatozoon:

The motor apparatus of the spermatozoon tail is the axoneme or axial filament complex which consists of the usual central pair or axial fibril (or microtubules) surrounded by an inner row of nine evenly spaced doublet microtubules, each with two rows of arms that project towards the adjacent double tubule, one row of radial spokes that radiate inwards towards the central pair of microtubules, with outer ring of nine coarse longitudinal fibres (Guraya, 1987).

Actually, all the structural components of the flagellum, which include the connecting piece, 9 + 2 axoneme, fibrous sheath and outer dense fibres, are structurally interlocked into one functional unit.

A central sheath, made up of projections has been described surrounding the two central tubules. It is connected by nine spokes, radial links to the nine doublets, which are also connected to each other by inter doublet links. (Fig. 8 d).

From morphological point of view tail consists of the following sub divisions:

1. Neck:

The neck is a short, slightly constricted segment made up of projections located between the base of the head and the first gyre of the mitochondrial helix of the middle piece. The neck differs clearly from the head and also from the rest part of the tail (i.e. midpiece, principal piece and end piece.) in certain morphological features of plasmalemma, a sharp demarcation of its upper limit by the posterior ring, and lack of continuity between the segmented columns and the outer dener's fibers of the tail (Fig- 8 A). The two Centrioles lie at right angles to each other are proximal and distal Centriole.

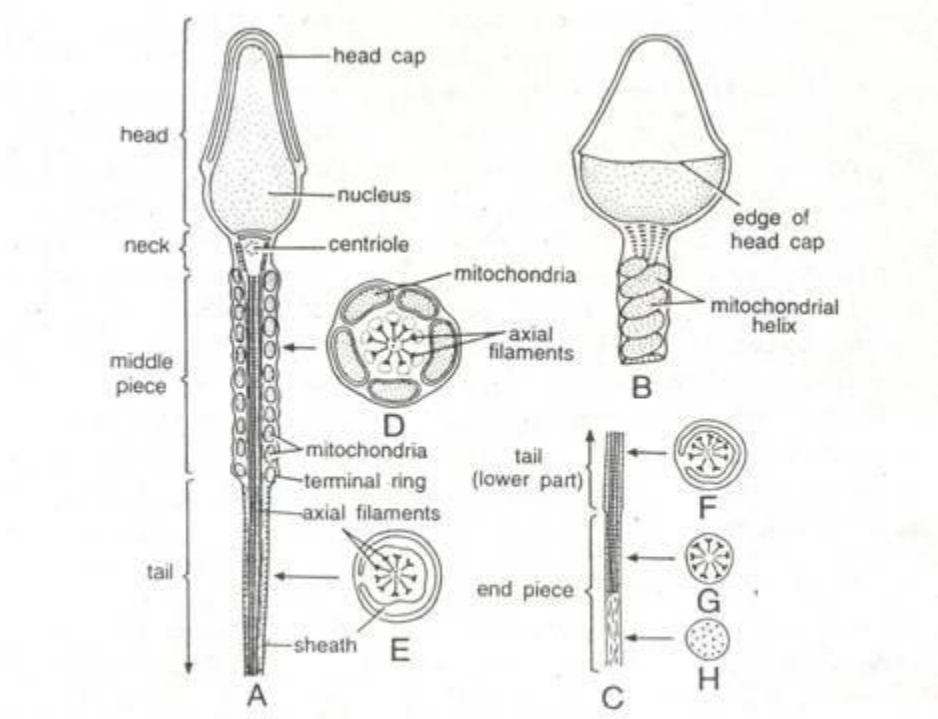


Fig. 8. Electron micrographs of human spermatozoa showing the structure A—L.S. of spermatozoa, B—Head, together with neck and middle piece, C—terminal part of tail proper and endpiece and D-H— highly magnified T.S. of middle piece and tail at various levels.

The distal Centriole forms and gives attachment to the axial filament of the sperm tail; the proximal Centriole has no active function in the spermatozoon but is a potential activist within an egg during first cleavge division of the fertilized egg.

Two or three mitochondria are also present in the neck. These generally establish close relationship with either end of the proximal Centriole by wrapping around the lateral surface of the latter. These mitochondria are continuous with the uppermost mitochondria of the mid piece helix.

2. Middle piece:

Anatomically, the mitochondrial sheath and the outer ring of coarse fibres characterize the mammalian sperm mid piece. It is that part of the flagellum which lies between the neck and annulus and forms the most important site for various metabolic activities of the sperm.

The axoneme of the mammalian sperm is surrounded by nine outer dense fibers which are also called the coarse or accessary fibres. These run for the major part of its length, thus constituting a 9+9+2 cross-sectional pattern (Fig. 2.8 d).

The mitochondria of the mid piece arranged end to end constitute a helix around the longitudinal fibrous elements of the tail. The end on junctions of mitochondria are generally seen at random along the course of the helix.

The mitochondrial sheath is believed to be the source of energy (ATP) for sperm motility. However, this energy is limited and once utilized cannot be renewed, except in mammals and in those animals where spermatozoa remain alive within maternal body because there are energy sources available to the spermatozoon.

At the junction of mid piece and principal piece is present the annulus which is also known as the ring Centriole or Jensen's ring. The annulus is composed of the closely packed filamentous subunits, 3 to 4 nm in diameter. It develops in close association with the plasma membrane and remains firmly adhered to it. The functional significance of annulus is still not clear but according to some scientists the function of the annulus could be to prevent displacement of the mitochondria.

3. Principal piece:

The main piece or principal piece of mammalian spermatoza is surrounded by a fibrous sheath which shows a similar basic organization in different species of mammals. Fibrous sheath is composed of a series of circumferentially oriented ribs that extend half way around the tail end in two longitudinal columns which run along opposite sides of the sheath for its whole length. The sheath is not attached to the plasma membrane.

The longitudinal columns extend in the principal piece along the whole length of the fibrous sheath in its dorsal and ventral surfaces. These are composed of 15 to 20 nm thick longitudinal subunits attached to the axoneme during sperm movement.

The plasma membrane is independent of this complex. Towards the end of the piece, the longitudinal columns progressively reduces in size. Meanwhile the ribs become slender. The abrupt ending of the fibrous sheath marks the junction of the principal and end piece.

4. End piece:

The end piece consists of a central pair of axial fibrils and ring of nine doublet fibres, which are surrounded by the plasma membrane. The 9+ 2 pattern of axial filament complex extends through most part of the tail including the end piece, but the arrangements of the fibres in the tip of the end piece is changed and decreasing number of fibres suggests a successive termination of the single subfibres (Fig. 8 F-H).

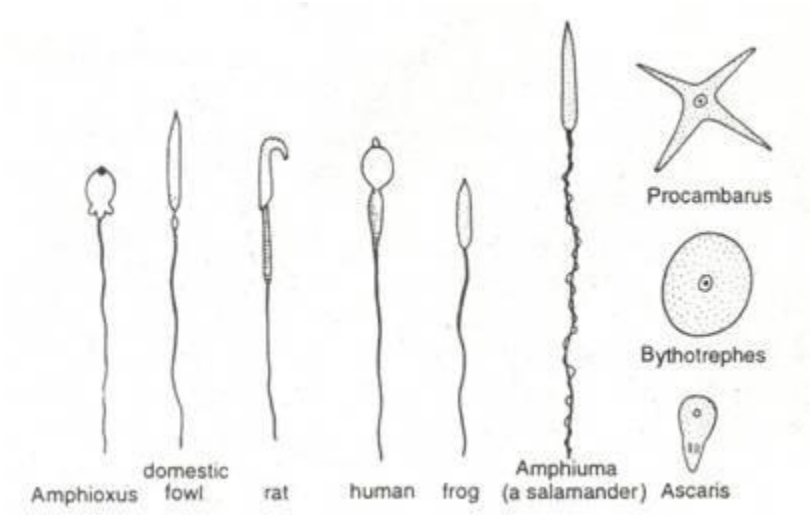


Fig. 9. Sperm of some animals. **Procambarus** and Bythotrephes are **crustaceans.**

Functions of the spermatozoon:

The main function of the spermatozoon is to carry the paternal genetic dowry and to activate the ovum.

Types of sperm

The type of the sperm produced varies from species to species. The size of the sperm may be as little as 0.018 mm in Amphioxus or as large as 2.25 mm or more in toad. The sperm head is, however, species specific. It may be spheroidal (teleosts), rod or lance-shaped (amphibians), spoon-shaped (man and many other mammals), or hooked (mouse and rat).

The sperm types are again divided into two main types found in animals-flagellate spermatozoa, which posses a flagellum or tail like biflagellate (in Opsanus, a toad fish) spermatozoa. The non-flagellate spermatozoa lack flagella and are found in Ascaris, crab etc.

Steps of Fertilization

As a result of copulation, semen containing sperms is ejaculated into the vagina. Once the semen (mixture of spermatozoa and accessory fluids) is deposited into the vaginal passage of the female, the spermatozoa start their journey through the uterus to reach the oviducts. On an average, the spermatozoa can swim several millimetres each second. Apart from this speed, they need to increase their mobility.

Spermatozoan mobility is aided by:

a) Ejaculation of the semen into the vagina itself triggers mobility

b) Muscular contractions of the wall of the uterus and the oviducts

c) Secretion of a viscous liquid from the secretory cells of the epithelial lining of oviductal mucosa

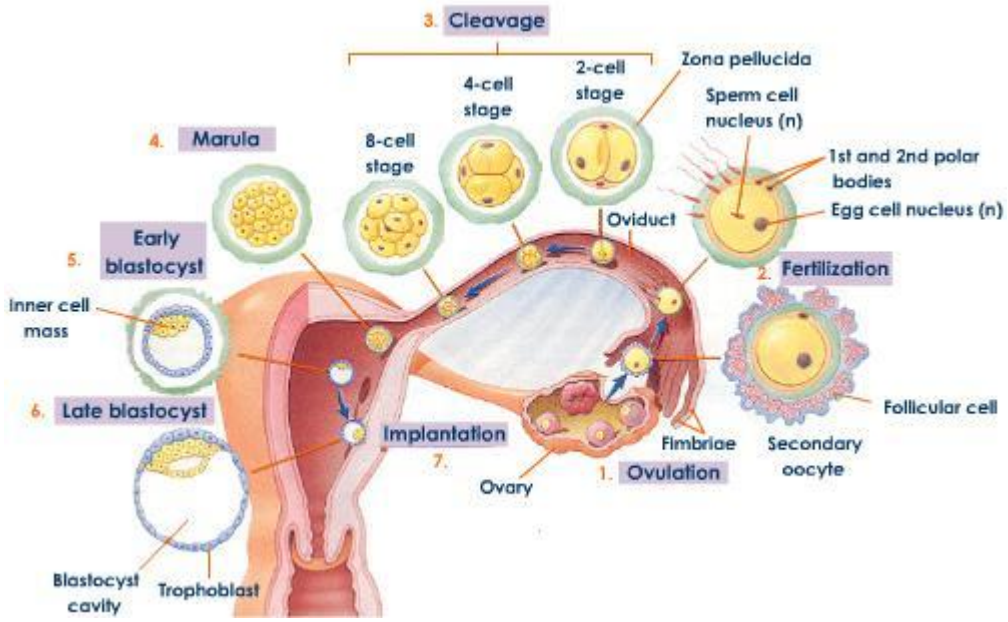
This phenomenon of sperm activation in mammals is known as capacitation, which takes about 5 - 6 hours.

The sperms migrate up through the uterus in to the fallopian tube. The fusion of the sperm nucleus with egg nucleus is called fertilisation and a zygote is formed in the fallopian tube.
In all mammals fertilisation is internal. In fishes and amphibians fertilisation is external as the egg is fertilised outside the body.

Fertilization

Approach of spermatozoon

During copulation, the male inserts its erectile penis into the vagina of the female and releases about 3.5 ml of seminal fluid. The semen contains millions of sperms. This process is called insemination.



Site of Fertilization of Egg and Implantation of Embryo in a Mammal

The sperms travel a great distance through the female genital tract beset with chemical hazards in the form of strong acid secretions from the female tract. They also meet with mechanical obstacles while passing through crooked and compressed tract which often gets narrowed or occluded.

In an ejaculate of semen, the number of sperms is about 200,000,00. This high number ensures that at least a few sperms reach the fallopian tube which is the site of fertilisation. Actively motile sperms swim like tadpoles in the fluid medium at the rate of 1.5 - 3mm per minute to reach the site.

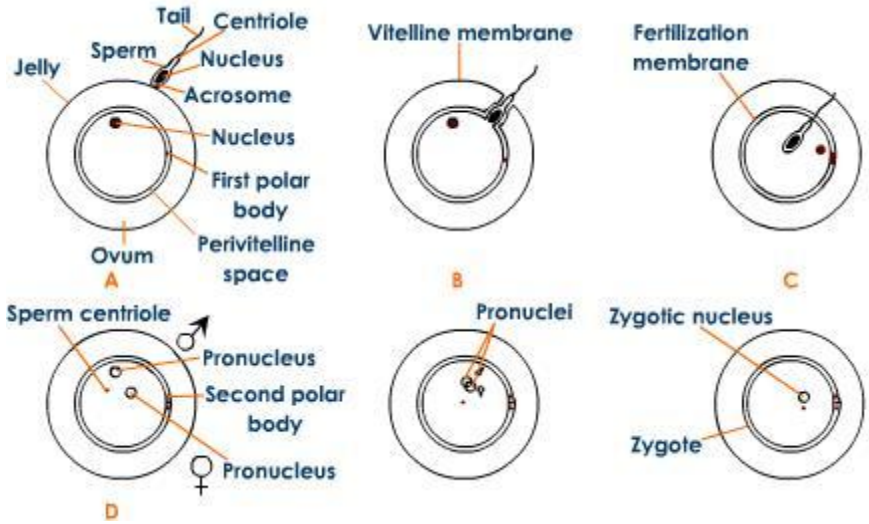
The fertilisability of human sperm in the female genital tract is of 12 - 24 hrs while they survive upto 3 days. Since the ovum is non motile and the energy content of the sperm is low, the sperm must approach the egg as quickly as possible. So it is only about 24 hours.

Penetration of Sperm

The sperm generally comes in contact with the ovum at the animal pole. A number of sperms surround the ovum, but only one of them fuses with the egg.

Fertilisation of the egg with only one sperm is known as monospermy.

Fertilisation occurs in the ampulla of the oviduct.



Reaction of egg

The acrosome of the sperm produces an enzyme hyaluronidase which acts as the sperm lysin. It dissolves the mucus and egg membranes, making the passage for the sperm nucleus into the egg cytoplasm.

Essence of activation

Activation of Ovum

The penetration of the sperm activates the egg and initiates a series of changes in the egg cortex and in the egg cytoplasm. Ovulation in the human female occurs at the secondary oocyte stage in which meiosis - I has been completed and the first polar body has been released but second meiotic division has yet to be completed. When the sperm lysin dissolves the egg membrane, the ovum is activated to release the second polar body and begin cleavage.

Amphimixis

The fusion of the male and female nuclei is called amphimixis. This restores the original diploid condition in the zygote nucleus. Moreover it bring about the intermingling of the paternal and maternal hereditary material so that the resulting offspring receives both paternal and maternal characteristics.

The entry of the sperm invokes a chemical signal in the egg cell. This makes the egg imperviou for the entry of any more sperms, thus preventing polyspermy. The signal is transmitted to the egg surface in capacitating hundred of sperms in the vicinity of the egg cell.

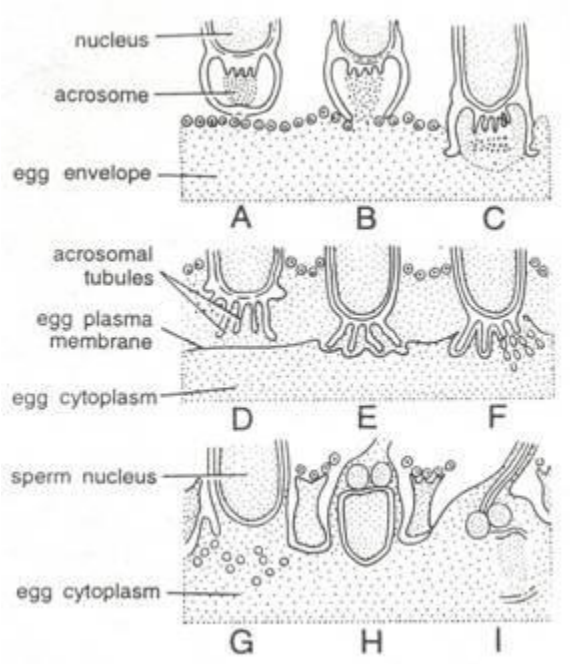
Changes in egg cytoplasm during fertilization

The plasma membrane of both gametes (sperm and ovum) becomes continuous and forms a single cell, called zygote. At this very time, certain very important changes occur in the cytoplasm of egg:

(1) Fertilization cone formation.
(2) Cortical reactions and formation of fertilization membrane.
(3) Metabolic activation.

1. Fertilization cone formation:

Immediately after the acrosomal filament of spermatozoon touches the surface of the egg, the cytoplasm of the egg bulges forward at the point of contact, producing a process of hyaline cytoplasm called the fertilization cone.



Fertilization cone develops or appears in many forms. It may be in the form of a more or less simple conical protrusion or it may consist of several irregular pseudopodium like processes, or in some cases it may take the form of a cytoplasmic cylinder stretching forward along the acrosomal filament or tubule whatever its shape, the fertilization cone gradually engulfs the spermatozoon and then begins to retract.

Here one point should be clear, that normally, the spermatozoon does not enter the egg cytoplasm intact, nor, it is swallowed but the sperm nucleus and other sperm structures (periacrosomal material, proximal Centriole and mitochondria of mid-piece of spermatozoon) pass to the fertilization cone of the egg. The plasma membrane of sperm becomes one entity of plasma membrane of the egg.

Further during the whole penetration process of sperms into the egg, acrosomal granule never makes its entry into the egg, but only the periacrosomal material is injected into the egg

cytoplasm along with other contents of the sperm. Some workers suggest that this periacrosomal material is responsible for the activation of egg.

There exists some variation in different animals, as to how much of the spermatozoon is taken into the interior of egg during fertilization. In mammals, complete structures of spermatozoon (viz., nucleus, mid piece, tail etc.) penetrate into the egg cytoplasm.

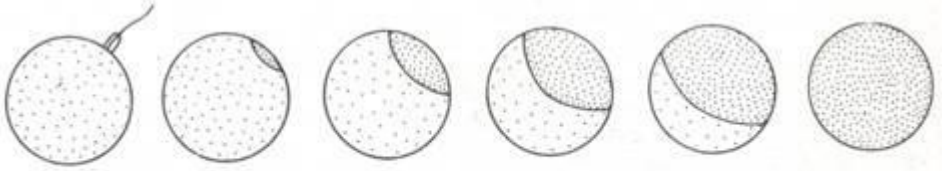


Fig. 5. Spreading of cortical change (black) in a sea urchin egg after fertilization. The figure at the left represents the moment when the spermatozoon contacts the egg surface.

In echinoderms, sperm tail remains exterior to the vitelline membrane; in Neries only sperm head and proximal Centriole enter the egg cytoplasm. In most animals, however, the sperm nucleus and mid piece make their entry in the egg as a rule.

2. Cortical reactions and formation of fertilization membrane:

Even before the fertilization cone is formed and the spermatozoon penetrates into the interior of egg, a chain of physio-chemical reactions is set in the egg cortex. All these reactions are collectively called cortical reactions.

These reactions may differ from one group of animals to another, but in most groups, the cortical reactions lead to the formation of fertilization membrane outside the egg plasma membrane. This membrane blocks the entry of the late arriving spermatozoa in the egg interior, and thus avoids polyspermy. The process of cortical reactions and fertilization membrane formation in different groups of animals is as under:

(a) Sea urchins:

In sea urchins, as soon as the apical end of acrosomal tubule touches the surface of egg, from the site of contact, a wavelike color change from yellow to white (under dark field microscopy) travels rapidly around the egg cortex and is shortly followed by the elevation of fertilization cone from the egg surface and the formation of fertilization membrane around the egg plasma membrane.

Electron micrographs of sea urchins unfertilized eggs show that the egg cortex is bounded by two membranes (i) an outer 30 A° thick, vitelline membrane, and (ii) an inner, 60 A° thick plasma membrane.

Beneath the plasma membrane occurs a layer or cortical granules. A fertilization membrane is formed in the following stages:

The outer vitelline membrane separated from the plasma membrane, undergoes expansion and becomes the outer layer of the fertilization membrane. The cortical granules explode and release the following three components:

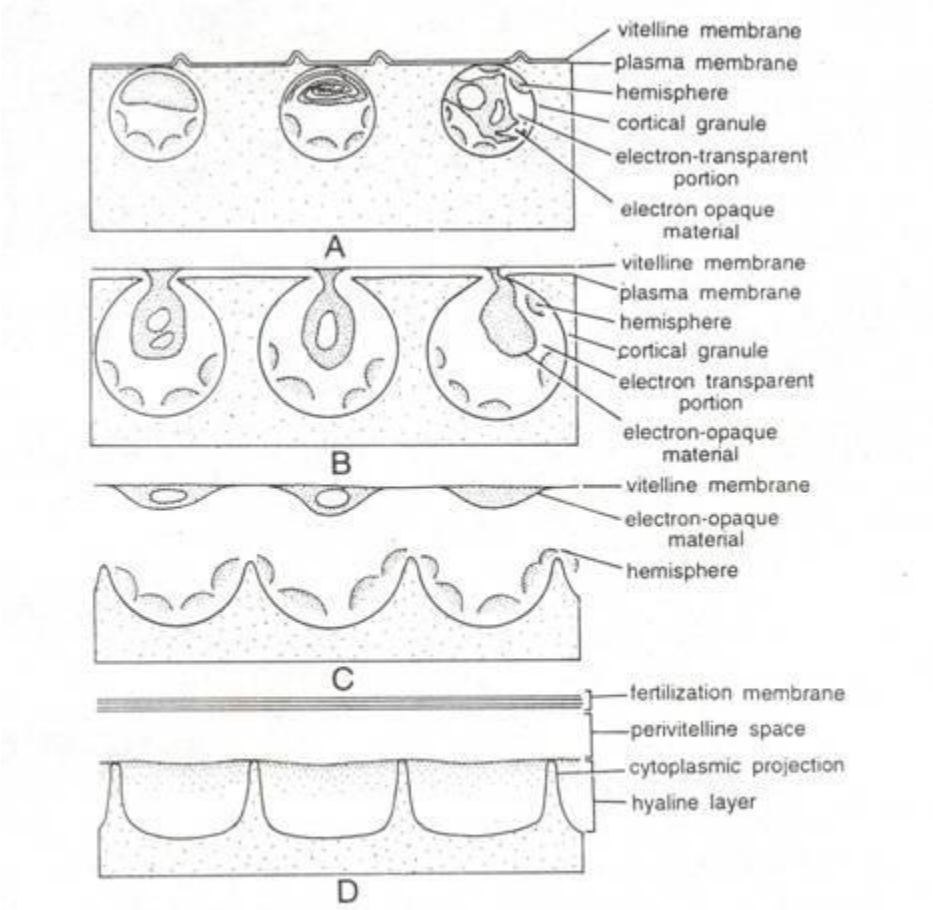


Fig 6. Cortical reaction andfertilization membrane formation in the sea urchin *Clypeasterr japonicus*. A—unfertilized egg, B—fusion of egg plasma membrane and membrane of cortical granules, C—adhesion of electron opaque material to the vitelline membrane, D—egg surface after completion of cortical events.

(1) Dark, denser, lamellar and folded parts of the granules—these lamellar bodies unfold and fuse with the inner side of the already elevating membrane, the vitelline membrane.
(2) Globules, which fuse together and build up a new surface of the viscuous hyaline layer, just at the outer side of the egg plasma membrane. The hyaline layer adheres closely to the surface of the egg and during clevage; it helps to keep the blastomeres together.
(3) The liquified component of the cortical granules fills the perivitelline space between the new egg surface and now the completed and elevated fertilization membrane. It contains mucopolysaccharides and abundant water.

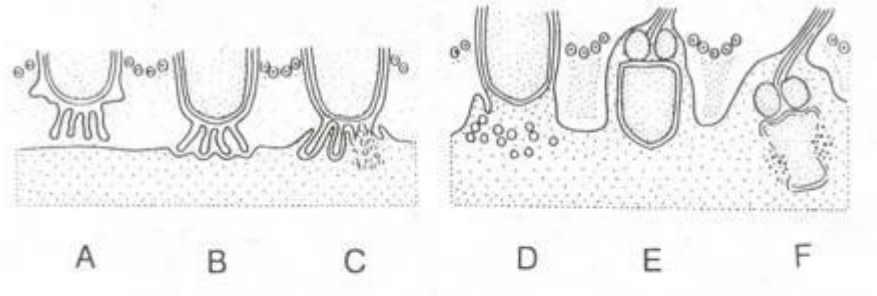


Fig. 7. Stages in the association ofegg and sperm in the polychaete Hydroides. A–acrosomal tubules lead sperm penetration, B–acrosomal tubules initiate contact with egg plasma membrane, which forms mirovilli, C–fusion of the plasma membranes of egg and sperm, and release of the contents of acrosome, D-F– successive stages in the engulfment of the sperm by the fertilization cone.

All these structures, namely, vitelline membrane and contents of cortical granules, thus form a fertilization membrane, which is much thicker (upto 900 A°) and stronger.

Among other invertebrates, fertilization membrane formation has been observed only in certain annelids (e.g., Nereis). In others no structural but metabolic changes have been observed in egg cortex.

(b) Vertebrates:

In vertebrates the changes which occur in the cortex are similar to sea urchins. In bony fishes and frog, the cortical granules are broken down immediately after sperm's penetration into the egg cytoplasm.

Their contents become liquified and extruded on the surface of the plasma membrane of the egg and fill the perivitelline space occurring in between the chorion and plasma membrane and in between the vitelline membrane and egg plasma membrane in the frog.

In both cases, the vitelline membrane or chorion itself does not transform into the fertilization membrane as in sea urchins. The chorion in fishes becomes hardened or "tanned" after fertilization and no new membrane is formed in either of the two animals.

In some mammals (man, rabbit, etc.) the cortical granules burst and release their contents into the perivitelline space created between the egg plasmalemma and the zonapellucida.

In urodele amphibians and some mammals, which lack cortical granules, neither any cortical reaction nor fertilization membrane formation occurs.

3. Metabolic activation:

After the sperm penetrates the unfertilized egg a series of diverse cytoplasmic reactions are initiated. Following metabolic changes occur in the egg at fertilization.

(a) Changes in plasma membrane:

The permeability of plasma membrane increases for the molecules of water and certain other chemicals like ethylene glycol, phosphate, K^+etc. At fertilization, the electrical potentiality of plasma membrane becomes more positive and it gradually becomes more negative. The change in the potentiality of the membrane is governed by the unequal distribution of chloride ions.

Besides this a plasma membrane enzyme, adenylcyclase becomes activated at the time of fertilization and it starts the formation of a chemical molecule $3^1 – 5^1$ cyclic AMP, which is supposed to activate most of the metabolic reactions in a fertilized egg.

(b) Ionic changes:

Certain intracellular changes occur in the concentration of cations, especially those of sodium, potassium and calcium. The change in calcium ion concentration in a fertilized egg has great significance in the metabolic activation of the egg.

(c) Changes in the rate of respiration:

In a fertilized egg, the rate of respiration either increases (e.g. sea urchins) or decreases (e.g. Chaetopterus and Molluscs, Cumingia) or remains tatic (e.g. Bufo and Fundulus). There appears to be a relation between the post-fertilization oxygen consumption and the stage of maturation of the egg at fertilization.

Because at the time of fertilization the sea urchin egg has completed maturation, the egg of Bufo is at second maturation division stage and the egg of Chaetopterus is at the first maturation division stage.

The increased oxygen consumption is related with the oxidation of glycogen and other food stuffs of the egg and synthesis of numerous ATP molecules.

(d) Co-enzyme changes:

The primary action of the spermatozoon consists of the release or activation of the oxidative enzymes of the egg and that the ensuing increase in oxidation provides the energy necessary for the performance of other changes in the egg and for the development of the egg in general.

In a fertilized egg inter conversion of pyridine coenzyme, NAD into another co-enzyme NADP and also NADPH due to phosphorylation of the NAD in the presence of a enzyme NAD Kinase takes place.

NAD + ATP → NAD Kinase NADP + ADP

There are ample evidences that NAD kinase enzyme, though present in the unfertilized egg, it exists in an activated state. It is activated only at the time of fertilization. The increased NADP and NADPH contents may initiate many synthetic pathways of fertilized egg.

(e) Changes in the rate of protein synthesis:

The cytoplasm of a mature unfertilized egg, though contains complete machinery for protein synthesis, such as DNA molecules, tRNA, mRNA, ribosomes and proteolytic enzymes required during protein synthesis, none or very little protein synthesis occurs because the mRNA of unfertilized egg remains "masked".

There are evidences that during later phases of oogenesis some inhibitor or repressor proteins are manufactured in sea urchins egg which inactivate chromosomal genes, mRNA molecules, ribosomes etc.

During fertilization there is an increase in proteolytic activity of the egg immediately following the penetration of spermatozoon which removes these inhibitor proteins from them and unmasks the mRNA and active protein synthesis is started. In the egg of frog, however, the rate of protein synthesis is increased quite early at the stage of ovulation itself.

(f) Initiation of mitosis:

The initiation of mitosis for cleavage is the most significant aspect of egg activation. The initiation of mitosis occurs because (i) The rate of DNA synthesis increases with great pace immediately after fertilization; (ii) the unfertilized egg cytoplasm although possesses a Centriole, yet this Centriole is incapable of division and also to form a mitotic spindle. Thus sperm stimulates the first mitotic division (cleavage) of fertilized egg by contributing its Centriole to the egg.

In some animals after fertilization, considerable amount of acid is formed during first several months.

Components of spermatozoon in the egg interior:

Many variations have been observed in different group of animals, as to how much part of the spermatozoon is engulfed into the interior of egg, during fertilization. In most cases, the sperm nucleus, periacrosomal material, proximal Centriole and mitochondria make their entry as a rule. The plasma membrane of the sperm becomes one of the entity of plasma membrane of the egg. In mammals, complete structure of spermatozoon (i.e. head, middle piece and tail) penetrates into the egg cytoplasm.

In echinoderms the sperm tail remains exterior to the vitelline membrane while in Nereis, only sperm nucleus and proximal Centriole enter the egg cytoplasm. There is no definite proof that any constituent of the spermatozoon except for the nucleus and Centrosome play any active part in subsequent development. The mitochondria have been observed in some cases to scatter in the cytoplasm of egg, but it is not known how long they maintain their existence there.

In vertebrates, as a rule the egg completes its first meiotic division in the ovary and reaches the metaphase stage of the second meiotic division. At this stage all further progress is arrested, ovulation takes place and the egg may become fertilized.

The second polar body gets extruded only if the egg is fertilized by a spermatozoon or activated in some other way. In ascidians, the egg reaches only the metaphase of the first meiotic division when it becomes ripe and if fertilized, only then the egg completes the first reduction division and carries out the second meiotic division.

Migration of pronuclei and amphimixis:

At the time of penetration of spermatozoon inside the egg cytoplasm, the sperm nucleus remains compact and its mitochondria and Centriole remain situated behind it. To perform the act of amphimixis, the sperm nucleus has to undergo two activities (i) it has to become pronucleus, and (ii) it has to migrate from the site of amphimixis.

As the sperm nucleus moves inwards from the site of fertilization cone, it soon rotates through an angle of 180°C, so that its mitochondria and Centriole assume the leading position. Besides this rotation, the sperm nucleus starts swelling and its chromatin, which is very closely packed, becomes finely granular. It ultimately becomes vesicular and has an appearance like the interphase nucleus and is called male pronucleus.

At the same time, the sperm aster forms around the proximal Centriole of the sperm in the egg cytoplasm. As the male pronucleus develops and migrates towards the site of amphimixis, the sperm aster seems to lead it.

The site of amphimixis lies either near the centre of microlecithal and mesolecithal eggs or in the centre of the active cytoplasm at the animal pole of macrolecithal and Teleolecithal eggs. As the sperm pronucleus and Centriole move inward, it may be accompanied by some cortical and subcortical cytoplasm.

If the latter is heavily pigmented, as in amphibian eggs, the trajectory of the sperm pronucleus may be marked by pigmented granules trailing along its path. This is called penetration path. This movement of the sperm appears to be directed and some investigators feel that it is due to a chemotoxic effect of chemicals liberated by the female pronucleus. During this movement toward the female pronucleus, the sperm may have to deviate from its penetration path. If it does, the new pathway is taken. This is referred to as copulation pathway.

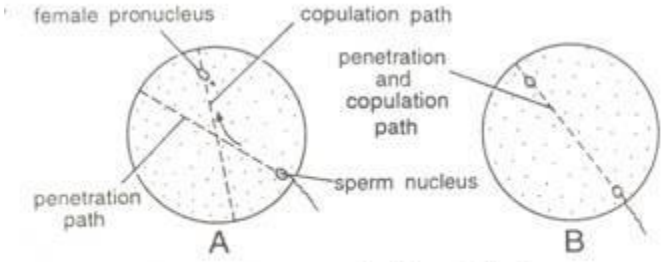


Fig. 8. Possible sperm paths during fertilization.

In some cases the sperm need not alter its direction. In these cases the penetration and copulation paths would be identical. The point of entrance, penetration path and copulation path are all believed to be responsible for establishing the primary plane of bilateral symmetry in the embryo.

Before amphimixis the nucleus of the egg also undergoes certain changes like the sperm nucleus. After the completion of the second meiotic division, the haploid nucleus of the egg is located near the surface of the egg in the form of several vesicles, known as karyomeres In a fertilizing egg, these karyomeres fuse together to form a female pronucleus which swells increase in volume and becomes vesicular. It also migrates towards the site of Amphimixis.

Amphimixis:

The fusion of male and female pronuclei is called as amphimixis. The actual fusion of pronuclei may differ in different animal.

(1) In sea urchins and vertebrates, the nuclear membranes of both pronuclei are broken down at the point of contact and their content unite in one mass surrounded by a common nuclear membrane. At the approach of first cleavage of fertilized egg, the nuclear membrane dissolves, chromosomes of maternal and paternal origin become arranged on the equator of the achromatic spindle.

(2) In Ascaris, some molluscs and annelids, the male and female pronuclei don't fuse but the nuclear membranes in both dissolve and the chromosomes become released. In the meantime, the Centrosome of the spermatozoon has divided into two and the Centrosome derived from male and female pronuclei become attached to the spindle.

Only after the completion of the first division of the fertilized egg, the paternal and maternal chromosomes become enclosed by common nuclear membrane to form the nuclei of two blastomeres into which the egg has been divided.

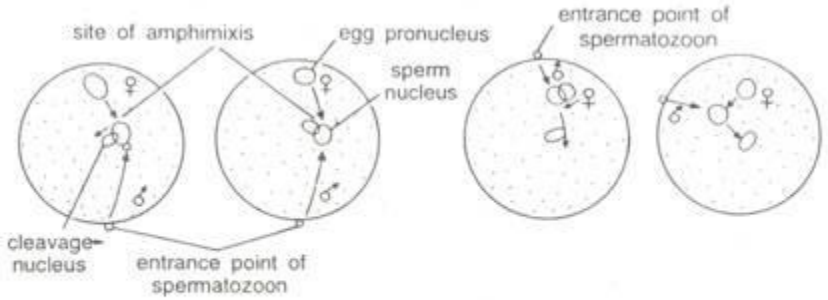


Fig. 9. Amphimixis in sea-urchin. Tracks of the pronuclei in their movements toward each other and to the site of the cleavage nucleus, with sperm entering at opposite side of egg from the site of egg nucleus and from the same side, respectively.

In both these types (1 and 2), the chromosomes of maternal and paternal set retain, of course, their individuality.

(3) In the Copepod Cyclops, the paternal and maternal nuclear components remain separate for some time even after cleavage has started, so that each blastomere has a double nucleus consisting of two parts lying side by side and each surrounded by its own nuclear membrane.

Post-fertilization changes in the egg cytoplasm:

The penetration of spermatozoa into the egg causes far-reaching displacements of the cytoplasmic constituents. As a result, the distribution of various cytoplasmic substances and inclusions in a fertilized egg may be very considerably different from that in the unfertilized egg and even quantitatively new areas may appear.

As a result of the extrusion of cortical granules, a large part of the original outer egg cell surface is replaced by the inner surface which surrounds the cortical granules and now are averted on the exterior. Most spectacular post-fertilization displacements in the egg cytoplasm have been observed in Ascidian, Styela partita and in frog. In both these animals, there establishes a bilateral symmetry in the cytoplasm of fertilized eggs.

Displacements of cytoplasmic substances in the egg of Ascidian Suela partita are as under:

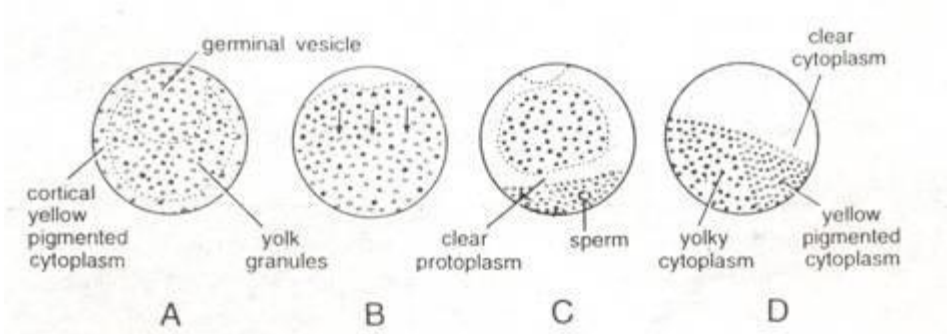


Fig. 10. Displacements of cytoplasmic substances in the egg of the ascidian, *Styela partita* following fertilization. A—Ripe unfertilized egg ; B—Egg soon after the entry of spermatozoon; C-D—Male pronucleus moves to meet female pronucleus and the formation of cytoplasmic territories.

The mature egg is covered by a layer of cortical cytoplasm containing yellow granules. The moment spermatozoon enters the egg near the vegetal pole, the yellow cortical cytoplasm falls into violent commotion.

The yellow cytoplasm begins to stream down along the surface of the egg toward vegetal pole, and accumulated as a cap. As the male pronucleus penetrates deeper into the cytoplasm and moves toward female pronucleus, the yellow cytoplasm, reverses its movements and streams upward only on the side where the spermatozoon entered the egg. Just before the first cleavage, the yellow cytoplasm forms a cresentic area (mesodermal crescent) just below the equator of the egg.

Simultaneously, a crescent of light gray cytoplasm (notochordal crescent) appears subequatorially on the opposite side of the egg. Thus, in a fertilized egg, four different kinds of cytoplasmic regions are now present-(i) The yellow cytoplasm on one side, and (ii) the light cytoplasm on the other side. These two together form a belt surrounding the egg just below the equator. Below this zone toward the vegetal pole, (iii) the cytoplasm in salty grey colour contains abundant yolk granules and in the sub cortical layer, there are a large number of mitochondria. But the cytoplasm in (iv) the animal hemisphere contains less yolk and a few mitochondria and appears more transparent. Thus, the cytoplasmic displacements following fertilization not only bring some kind of cytoplasm to more restricted areas, but also give the egg a distinct bilateral structure.

Monospermic and polyspermic fertilization:

Monospermic:

When only one spermatozoon penetrates into the egg to fertilize it, the type is called Monospermic fertilization, as in most classes of animal kingdom (e.g. coelenterates, echinoderms, bonyfishes, frog and mammals.)

Polyspermic:

It is of two types—(a) Pathological polyspermy. This type occurs in pathological conditions when many spermatozoa penetrate the ova of a normally Monospermic animal.

(b) Physiological polyspermy:

When many spermatozoa penetrate ova as a whole, such kind of fertilization is called polyspermic fertilization. This type of fertilization occurs in animals having yolky eggs like molluscs, selachians, urodeles, reptiles and birds. In such eggs only one spermatozoon participates fully in the development of the embryo, the rest degenerate sooner or later. This type of polyspermy, because it has some physiological significance, is called physiological polyspermy.

Significance of fertilization:

The process of fertilization has following significances:

(1) The fertilization ensures the usual specific diploidy of the organisms by the fusion of the male and female pronuclei.

(2) It introduces genetic variations in the species.

(3) It activates the egg to start cleavage.

Possible questions

1. Discuss on the significance of different stages of gametogenesis.
2. Discuss in brief about fertilization.
3. Write a note on the essence of activation upon fertilization.
4. Discuss about maturation of oocyte and egg envelopes.
5. Explain the changes in egg cytoplasm during fertilization.
6. Write a note on sperm structure and its types.
7. Comment on spermatogenesis in detail.