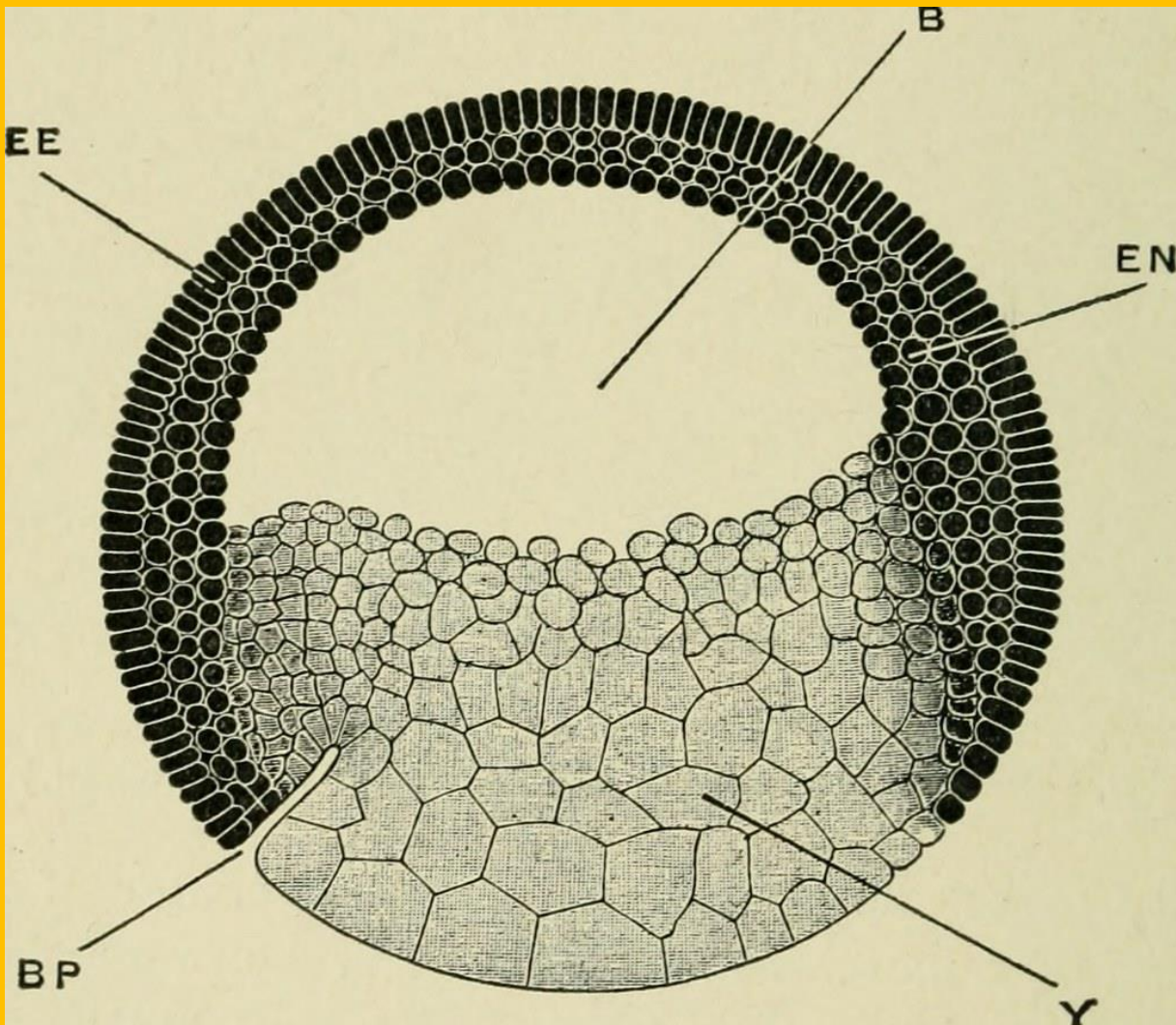**M.Sc. III Semester**

DEVELOPMENTAL BIOLOGY

DEPARTMENT OF ZOOLOGY
SCHOOL OF SCIENCES
UTTARAKHAND OPEN UNIVERSITY

Developmental Biology
(MSCZO-602)

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COURSE II: DEVELOPMENTAL BIOLOGY   **Credit: 3**

Course code: MSCZO- 602

UNIT 1: CONCEPT OF DEVELOPMENTAL BIOLOGY

CONTENTS

1.1 OBJECTIVES

After studying this module, you shall be able to learn and understand about:

i.   Principal features and patterns of development

ii.   Coelom,

iii. Segmentation,

iv.   Somites

v.   Diploblast

vi.   Protostomes and deuterostomes

1.2 INTRODUCTION

From the patterns in development, developmental biology evolved and became one of the old and young disciplines in biology; it originated in the 1950 and formally formed an independent discipline in the 1970.

A new discipline gradually was formed in the process of learning molecular embryology which also comprehensively strengthened and further developed this discipline

Since the 1980s, due to the development of disciplines such as genetics, cell biology, and molecular biology, a large number of new research methods were also applied, and developmental biology made great progress.

The research content of this subject includes the occurrence and formation of gametes, fertilization process, cell differentiation, and morphogenesis. It also includes how different cell groups in the development process are reconfigured and specialized.

The emergence of various cell types, the appearance of the final organ phenotypic characteristics, the establishment of special functions, the expression, control, and regulation of genes at different developmental stages, the causal relationship between genotype and phenotypic expression, the relationship between nucleus and cytoplasm during development, interrelationships between cells, and the effects of external factors on embryonic development were predominantly seen. Among them, cell differentiation became a core problem in the process of developmental biology.

1.3 HISTORY OF DEVELOPMENT

It originated in the 1950 and formally formed an independent discipline in the 1970. A new discipline gradually formed in the process of learning molecular embryology which is also a comprehensive and further developed discipline.

The multidisciplinary approach to the study of development first arose before the turn of the Twentieth Century as integration of embryology (initially the descriptive study of embryonic development) with cytology (the study of cellular structure and function) and later with genetics (the study of inheritance).

The leading cytologists of that time (primarily E.B. Wilson at Columbia University in New York City) recognized that the development of the embryo is a manifestation of changes in individual cells and that an understanding of the fundamental principles of development would come from studying cellular structure and function.

Wilson recognized that the characteristics of an organism gradually emerge by utilization of the inherited information that is located on the chromosomes. Therefore, it was important to comprehend the nature of that information and how it is utilized during development. However, in the absence of concrete evidence, there was a great deal of rampant speculation as to how the chromosomes participate in development. The German embryologist Wilhelm Roux was the source of much of this speculation.

Roux believed that the fertilized egg receives substances that represent different characteristics of the organism, which - as cell division occurs - become linearly aligned on the chromosomes and are subsequently distributed unequally to daughter cells.

This "qualitative division" fixes the fate of the cells and their descendants because some of the determinants are lost to a cell at each division.

Roux (1888) appeared to have confirmed his theories through an experiment he conducted on frog eggs.

Another German embryologist, Hans Driesch (1892), approached the problem differently with sea urchin embryos. Instead of destroying one of the cells of the two-celled embryo, he separated the cells from one another and found that isolated cells at the four-cell stage also develop normally. Thus, Driesch concluded that each cell retains all the developmental potential of the zygote.

The conflict between these two opposing views of development has been settled in favor of Driesch's interpretation by numerous cell separation experiments.

The Role of the Hereditary Material in Development

Although the equal distribution of hereditary information to all cells had been established in the late 1800s, its role in development remained an enigma. Two key contributions at the dawn of the Twentieth Century provided the impetus for additional progress:

• In 1900, the significance of Gregor Mendel's work on heredity was finally appreciated.

• The other contribution was made by Theodor Boveri, who in a paper published in 1902 demonstrated that normal development is dependent upon the normal combination of chromosomes. Each chromosome must have qualitatively unique effects on development.

Developmental biology is one of the important basic branches of biological sciences. The research content is infiltrated by many other disciplines, especially genetics, cell biology, and molecular biology.

It uses modern science and technology to study and analyze the processes and mechanisms of organisms from spermatogenesis and egg development, fertilization, growth, aging, and death from the molecular level, sub-microscopic level, and cellular level.

Although there are many species of animals, the development of embryos still has a similar pr ocess, which can be divided into stages of fertilization, cleavage, morula, blastocyst, gastrula, and organ formation.

In addition, during the embryonic development of vertebrates, the characteristics common to various animals will appear first (such as the skin), and then specialized structures (such as fish scales) will be developed.

In general, the ectoderm forms the epidermis and nerve tissue. The endoderm forms the intestinal epithelium and the digestive gland epithelium, which forms bone, muscle, blood, lymph, and other connective tissues. Others are derived from the mesoderm. But there are exceptions: the sphincter of the eye iridescence does not come from the mesoderm, nor from the mesenchyme, but from a part of the retina, that is, from the ectoderm.

The smooth muscle of the sweat gland is not from the mesoderm, but from the ectoderm; the mesenchyme itself is unclear, as it may come from the ectoderm or the mesoderm, or even from the endoderm. Research on developmental biology needs further advancement, which will help to understand the developmental mechanisms of organisms.
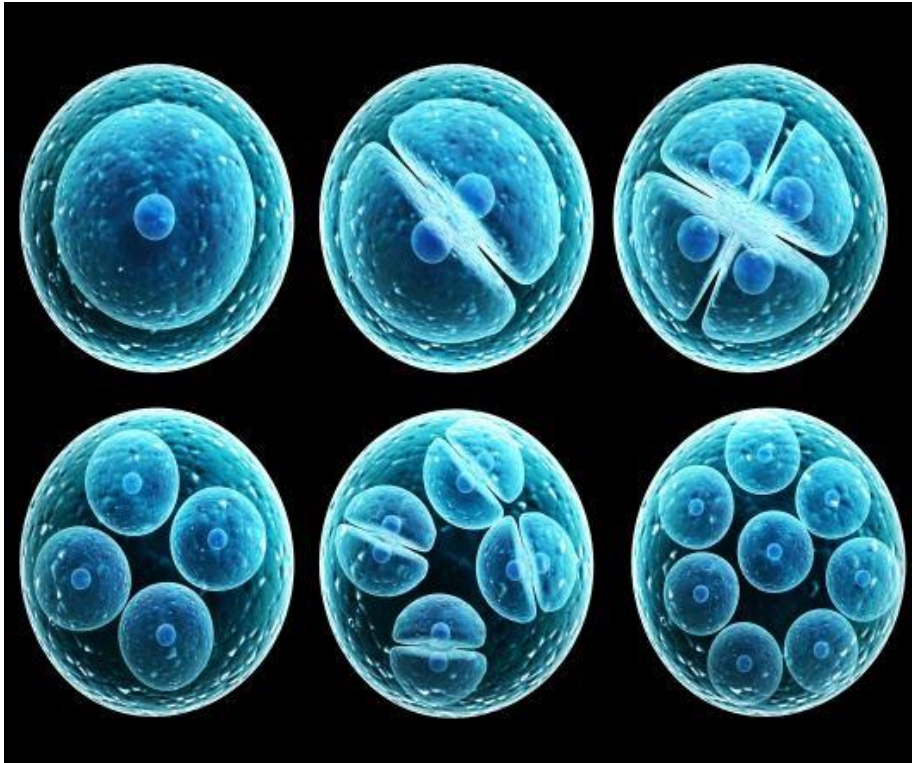
Fig. 1.1: Developmental stages

1.4 PRINCIPLE FEATURES AND PATTERNS OF DEVELOPMENT

Developmental biology is the study of the process by which animals and plants grow and develop. Developmental biology also encompasses the biology of regeneration, asexual reproduction, metamorphosis, and the growth and differentiation of stem cells in the adult organism.

Developmental biology includes the production of gametes, fertilization, and development of the embryo, emergence of the adult organism, senescence, and death. Developmental biologists in the department attempt to understand the molecular, genetic, cellular, and integrative aspects of building an organism.

The main processes involved in the embryonic development of animals are tissue patterning (via regional specification and patterned cell differentiation); tissue growth; and tissue morphogenesis.

The regional specification refers to the processes that create a spatial pattern in a ball or sheet of initially similar cells. This generally involves the action of cytoplasmic determinants, located within parts of the fertilized egg, and of inductive signals emitted from signaling centers in the embryo.

The early stages of the regional specification do not generate functional differentiated cells, but cell populations committed to developing to a specific region or part of the organism. These are defined by the expression of specific combinations of transcription factors.

Cell differentiation relates specifically to the formation of functional cell types such as nerve, muscle, secretory epithelia, etc. Differentiated cells contain large amounts of specific proteins associated with cell function.

Morphogenesis relates to the formation of three-dimensional shapes. It mainly involves the orchestrated movements of cell sheets and individual cells. Morphogenesis is important for creating the three germ layers of the early embryo (ectoderm, mesoderm, and endoderm) and for building up complex structures during organ development.

Tissue growth involves both an overall increase in tissue size, and also the differential growth of parts (allometry) which contributes to morphogenesis. Growth mostly occurs through cell proliferation but also through changes in cell size or the deposition of extracellular materials.

The development of plants involves similar processes to that of animals. However, plant cells are mostly immotile so morphogenesis is achieved by differential growth, without cell movements. Also, the inductive signals and the genes involved are different from those that control animal development.

1.4.1 COELOM

All animal has a cavity. Different animals use these cavities for different purposes. A vast fluid-filled area between the body wall and the internal organs is typically referred to as a body cavity. The alimentary canal is located between the body wall and the coelom, a perivisceral cavity. The coelom develops during embryonic development as a split in the mesoderm, which divides into two layers: a somatic layer that surrounds the endoderm and a splanchnic layer that lies close to the epidermis.

The coelomic epithelium, which secretes coelomic fluid, surrounds the coelom. On one end, the excretory organs open into the coelom, and on the other, they open to the outside. Coelomoducts, which transport sperms or eggs from the coelom to the outside, are produced by the coelom wall, which also gives rise to reproductive cells. The per visceral cavity, also known as the splanchnocoel, is formed by the majority of the coelom and houses the visceral organs. The gonocoel and nephrocoel, whose coelomic character can only be appreciated if their developmental histories are followed, are examples of confined cavities that are formed when specific areas of the perivisceral cavity are cut off from it. The first animals with a real coelom are annelids.

Coeloms evolved from acoelomates, then pseudocoelomates, and finally coelomates. The absence or presence of peritoneum or epithelial lining distinguishes a pseudocoelomate from a coelomate animal. It exists in coelomate animals but not in pseudocoelomate ones. A real coelom might have different embryological origins. It is referred to as schizocoelous if it arises from a split in mesoderm cells. It is referred to as enterocoelous if it arises from out pocketing from the embryonic gut.

According to the mode of coelom formation, there are generally two types of animals:

(A) Schizocoelomate: When coelom arises by the splitting of mesodermal bands or masses during embryonic development, it is called schizocoel, and animals are called schizocoelomates (Fig. 1.2). The animals belonging to the phylum Mollusca, Annelida, Arthropoda, and Onychophora are schizocoelomates.
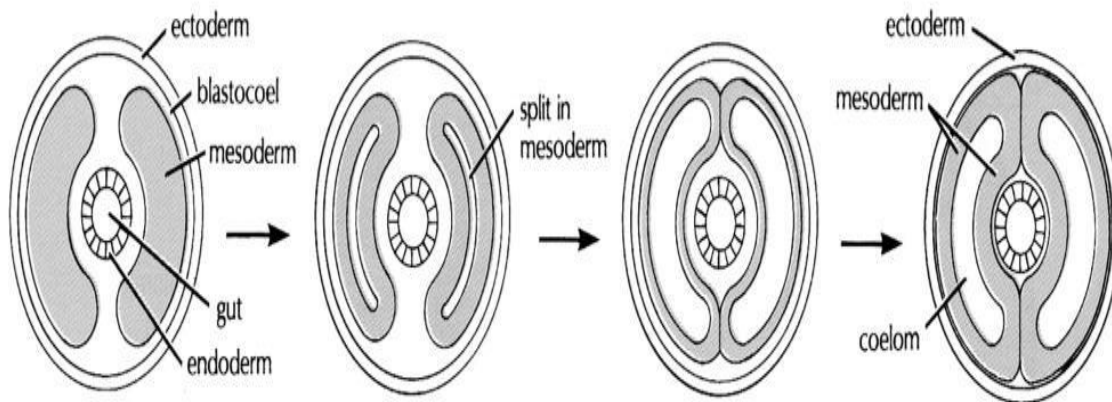
Fig.1.2 Coelomformation by the splitting of mesoderm

(B) Enterocoelomate: When coelom is formed by the evagination from the embryonic archenteron and the pouch-like structures are detached from the archenteron and gradually occupy the whole body by enlargement, called enterocoel (Fig. 1.2). The animals having enterocoel are called enterocoelomate. The animals belonging to phylum Echinochordata, Hemichordata, and Chordata are enterocoelomates.
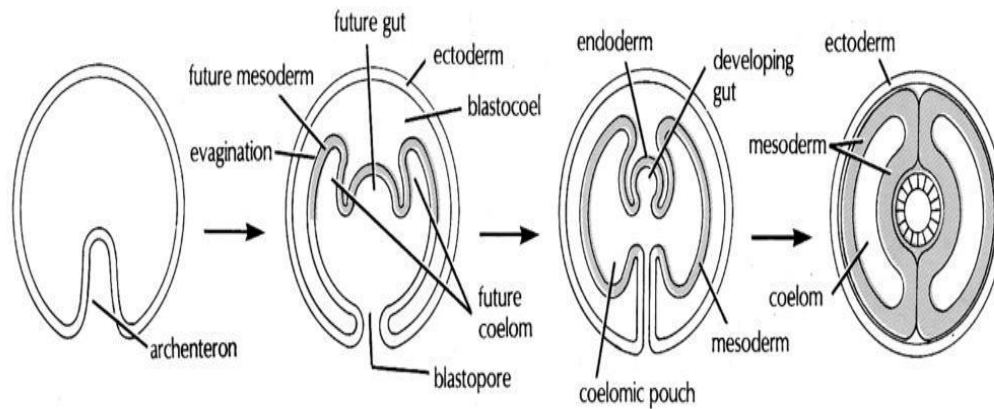
Fig 1.3 Coelom formation by out pocketing of the primitive gut

1.4.2 SEGMENTATION

Segmentation is a difficult process to satisfactorily define. Many taxa (for example the mollusks) have some form of serial repetition in their units but are not conventionally thought of as segmented. Segmented animals are those considered to have organs that were repeated, or to have a body composed of self-similar units, but usually, it is the parts of an organism that are referred to as being segmented.

Segmentation in animals typically falls into three types, characteristic of different arthropods, vertebrates, and annelids. Arthropods such as the fruit fly form segments from a field of equivalent cells based on transcription factor gradients. Vertebrates like the zebra fish use oscillating gene expression to define segments known as somites. Annelids such as the leech use smaller blast cells budded off from large teloblast cells to define segments.

Arthropods: Although *Drosophila* segmentation is not representative of the arthropod phylum in general, it is the most highly studied. Early screens to identify genes involved in cuticle development led to the discovery of a class of genes that were necessary for proper segmentation of the *Drosophila* embryo.

To properly segment the *Drosophila* embryo, the anterior-posterior axis is defined by maternally supplied transcripts giving rise to gradients of these proteins. This gradient then defines the expression pattern for gap genes, which set up the boundaries between the different segments. The gradients produced from gap gene expression then define the expression pattern for the pair-rule genes. The pair-rule genes are mostly transcription factors, expressed in regular stripes down the

length of the embryo. These transcription factors then regulate the expression of segment polarity genes, which define the polarity of each segment. Boundaries and identities of each segment are later defined.

Within the arthropods, the body wall, nervous system, kidneys, muscles, and body cavity are segmented, as are the appendages (when they are present). Some of these elements (e.g. musculature) are not segmented in their sister taxon, the Onychophora.

1.4.3 SOMITES

The somites (outdated term: primitive segments) are a set of bilaterally paired blocks of paraxial mesoderm that form in the embryonic stage of somitogenesis, along the head-to-tail axis in segmented animals. In vertebrates, somites subdivide into the sclerotomes, myotomes, syndetomes, and dermatomes that give rise to the vertebrae of the vertebral column, rib cage, part of the occipital bone, skeletal muscle, cartilage, tendons, and skin (of the back).

The word *somite* is sometimes also used in place of the word *metamere*. In this definition, the somite is a homologously-paired structure in an animal body plan, such as is visible in annelids and arthropods.

The mesoderm forms at the same time as the other two germ layers, the ectoderm and endoderm. The mesoderm at either side of the neural tube is called the paraxial mesoderm. It is distinct from the mesoderm underneath the neural tube which is called the chordamesoderm that becomes the notochord. The paraxial mesoderm is initially called the "segmental plate" in the chick embryo or the "unregimented mesoderm" in other vertebrates. As the primitive streak regresses and neural folds gather (to eventually become the neural tube), the paraxial mesoderm separates into blocks called somites.

Formation: The pre-somitic mesoderm commits to the somitic fate before the mesoderm becomes capable of forming somites. The cells within each somite are specified based on their location within the somite. Additionally, they retain the ability to become any kind of somite-derived structure until relatively late in the process of somitogenesis.

The development of the somites depends on a clock mechanism as described by the clock and wavefront model. In one description of the model, oscillating Notch and Wnt signals provide the clock. The wave is a gradient of the FGF protein that is rostral to caudal (nose to tail gradient). Somites form one after the other down the length of the embryo from the head to the tail, with each new somite forming on the caudal (tail) side of the previous one.

The timing of the interval is not universal. Different species have different interval timing. In the chick, embryo somites are formed every 90 minutes. In the mouse, the interval is 2 hours.

For some species, the number of somites may be used to determine the stage of embryonic development more reliably than the number of hours post-fertilization because the rate of development can be affected by temperature or other environmental factors. The somites appear on both sides of the neural tube simultaneously. Experimental manipulation of the developing somites will not alter the rostral/caudal orientation of the somites, as the cell fates have been determined before somitogenesis. Somite formation can be induced by *Noggin*-secreting cells. The number of somites is species-dependent and independent of embryo size (for example, if modified via surgery or genetic engineering). Chicken embryos have 50 somites; mice have 65, while snakes have 500.

As cells within the paraxial mesoderm begin to come together, they are termed somitomeres, indicating a lack of complete separation between segments. The outer cells undergo a mesenchymal-epithelial transition to form an epithelium around each somite. The inner cells remain as mesenchyme.

1.4.4 DIPLOBLAST

Diploblasty is a condition of the blastula in which there are two primary germ layers:

the ectoderm and endoderm. Diploblastic organisms are organisms that develop from such a

blastula and include cnidaria and ctenophore, formerly grouped in the phylum Coelenterate, but

later understanding of their differences resulted in their being placed in separate phyla. The

endoderm allows them to develop true tissue. This includes tissue associated with the gut and

associated glands. The ectoderm, on the other hand, gives rise to the epidermis, the nervous tissue,

and if present, nephridia.

Simpler animals, such as sea sponges, have one germ layer and lack true tissue organization.

All the more complex animals (from flatworms to humans) are triploblastic with three germ layers

(a mesoderm as well as ectoderm and endoderm). The mesoderm allows them to develop

true organs. Groups of diploblastic animals alive today include jellyfish, corals, sea anemones, and

comb jellies.

Features of the diploblastic animal

    a) They consist of jelly-like noncellular mesenchyma or coagulated mesoglea in the middle among ectoderm and endoderm.

    b) They show radial symmetry, biradial, or rotational symmetry.

    c) A lesser degree of specialization.

d) No proper transport system.

e) Absence of coelom.

f) Sac-like digestive system and gastrovascular cavity.

g) Diploblastic creatures might have cell types that serve different capabilities, for example, epitheliomuscular cells, which act as a covering as well as contractile cells.

h) The endoderm of diploblastic animals has true tissues and intestines. A non-living layer named mesoglea is present between the ectoderm and endoderm.

i) Mesoglea helps in protecting the gut lining and body.

j) These animals do not develop organs.

   Examples: Phylum Porifera and Cnidaria.

1.4.5 PROTOSTOMES AND DEUTEROSTOMES

The creation of the mouth first or the anus can be used to separate the bilateral metazoans into two major assemblages. Protostomes are metazoans in which the animal's anus and mouth are formed largely by the blastopore, and Deutrostomes in which the animal's anus and mouth are formed mostly by the blastopore. The next subsections will provide your study material on these two groupings.

Protostomia: The metazoans in which the mouth is derived from blastopore on the anterior end and anus that appears later to complete the alimentary canal are included in Protostomia. As the mouth forms first, their animals are included in the 'Protostomia" (Mouth first) division of the animal kingdom. The nerve cord is ventral in protostomes.
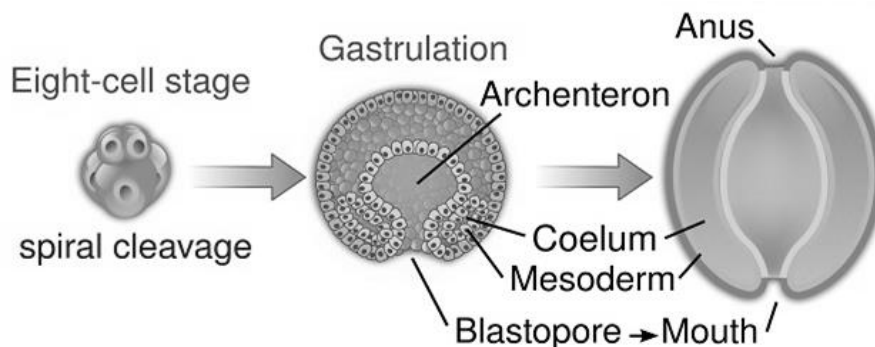
Fig. 1.4 Diagram showing the development of mouth from the blastopore

The developmental characteristics of protostomes are as follows.

1. **The pattern of embryonic cleavage:** Cleavage is spiral in protostomes, i.e., the axis of the cleavage plane is oblique, so blastomeres have a spiral arrangement in which one tier of cells alternates with the next tier of cells. The spiral cleavage is masked at the 6th cleavage 64-cell stage.

2. **The fate of embryonic blastomeres:** The fate of blastomeres is determined very early during holoblastic cleavage. This is called determinate or mosaic cleavage, which means blastomeres are destined to form a particular organ in the very early stage of cleavage. In Figure 1.4 just after the first cleavage ablation of one of the cells takes place it leads to the loss of head structure in the embryo that drives from it. Such a type of development is said to be mosaic.

3. **Fate of blastopore:** The blastopore either becomes mouth (e.g., Mollusca) or gives rise to both mouth and anus (e.g., some mollusks, polychaetes, and onychophorans) in adults.

4. **Formation of mesoderm:** Mesoderm originates from the fourth cell, named mesentoblast (also called as '4d' cell) which increases in number by proliferation.

5. **Formation of coelom:** Coelom originates from the splitting of the mesodermal cell mass. This process of coelom formation is known as schizocoely and coelom are called schizocoelom ('schizo' means split). **Examples:** Coelomate protostomes include Sipuncula, Echiura, Annelida, Pogonophora, Mollusca, Onychophora, Tardigrada, Pentastomida, and some groups of arthropods.

Deuterostomia: The metazoans in which anal opening are derived from blastopore during embryonic development and represents the posterior end of the body and mouth are formed later are included in deuterostomia. As the anus forms first and the mouth is formed secondarily, these animals are grouped in deuterostomia (Mouth second). The nerve cord is dorsal in deuterostomes.
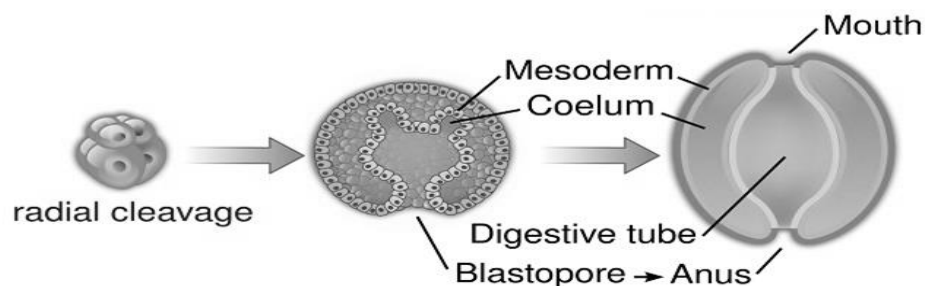

Fig. 1.5 Diagram showing the development of the anus from the blastopore

The developmental characteristics of deuterostomes are as follows.

1. Pattern of embryonic cleavage: The radial pattern of embryonic cleavage occurs in which the cleavage plane is either parallel or at a right angle to the polar axis. Blastomeres are arranged directly above or below one another.

2. Fate of embryonic blastomeres:

Cleavage is indeterminate and if blastomeres are separated at 4 cell stages, each one will develop into a complete individual. Cleavage is regulative because each of the blastomeres if separated can regulate its development (Fig 1.6). In figure 1.6, if ablation of one cell takes place, then the descendants of the remaining cell can give rise to the structure in the embryo that would have developed from the lost cell. In this case, the green cell can regenerate the head structure as well as the trunk region. Such development is said to be regulative.

3. Fate of blastopore: Blastopore becomes the adult anus and then the formation of the mouth takes place from a second opening on the dorsal surface of the embryo.

4. Formation of mesoderm: Mesodermal tissue is formed by the outgrowth of the endodermal wall of the archenteron.

5. Formation of coelom: Coelom is formed by evagination of pouches from the wall of the archenteron and each diverticulum becomes separated from the archenteron and develops an independent coelomic pouch. This process of formation of the coelom is called enterocoely and the coelom is called enterocoelom.

Examples: Deuterostomes include Echinoderms, Chordates, Pogonophores, Hemichordates, and some minor phyla.

1.5 TERMINAL QUESTIONS AND ANSWERS

Q.1 Explain in detail about Coelom.

Q.2 what do you understand by Segmentation? Explain in detail.

Q.3 Write a short note on Somites.

Q.5 Define the Diploblastic Animal.

Q.6.Explain in detail the Protostomes and Deuterostomes ?

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UNIT 2: GAMETE AND FERTILIZATION

CONTENTS

2.1 OBJECTIVES

The study of this unit will let the students understand the following:

- The structure of Gametes

- Difference between egg and sperm

- The concept of Fertilization.

- Preparation of cell for the fertilization

- Significance of fertilization.

- Elucidate the type of Fertilization.

- Elucidate the various types of organization in insects.

- Various factors involved in Fertilization.

- The Bio-Chemistry involved during fertilization.

- Difference between prefertilization and postfertilization events.

2.2 INTRODUCTION

A cell is the fundamental Unit of Life. Every Living Being is a combination of several Cells combined as tissue, muscles, and organs. These cells undergo drastic changes during their entire life, generating, regenerating, dying, etc. Evolotion in the cells apart from the rest of the world was quiet evident and remarkable. This course of evolution leads to certain cells becoming specialized for reproduction that came to be known as Gamete.

Gametes have evolved according to their sex named spermatozoa and Ova for Male and females respectively. These gametes carry a single copy of the genetic material which fuses with themselves (ova and sperm). They have unique structures that evolved to facilitate their fusion.

The entire process of fusing is known as fertilization. This fertilization undergoes two main processes namely pre-fertilization and post-fertilization. Fertilization had a huge significance in maintaining diploidy in the race and genetic variation. The fertilization is subjected to a lot of factors that influence its timing, structure, etc. A set of events happens before fertilization and a lot of changes happen in the cytoplasm of the egg after the sperm penetrates its membrane. The process of fertilization has been studied extensively in marine invertebrates like sea urchins, echinoids, amphibians, mammals, and vertebrates also.

2.3 ULTRA STRUCTURE OF GAMETE

Gamete means the reproductive cell or sex cells. These gametes are Female gametes and male gametes. Female gametes and Male gametes are known as ova or egg cells and sperm respectively. All Gamete cells are haploid cells which mean that they carry only one copy of a chromosome. The Male and Female gamete fuse them to form a new cell known as a zygote. Sperm cells or spermatozoa are small and motile whereas egg cell or ovum is relatively large and non-motile. Ova mature in the ovaries of females and sperm in the testes of males. Spermatozoon and ovum fuse during fertilization to form a new diploid organism. Gametes carry half the genetic information of an individual, one ploidy of each type. They are created through the process of meiosis. A germ cell undergoes two fissions, resulting in the production of four gametes.

2.3.1 SPERMS

Sperms are male gametes developed in the testis through a process of spermatogenesis. Spermatogenesis forms spermatids. These spermatids produced a specialized structure called sperm.

2.3.1.1 STRUCTURE OF SPERM

The sperm's body is covered by the plasma membrane. Their body is divided into head, neck, Middle-piece, and tail.

The Head contains the nucleus which has the genetic code, which is the main contribution to the new offspring. Heads have the acrosome which contains digestive enzymes needed to penetrate the ovum.

Mid-piece is posterior to the head. It contains mitochondria to provide energy to the sperm. These mitochondria convert fructose and other energy substrates into high-energy compounds.

The tail joins the head at the proximal centriole and is called the implantation region. The head and tail get separated at this point during fertilization.

A tail is a flagellum that causes the sperm to swim. A major feature of the tail is the axial filament. The axial filament starts at the proximal centriole and runs through the entire body of the tail and is made up of a small bundle of tiny fibrils.
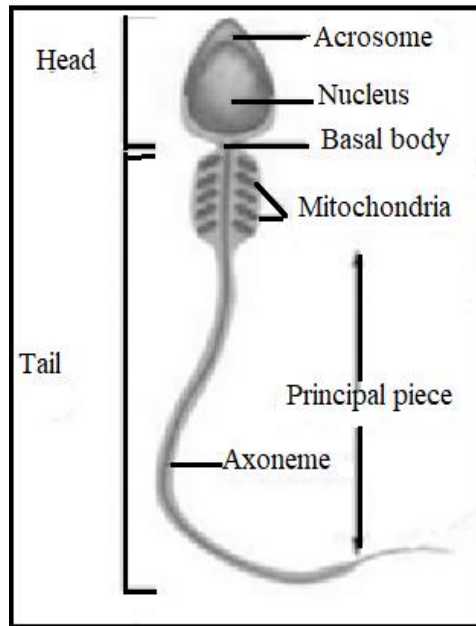
Fig 2.1 Diagram of Sperm

2.3.2 EGG

The egg is the Female gametes developed in the ovary through a process of oogenesis. Eggs are single cells that are capable to produce a new organism when fused with sperms. These Eggs are released from an ovary and move to the uterus waiting for the sperm to fuse and produce a new organism.
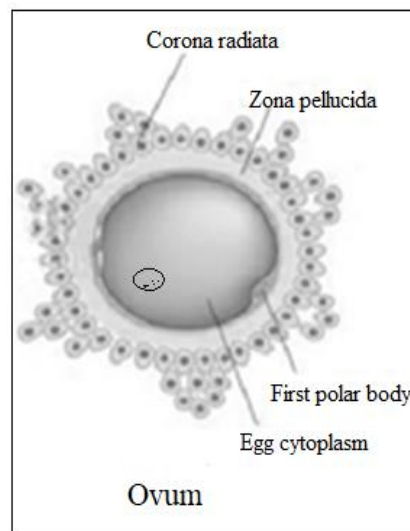
Fig 2.2 Diagram of Egg

In shape, the eggs are spherical or oval and are non–motile. Their sizes vary in different animals with the smallest size found in mammals and the largest in birds. These cell sizes are greater than other body cells. Inside the Egg spherical boundary, a large amount of cytoplasm exists which is called ooplasm, a big nucleus exists within the cytoplasm known as a germinal vesicle. The side of the egg containing the nucleus is called the animal pole and another side is known as the vegetal pole. The nucleus is surrounded by a plasma membrane known as oolemma. This oolemma leads to the rise of microvilli which is responsible for absorbing the food material to facilitate the growth of the cell. Three egg membranes namely outer corona radiata, middle zona pellucida, and inner plasma membrane cover the cell. Follicle cell forms corona radiata and is the outer layer of the cell-attached with zona pellucida. Zona pellucida forms the vitelline membrane of the cell that surrounds the egg. This membrane is thick and transparent. The plasma membrane is the innermost layer. There is some space between zona pellucida and plasma membrane known as perivitelline space.

2.4 MECHANISM OF FERTILIZATION

Fertilization involves the fusion of two haploid cells namely sperm and ova to produce a new diploid organism.

Types of Fertilization: There are two different types of fertilization:

1. External Fertilization: The fusion happens outside the body. It occurs in the environment. Various physical conditions like temperature, water, soil, humidity, etc., affect the fertilization process.

2. Internal Fertilization: The fusion happens inside the female body. This fertilization is free from changes in physical conditions like temperature, water, soil, humidity, etc. of the environment.

FACTORS INVOLVED IN FERTILIZATION:

1. Chemotaxis: Some animal eggs attract the sperms by the release of certain chemicals. These chemicals are found in the chorion lining the micropyle. Once the chorion is removed the activities of sperm are ceased. This is found in coelenterates, fishes, insects, etc.

2. Life Span of Gametes: In External Fertilization life span is short whereas in internal Fertilization is long which can vary from 17 hrs in Rats to 4 years in Turtles inside the female genital tract.

3. Production of an enormous number of sperms: A large number of sperms are produced to fertilize the lesser number of the egg.

4. Mechanical Juxtaposition of Gametes: This refers to the mechanical position by which sperm can reach the ovum. It is different from animal to animal like *Sepia* uses an arm to transfer their sperm, copulation in mammals, etc.

5. Synchrony in the Production and Release of Gametes: The synchronization of male and female maturity and their release to facilitate fertilization.

The fertilazation process involves two different phases namely Pre-Fertilization and Post Fertilization

Step 1: Pre –Fertilization: This refers to the process which occurs before the fusion of gametes.

Step 2: Post- Fertilization: This refers to the process after the fusion of gametes Fusion of male and female gametes produces a new organism in the fallopian tube and its attachment to the uterus of the female body.
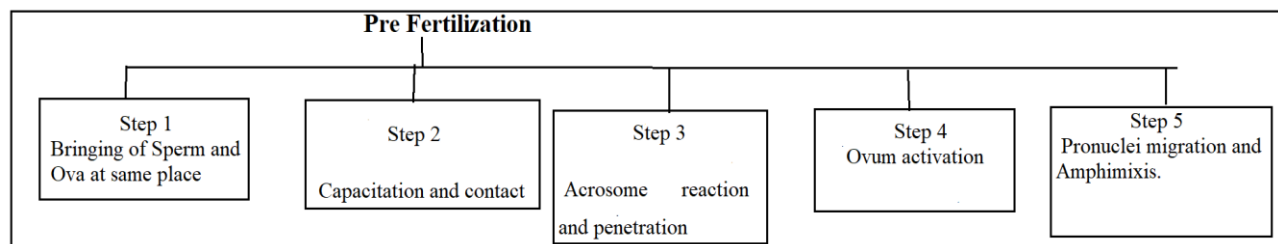
Fig 2.3 Steps of Pre- Fertilization

2.4.1 PRE- FERTILIZATION

Pre Fertilization comprises the five stages:

STEP 1: Bringing of sperm and ova to the same place: Most Invertebrates and vertebrates maintain a close distance between spermatozoa and ovum by the act of some behavior. Spermatozoa and ovum need to be in a liquid medium at the same time for fertilization. There are two types of fertilization based on nature and place. In External Fertilization, eggs are released on a liquid medium followed by the release of sperm near them. In Internal Fertilization they are brought together by an act of copulation. In some animals especially where the fertilization is external Sperm are attracted to eggs by the release of chemicals called Chemotaxis. These Chemotaxes are released by the egg. These chemicals are present in the chorion lining the micropyle.

STEP 2: Capacitation and contact: Capacitation refers to the act where the sperm fertilizes the egg of the same species. There exists a set of chemicals i.e fertilizins and antifertilizins which

ensure that fertilization will occur between the gametes of the same species. These chemicals also ensure that only one sperm can fertilize the egg.
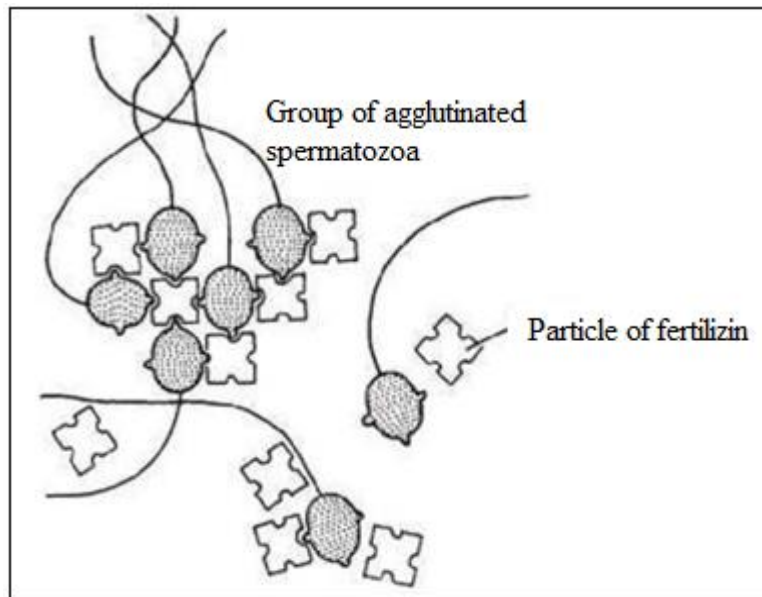
Fig. 2.4: Mechanism of Fertilizin and Anti fertilizin Reaction

Fertillizin – antifertilizin reaction: F.R. Lillie postulated the Fertilizin and Anti Fertilizin Theory. This reaction between Fertilizin and Anti Fertilizin ensures the sperm and ova of a certain species can only mate. Fertilizin is the chemical found on the surface of the egg. These Fertilizin molecules have many receptors or binding sites to bind with the sperm. These receptors are species-specific to fuse with the sperms of the same species. These glycoproteins molecules are embedded with the jelly coat or plasma membrane of the ovum.

Anti-Fertilizin is the chemical found on the surface of sperm. They are acid proteins with a smaller molecular weight than fertilizin. This anti-fertilizin is also species-specific. The reaction between Fertilizin and Anti Fertilizin is quite analogous to the lock and key mechanism of enzymes.

Mechanisms of Fertilizin and Anti fertilizin Reaction:

1. Sperms identify the eggs and react by identifying with Fertilizin molecules.
2. The first attachment between egg and sperm is due to the link between fertilizin and anti-fertilizin particles.
3. Eggs are released in a liquid medium. Due to the liquid medium, few molecules of fertilizin combine with the medium. These start to attract all the sperms currently in the same medium.

4. This aggregation of sperms leads to agglutination. Only a few sperms reach the surface of the egg. This mechanism helps to reduce polyspermy.

5. This action ensures that fertilization will be done within species.

6. Fertilizin activates the sperm to initiate the acrosome reaction to dissolve the egg membrane.

STEP 3: Acrosome reaction and penetration: Eggs (Ovum) are covered with one or more layers of membrane or gelatinous or follicle cell to prevent the egg from getting fertilized except in Porifera and Coelenterates. Spermatozoa need to break these layers to make the process of fertilization. Spermatozoa get attached to these layers and become motionless. The acrosome found in the head of the sperm performs physiochemical activity to break these layers. Acrosome releases a certain enzymatic protein called sperm lysins. In mammals corona radiata acts as a barrier that prevents spermatozoa to reach the ovum. To facilitate the sperm acrosome has an enzyme called hyaluronidase which aids in dissolving the adhesive layer and cell disbursement. The acrosome undergoes morphological change and transforms into a filament to help the release of sperm inside the egg.
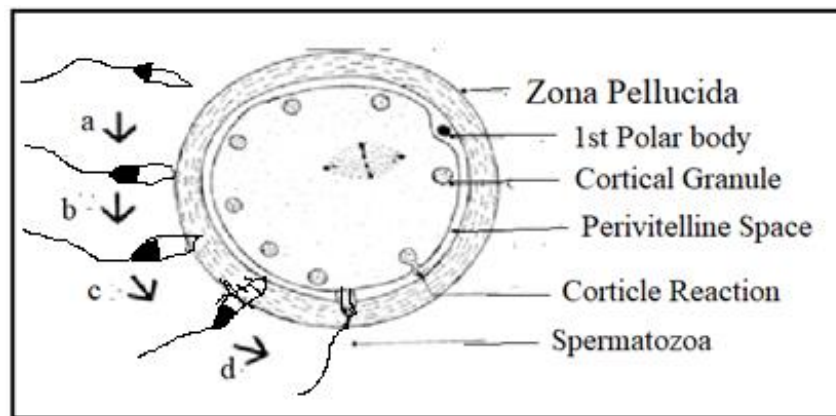
Fig. 2.5: Stages of the sperm-egg association during fertilization

Acrosome reaction and penetration in *Saccoglossus*: Colwin and Colwin, 1967 described the acrosome reaction and penetration in *Saccoglossus*. In *Saccoglosus* spermatozoon have a spherical nucleus, a tail, and an acrosomal vesicle at the front. These acrosomal vesicles are surrounded by acrosomal granules except at the apex. Apex has a space filled with a material called peri acrosomal material.

The following events occur when sperm comes in contact with an egg. They are

1. **Bursting of acrosome:** Acrosomal apex bursts and expose acrosomal vesicles.

2. **Lytic enzymes are released:** Acrosomal vesicles touch the egg layer and release Lytic enzymes to make passage through the layer.

3. **Acrosomal tubules are formed:** Acrosomal membrane starts to move towards the nucleus and form a long slender tubule.

4. **Fusion of acrosomal tubule with egg membrane:** Acrosomal tubule enters the egg after Lytic enzyme has made a passage through the membrane.

Sperm Contents are passed: Acrosomal tubules dissolve and the other material of the sperm gets mixed with the egg cytoplasm.

1. The nucleus of the spermatozoon moves towards the fertilization cone.

This process remains the same for other animals. It is the number and size of acrosomal tubules which vary from species to species.

STEP 4: Ovum activation: This process starts when the sperm touches the surface of the egg plasma membrane facilitated by acrosome filament. The fusion of the two eggs takes place and a single mosaic membrane is formed. Thus plasma membrane of both cell become a single cell called zygotes. A lot of changes occur in the cytoplasm of the egg. These changes have been summed up in the following steps:

1. Fertilization Cone Formation.

2. Formation of membrane and cortical reaction.

1. Fertilization cone formation: The acrosomal filament of spermatozoa touches the egg membrane, and the cytoplasm of the egg moves forward at the point of contact and forms a structure that appears like a simple conical protrusion. The cytoplasm started to engulf the spermatozoa. Spermatozoon doesn't enter the egg cytoplasm intact instead they are spread over. The Sperm nucleus and other sperm structures move towards the fertilization cone. The plasma membrane of spermatozoa mixes with the egg plasma membrane to form a single entity. The acrosomal granule stays outside the egg membrane; only peri acrosomal material enters the egg cytoplasm.

Some variation exists in how the spermatozoon is taken into the interior of the egg. In mammals, the entire spermatozoon penetrates the egg cytoplasm. In *Nereis* only the head and proximal centriole enter the egg cytoplasm. However, as a golden rule in the majority of animals, the sperm nucleus and mid-piece enter the egg.
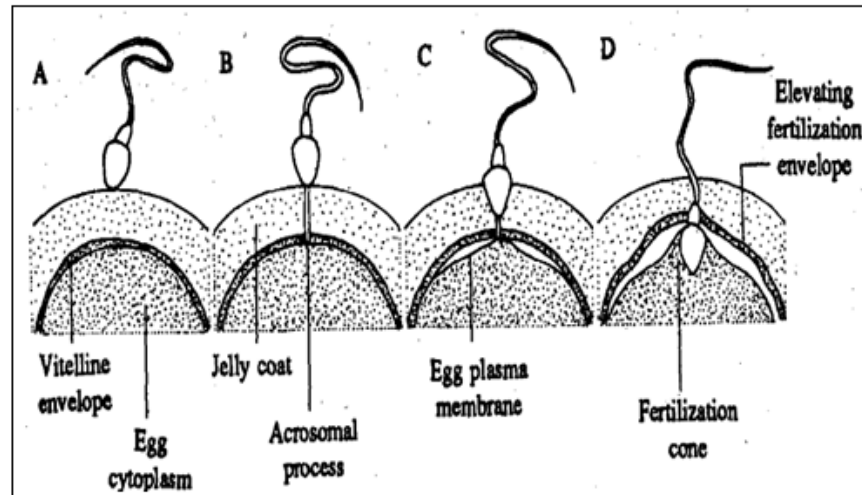
Fig. 2.6: Fertilization cone formation

2. Formation of membrane and cortical reaction: A set of physical-chemical reactions occur inside the egg before the spermatozoa start to penetrate them. The set of physical-chemical reactions is known as a cortical reaction. This cortical reaction role is concerned with the formation of membrane outside the egg plasma with the sole objective to prevent the arrival of late spermatozoa inside the egg.

The process of membrane formation varies from animal to animal. A glimpse of cortical reactions and membrane formation in some animals has been discussed.

Sea Urchins: Upon touching of acrosomal tubule with egg surface, color changes from yellow to white (under dark field microscopy), which travels rapidly around egg cortex. It is followed by the formation of a fertilization cone and membrane around the egg plasma membrane. Sea Urchin unfertilized egg has egg cortex bound by two membranes namely vitelline and inner thick plasma membrane. A layer is formed below the plasma membrane. The fertilization membrane is formed in the following way:

Outer vitelline membrane is separated and undergoes an expansion to become the outer layer. During expansion cortical granules explode and release three components namely Dark, denser, lamellar, and folded parts, Globules, and the liquid component.

All the three component form different structures namely

1. Dark, denser, lamellar, and folded part: They fuse with the inner side of the vitelline membrane.

2. Globules: They form the surface of the hyaline layer. This hyaline help to keep the blastomeres together.

3. Liquid component: They fill the perivitelline space between the new egg surfaces. It contains mucopolysaccharides and water.

All three components together form a fertilization membrane (Slow block to polyspermy)

Vertebrates: Upon touching of acrosomal tubule with the egg surface, Lytic digestive enzymes are released by the acrosomal tubule to penetrate the egg membrane. The cortical granules are broken into egg cytoplasm and their content becomes liquefied and comes on the surface and fills the perivitelline space between the chorion and plasma membrane. In fishes chorion becomes hardened. In mammals, cortical granules fill the space between egg plasmalemma and the zona pellucida.

An animal that lacks cortical granules such as urodele amphibians, some lower order mammals. In those animals neither a membrane is formed nor does neither any cortical reaction take place.

Theory of Activation: Several Theories have been proposed to illustrate how sperm stimulates the eggs. They are

1. **Theory of Boveri:** Boveri proposed that the mature egg has no active division center. This division center is transferred into the egg by the sperm to initiate the process of cell division.

2. **Theory of Loeb:** Loeb, 1913 proposes two principles namely the lytic principle and cytolysis principle. The lytic principle states the factors which increase the oxidation process and the cytolysis principle deals with controlling the increased oxidation rate.

3. **Theory of Bataillon:** Bataillon proposed that the secretion of a substance in the perivitelline membrane and elevation of the fertilization membrane activate the egg to start fertilization.

4. **Viscosity Theory:** Heilbrunn postulated that calcium is released during fertilization which increases the viscosity of egg cytoplasm. This increased calcium concentration starts the development process.

5. **Deinhibition Theory:** Runnstrom and Brachet observe that metabolic inhibitors accumulate in the oocyte during the maturation process. These inhibitors are removed and eggs are deinhibited.

Step 5: Pronuclei migration and amphimixis: The sperm nucleus remains compact with mitochondria and centrioles behind it after penetrating the membrane of the egg. The sperm nucleus has to perform two functions to be able to perform amphimixis.

1: Becomes pronuclei.

2: Migrate from the site of amphimixis.

1: Becomes Pronuclei: After the penetration of the egg by spermatozoon, the nucleus starts to move inwards from the site of cone formation. The nucleus rotates by 180° to enable mitochondria and centrioles to assume the first position. The sperm nucleus starts to swell. The chromatin material inside the cell starts to become granular. It starts to look more like an interphase nucleus called a male pronucleus.

2: Migrate from the site of amphimixis: Sperm centriole is surrounded by sperm aster in the egg cytoplasm. Sperm aster starts to lead the nucleus to the site of amphimixis (fusion of male and female gametes).

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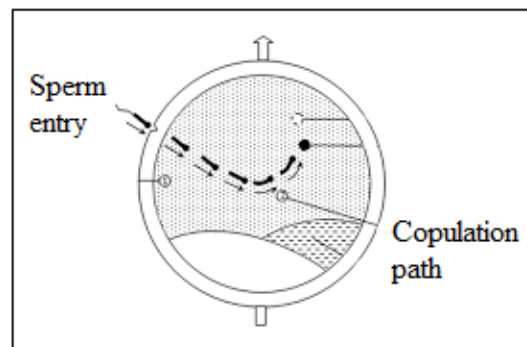
Fig. 2.7: Copulation Path

The egg nucleus also undergoes some changes. The Haploids nucleus of the egg fuses into one another and forms a female pronucleus. They also swell and become vesicular. The female pronucleus also starts to move towards the site of amphimixis.

The site of fusion lies near the center of the microlecithal and telolecithal egg or at the center of the active cytoplasm. The path that which male pronucleus follows depends upon the effect of the chemical released by the female pronucleus. The normal path followed by the male pronucleus is known as the penetration path and a new path subjected to some changes is known as the copulation path

2.4.2 POST FERTILIZATION

Spermatozoa penetration of the egg results in a significant change in the position of cytoplasmic constituents. Several new areas emerge within the egg. Cortical granules extrusion results in replacing the outer egg cell surface with the inner surface with everted on the exterior.

The most significant change in the cytoplasm has been observed in ascidian and frog. A bilateral symmetry has been established in the cytoplasm of both animals in the fertilized egg. A *Zygote* is formed after the rearrangement of the cytoplasm. Displacement has been quite remarkable in the ascidian, frog, and Styela partita. This need arise to rearrange the nucleus and genetic material equally.

Displacement of cytoplasmic material in ascidian *Styela partita*:

The mature egg of *Styela partita* is covered with a layer of cortical cytoplasm which contains yellow granules. As the spermatozoon enters the egg, the cytoplasm starts a violent commotion. The cytoplasm starts to move towards the vegetal pole and arrange itself into the shape of a cap. As sperm move towards the egg nucleus, the cytoplasm moves upward towards the direction from where spermatozoa entered. This start to enter four different regions of the cytoplasm. They are

1. Yellow cytoplasm on one side.
2. Light color cytoplasm on the other side.
3. Slaty grey color cytoplasm containing yolk granules and mitochondria.
4. Clear and transparent Cytoplasm

This displacement separates the cytoplasm and arranges them to start the process of cleavage.
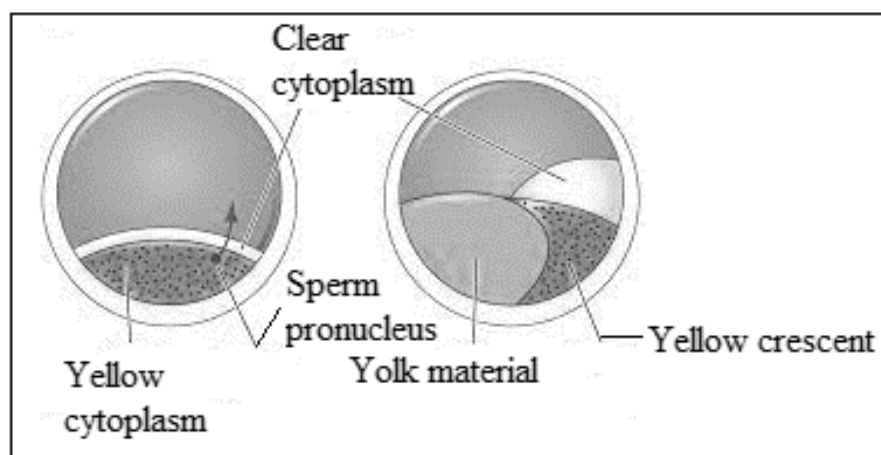
Fig. 2.8: Displacement of cytoplasmic material in ascidian Styela partita

Fertilization ensures that the cycle of cleavage can start, which will become the first step of embryo development. This is followed by morulation, Blastulation, Gastrulation and finally, a new multicellular organism is brought into the world.

2.4.3 BIOCHEMISTRY OF FERTILIZATION

Metabolic activation: A series of cytoplasmic reactions take place after the sperm penetrates the unfertilized egg. The steps involved are:

a. Changes in plasma membrane

b. Ionic changes

c. Changes in the rate of respiration

d. Co-enzyme changes

e. Rate of protein synthesis

f. Mitosis initiation

g. Breakdown of polysaccharides

h. Increase in hexose phosphate

i. Dehydrogenase

a. Changes in the plasma membrane: The sperm penetration has increased the movement of water and other chemicals like ethylene, glycol, phosphate, K^+, etc. This led to the increase of electrical potential in the plasma membrane. It became more positive, but slowly turns negative due to unequal distribution of chloride ions.

Adenyl cyclase, an enzyme of plasma membrane gets activated at the time of fertilization and initiates the formation of 3' – 5' cyclicAMP molecule. This molecule is responsible for activating metabolic reactions in a fertilized egg.

b. Ionic changes: A substantial level of changes in the intracellular composition of the fertilized egg takes place at the ionic level. This is especially in sodium, potassium, and calcium. This change in ion concentration had a great influence on the metabolism activation of the fertilized egg.

c. Changes in the rate of respiration: The rate of respiration varies from animal to animal. In some animals where fertilization is completed (Sea Urchin), it increases whereas in others it either decreases (Molluscs) or remains stable (*Bufo*).

The increased rate is associated with the increased oxygen demand for oxidation of glycogen and the release of numerous ATP molecules.

d. Co-enzyme changes: Spermatozoa contain an acrosome that has a lot of oxidative enzymes. These enzymes come inside the egg after fertilization and ensure an increase in oxidation. This increase in oxidation is to provide energy for the development of egg and supplement other changes necessary for it.

The following process takes place:

$$NAD + ATP \xrightarrow{NADKinase} NADP + ADP$$

e. Rate of protein synthesis: The cytoplasm of an unfertilized egg contains a lot of protein synthesis material like DNA molecules, tRNA, mRNA, ribosomes, and proteolytic enzymes. During fertilization, proteolytic activity increase to remove the inhibitor protein and mRNA is unmasked to start the process of active protein synthesis.

f. Mitosis initiation: The process of active protein synthesis increases the rate of DNA synthesis. The sperm initiate the first mitotic division (Cleavage) by contributing centriole to the egg. Though centriole is there in the unfertilized egg, they are incapable of performing mitotic division. This sperm centriole becomes the basics of the new organism gender as half of DNA material is contributed by sperm and another half by egg.

g. Breakdown of polysaccharides: An increase in lactic acid concentration takes place on account of the rapid breakdown of polysaccharides.

h. Increase in hexose phosphate: This chemical increases considerably after fertilization.

i. Dehydrogenase: The activity of the enzyme performing dehydrogenase increase significantly after fertilization.

SIGNIFICANCE OF FERTILIZATION:

1. Maintain diploidy in the race.

2. Bring genetic variation.

3. Egg is activated to start the process of Cleavage.

2.5 SUMMARY

Sexual reproduction has evolved to improve the chance of an individual or species. This reproduction has evolved by the fusing of male and female gametes. These gametes have evolved with the climatic situations, adaptation, and their habitat and habitat. The male gamete called spermatozoa has evolved as a structure where it can carry the genetic material, move through the liquid medium, have sufficient resources to penetrate the ovum, and the mechanism to provide energy while traversing to fertilize the egg. To facilitate them it has a head (for genetic material),

neck (to join the tail), Middle piece (to provide energy), and tail (traversing through liquid medium to meet ovum).

Ovum develops as a non-motile, spherical, or oval shape and it is larger than the sperms. A large amount of cytoplasm provides the energy required to fertilize an egg for its developmental journey to an embryo. A mechanism of protection where only one sperm of the same species can penetrate the egg and fertilize it. It also has villi for absorbing the food material to facilitate the growth of the cell.

Fertilization has evolved to maintain genetic variation and diploidy in the race. There are two types of fertilization: external fertilization and internal fertilization. A significant set of factor influence fertilization like life span of gametes, and the production of an enormous number of sperm. mechanical juxtaposition of gametes, their synchrony in production and release of gametes, and the mechanism by which sperm will fertilize the egg.

The process of fertilization has two different phases namely prefertilization and post fertilization. Prefertilization refers to the process which occurs before the fusion of gametes. This prefertilization is further made up of 5 steps namely: Bringing sperm and ova to the same place, Capacitation and contact, Acrosome Reaction, Ovum Activation, and Pronuclei migration and Amphimixis. Post Fertilization refers to the process which occurs after fertilization has been done. A lot of metabolic activities take place after the sperm penetrates the unfertilized egg. They involve changes in the plasma membrane and ionic changes. Changes in the rate of respiration, Co-enzyme changes, Rate of protein synthesis, and Mitosis initiation. Thus these activities lead to further development of cleavage, morulation, and gastrulation. These steps finally form a multicellular animal.

2.6 TERMINAL QUESTIONS AND ANSWER

2.6.1 MULTIPLE CHOICE QUESTIONS:

1. Which of the following animal has the longest duration of sperm survival in the female genital tract?

a. Rat

b. Garter Snake

c. Guppy

d. Turtle

2. Which of the following animal has the shortest duration of sperm survival in the female genital tract?

a. Rat

b. Hen

c. Rabbit

d. Bat

3. In Mammals _____ fertilization occurs.

a. Monospermic

b. Polyspermic

c. Depends On Species

d. Depends on climatic situation

4. Pre-Fertilization is made up _____ steps

a. 2

b. 3

c. 5

d. 4

5. A set of chemicals which are released by an egg to attract the opposite sex gametes

a. Fertilizins

b. Anti-Fertilizins

c. Glycoprotein

d. Sperm lysins

6. A set of chemicals that are released by sperm during fertilization.

a. Fertilizins

b. Anti- Fertilizins

c. Glycoprotein

d. Sperm lysins

7. A mechanism of protection where only one sperm of the same species can penetrate the egg and fertilize it.

a. Fertilization

b. Cortical reaction

c. Capacitation

d. Sperm lysins

8. Ova mature in the _____ of females.

a. Fallopian Tube

b. Uterus

c. Ovary

d. None of these

2.6.2 VERY SHORT QUESTION

1. Discuss the structure of sperm.

2. What is the difference between external and internal fertilization.

3. How fertilization helps in maintaining genetic variation and diploidy in the race.

4. Explain the basic structure of the ovum.

5. Illustrate the role of acrosomes in sperm.

6. Explain the process of formation of membrane and cortical reaction in fertilization.

7. Why a change in the rate of respiration occurs during fertilization?

8. Name the steps which the sperm nucleus has to perform to be able to perform Amphimixis.

9. How prefertilization is different from postfertilization.

10. Discuss fertilizin and anti fertilizin reactions mechanisms.

2.6.3 LONG QUESTIONS:

1. Give a details description of any four metabolic changes in the ovum followed by fertilization.

2. Explain the influence of physical factors like climate, temperature, humidity, etc. on fertilization.

3. What type of metabolic changes happens during fertilization?

4. Explain the significance of the mechanical juxtaposition of gametes during the process of fertilization.

5. Explain the process which occurs before fertilization.

6. Discuss the various type of fertilization.

7. Discuss the process of ovum activation during fertilization.

8. Explain the significance of capacitation in fertilization.

9. Explain how sperm structure has evolved to facilitate fertilization.

10. Explain the various metabolic activities that occurred during fertilization.

11. Explain the various physical, chemical, and cytological factors involved in fertilization.

Answers: 2.6.1

1(d) 2(c) 3(a) 4(c) 5(a) 6(b) 7(c) 8(c)

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2.8 GLOSSARY

Acrosomes: A part of a spermatozoon that is capable of releasing enzymes to break the ovum membrane.

Anti Fertilizin: A protein released by sperm to help them identify and attach with the same species of an egg.

Capacitation: The mechanism by which the sperm will fertilize the egg.

Corona radiate: The area of follicular cells surrounding the ovum.

Egg: The cell released by the ovary of the female which is capable to produce new organisms after fusing with the Male sex gametes.

Fertilization: The process in which two sex gametes namely sperms and ovum fuse together to form a new organism.

Fertilizin: A protein released by the ovum to stimulate the sperm to move towards them.

Microlecithal Egg; Egg that has little or no yolk.

Oxidation: The process by which energy-giving molecules or matter are oxidized to produce energy.

Proteolytic enzyme: Enzyme that is capable of producing or releasing protein.

Sperm: The Male sex gametes

Telolecithal Egg: Egg that has a significant amount of yolk which remain concentrates at one pole.

UNIT 3: CLEAVAGE, BLASTULATION AND GASTRULATION

CONTENTS

3.1 OBJECTIVES

After studying this module, you shall be able to learn and understand:

- Patterns of cleavage
- Understand how yolk influences cleavage
- Understand the significance of blastulation
- Elucidate the morulation and blastulation in frog, chick, and rabbit
- Classification of blastula
- Major events of gastrulation
- The significance of fate maps.
- Understand the morphogenetic movements in frogs, chick, and rabbits
- Understand the significance of gastrulation and exogastrulation
- Understand the different movements occurring during gastrulation.

3.2 INTRODUCTION

All sexually multicellular organism bodies have developed from a single cell formed after the fusion of male (spermatozoa) and female gametes (ovum). The process of further cell division starts called Cleavage just after fertilization. A series of mitotic cell divisions divide the single cell into 16-cell called mesomeres. A total of 4 mitotic divisions are followed which converts the single cell into 2, 4, 8, and 16 mesomeres. When the stages of 16 cells are attained they get transformed into a multicellular structure called morula. This division is further transformed into blastula by successive cell division. This blastula has a single layer of the blastoderm. No growth of ovum during blastula formation. The general shape doesn't change for the embryo except for a formation of a cavity called the blastocoel. The chemical change of glycogen and yolk into molecules of nuclear material like DNA, RNA, and nucleoproteins take place. The sudden cell division has increased the number of the nucleus which leads to the unfavorable ratio between the nucleus to the cytoplasm. This is brought to normal by the end of blastulation. Blastulation is followed by Gastrulation where the single layer of blastoderm is transformed into two and finally three germ layers namely ectoderm, mesoderm, and endoderm. The cell moves from one part to another and rearranges itself to start the process of development of several parts and organs of a multicellular

organism. Fate Map has been designed to understand which part of the blastula transformed itself into which part of a multicellular animal.

3.3 PATTERN OF CLEAVAGE

CLEVAGE: Cleavage is a process where a series of mitotic divisions happens after egg fertilization. The zygote undergoes a rapid succession so that the unicellular body will be transformed into a multicellular organism. The mitotic division led to numerous smaller nucleated cells called blastomeres which finally results in a hollow spherical body called a blastula.

CHARACTERISTICS:

1. Thousands of cells called blastomeres are formed.

2. Cleavage produces a hollow sphere of cells called a blastula.

3. Cleavage forms the cell to create tissue and organs.

4. Embryo shape and size don't change during cleavage.

5. Cleavage brings a proposition between nuclear and cytoplasmic material.

6. Conversion of yolk, and glycogen into the cytoplasm, then into a molecule of a nuclear substance.

PRINCIPLE OF CLEAVAGE: All cleavages follow common basic principles or laws.

They are as follows:

A. Sach's law–Sach in 1877 proposes two rules.

Rule 1: Cleavage divisions occur uniformly subjected to the uniform yolk stored in them. It will not be uniform if the yolk is unevenly stored in them.

Rule 2: Successive Division is at a right angle to its previous division.

B. Hertwig law -O. Hertwig in 1881 proposed 2 rules:

Rule 1: Nucleus and mitotic spindle is found at the center of blastomeres.

Rule 2: Spindle fibrosis is the longest axis of the egg.

C. Pfluger'sLaw - Eduard Friedrich Wilhelm Pflüger state that the formation of spindle fibers takes place in the region of less yolk.

D. Balfour's Law–Balfour in 1885 stated that the rate of cleavage is inversely proportional to the amount of yolk.
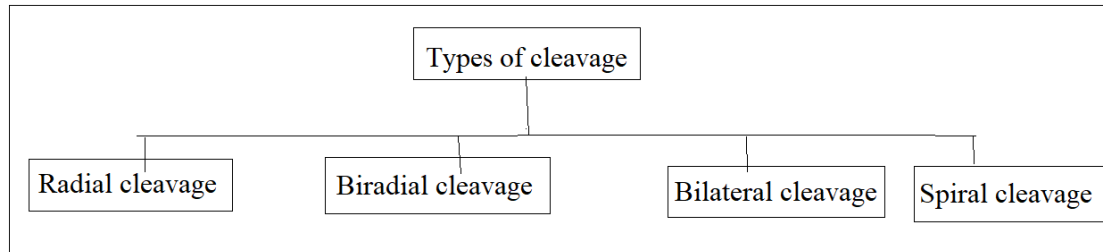
Fig 3.1: Types of Cleavage

Cleavages are of different types due to changes in egg organization. They are of the following types namely-

Radial Cleavage: Cleavage divides the Zygote in radial symmetry. Their cleavage divisions are at a right angle to their previous division. The successive divisions of the cell are placed just above the previous blastomeres. Thus, the new four blastomeres are arranged just above their four previous blastomeres. Thus, partitioning the blastula along any plane produce two identical halve. Example Echinodermata, Chordata, Frog.
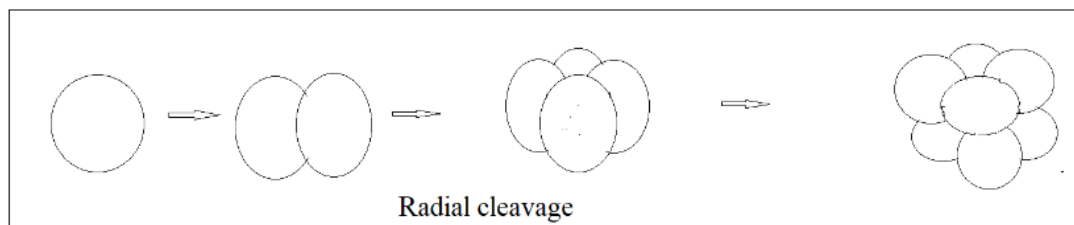
Fig 3.2: Radial Cleavage

Biradial Cleavage: Cleavage has two different patterns in the mitotic division. The   First two mitotic divisions are meridional and the third division is vertical. Thus the 8 blastomeres formed don't stand at a right angle to each other. Example: Polychaeta, Ctenophora.
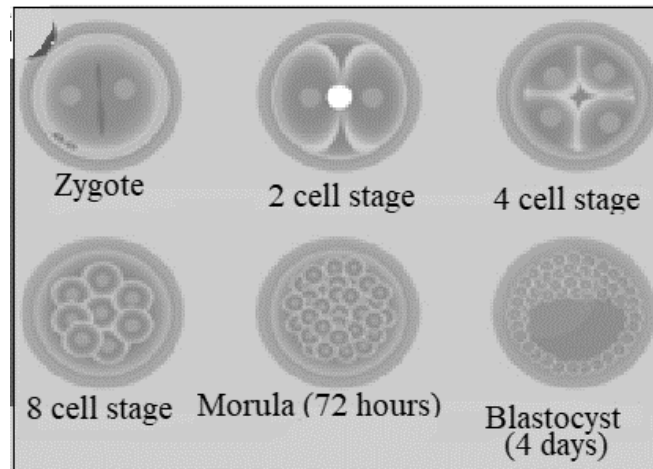
Fig 3.3: Biradial Cleavage

Bilateral Cleavage: This cleavage has two identical halves when the blastula is cut vertically. It can be right or left. This cleavage occurs due to unequal holoblastic (produce blastomere of unequal size) cleavage. The plane of bilateral symmetry is established by the plane of the first cleavage furrow. Example: Higher Mammals, ambiphians, tunicates.

Fig 3.4: Bilateral Cleavage

Spiral Cleavage: This cleavage has a rotational movement of cell parts around the north or south pole axis of the egg. This led to the inclination of the mitotic spindle concerning symmetry radii. Thus, each division produces one bigger cell (macromere) and a smaller cell (micromere). The following cleavage produces the increase in inclination which results in an arrangement in a spiral shape. If the rotation is in a clockwise direction then it is called a dextral or right-handedCleavage, else if in an anti-clockwise direction then it is called a sinistral or left-handed Cleavage. Examples: Nematoda, Rotifers, Annelids, Mollusca, annelids.
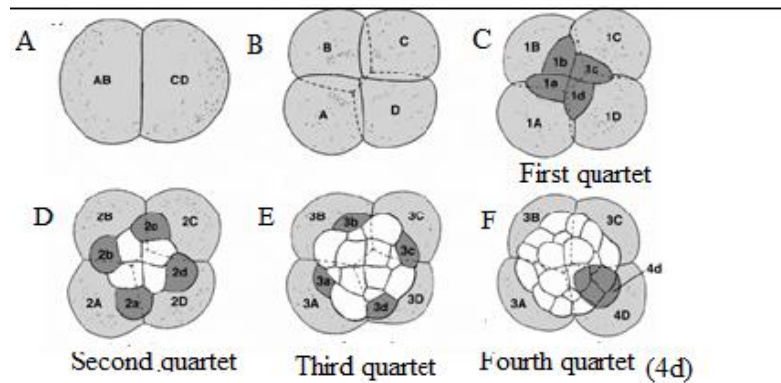
Fig 3.5: Spiral Cleavage

3.4 DETERMINATE AND INDERTERMINATE CLEAVAGE

Cleavages are also been classified according to the potential of the blastomeres to develop for future development. They are classified as determinate and indeterminate cleavage.

Determinate Cleavage: In this cleavage, the area has been marked for the development of a region from different parts of the egg quite early before the onset of Cleavage. In the ascidian egg, a region marked for the development of endoderm was removed. It resulted in the absence of endoderm when the embryo was formed later. Examples: Nematodes, Annelids, Mollusks, and Ascidian.

Indeterminate Cleavage: In this cleavage, there is no area marked for the development of a region. This Cleavage is quite flexible. A region generally used for the development of endoderm was removed from fertilized eggs of sea urchins. Yet the endoderm was developed in the final embryo. This cleavage simply cut the eggs into segments with each segment having the potential to develop any region. Example: All Vertebrates and some species of Echinoderms.

3.5 INFLUENCE OF YOLK ON CLEAVAGE

Cleavage has also been divided on the basis ofYolk and its distribution in an egg:
The following patterns have been observed:
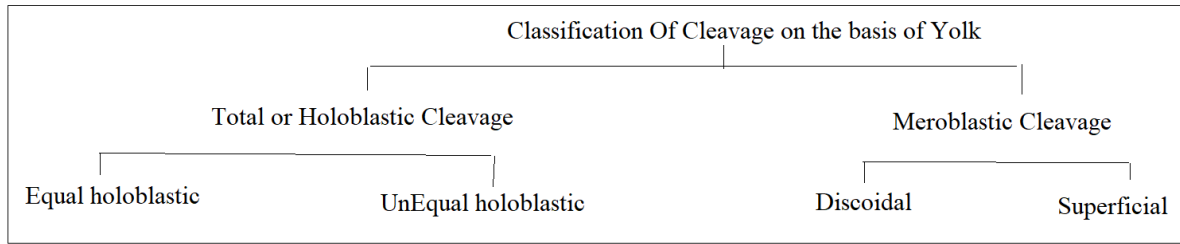
Fig 3.6: Classification of Cleavage based on Yolk

1. Total or Holoblastic Cleavage
2. Meroblastic Cleavage

1. Total or Holoblastic Cleavage: The entire cell is divided by each furrow.
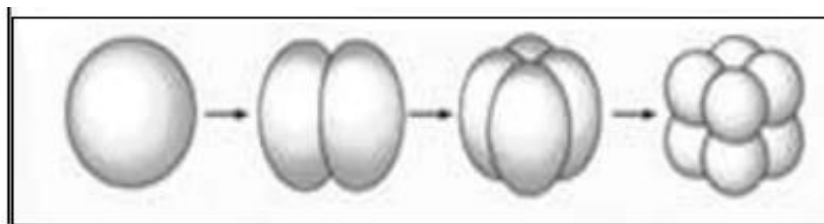
Fig 3.7 Total or Holoblastic Cleavage

This has been further divided into:

Equal holoblastic: This Cleavage produces blastomeres of equal size in any symmetry. This symmetry can be radial, biradial, spiral, and bilateral. This Cleavage is found in microlecithal and isolecithal eggs. Examples: Amphioxus, Marsupials, and placental mammals.
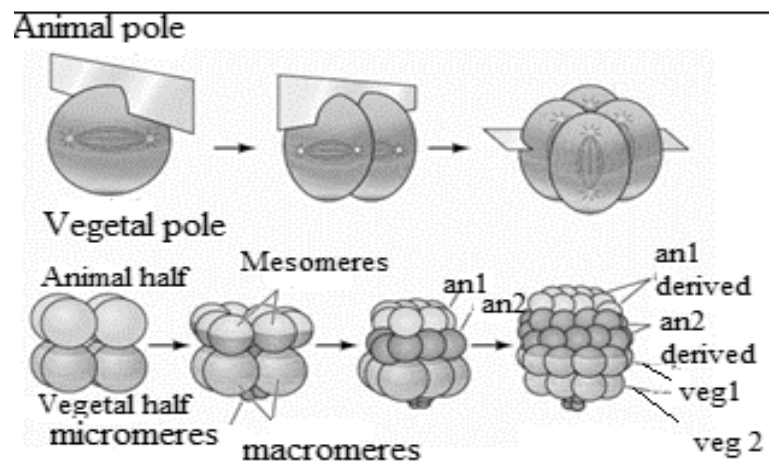
Fig 3.8 Equal holoblastic

Unequal holoblastic: This Cleavage produces blastomeres of unequal size in any symmetry. This symmetry can be radial, biradial, spiral, and bilateral. They produce small-size blastomeres called micromeres and large-size blastomeres called macromeres. This Cleavage is found in mesolecithal and moderately telolecithal eggs. Example: Lower Fish and amphibians.

(1) View from the animal pole at the end of first cleavage. (2) View from the animal pole during metaphase in the AB and CD blastomeres after second cleavage. (3) Animal pole during prophase of a 4-cell embryo (4) Vegetal pole of a 4-cell embryo (5) View of a 4-cell embryo from the 'A' quadrant. (6) View from the vegetal pole of an 8-cell embryo.
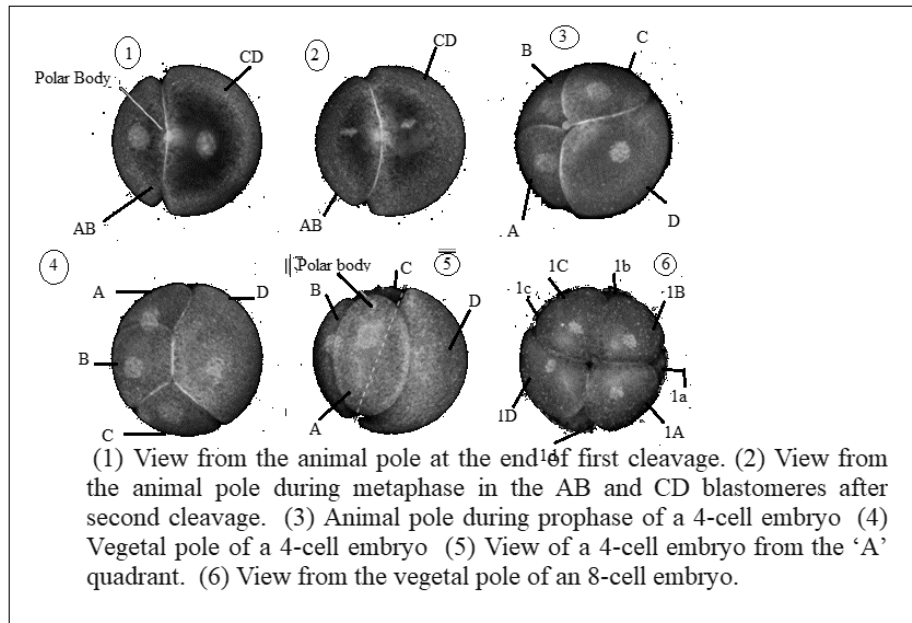
Fig 3.9: Unequal holoblastic

2. **Meroblastic Cleavage:** This cleavage divided the cell partially. This result in the creation of unequal size micromeres. This cleavage is found in those eggs which have a patch of yolk-free cytoplasm called blastodisc. The first two or three cleavage divides the blastodisc vertically, but never reaches the bottom of the blastodisc. The yolk part of the ovum is never cut by furrow.
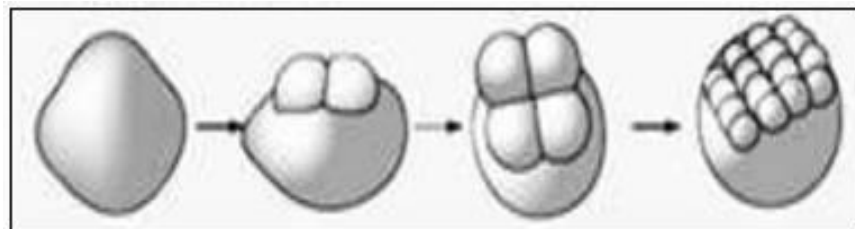
Fig 3.10: Meroblastic cleavage

This has been further divided into:

1. **Discoidal:** This type of Cleavage is found in macrolecithal and telolecithal eggs. The cleavage is restricted to the disc-shaped active cytoplasm of the animal pole. Example: bony fish, reptiles, birds.
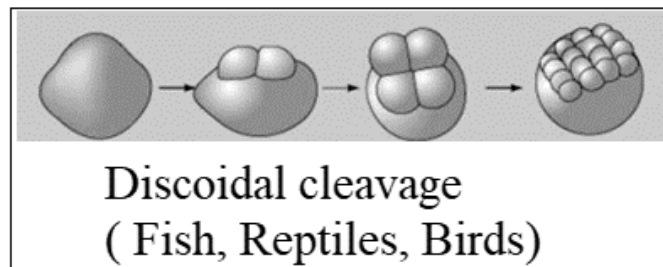
Fig 3.11: Discoidal cleavage

2. **Superficial:** This type of Cleavage is found in a centrolecithal egg. The cleavage is restricted to the peripheral cytoplasm of an egg. The zygote nucleus divides without cytoplasm division. So a large number of nuclei are formed and get embedded in the cytoplasm superficial layer. Example: Arthropoda, and Insects.
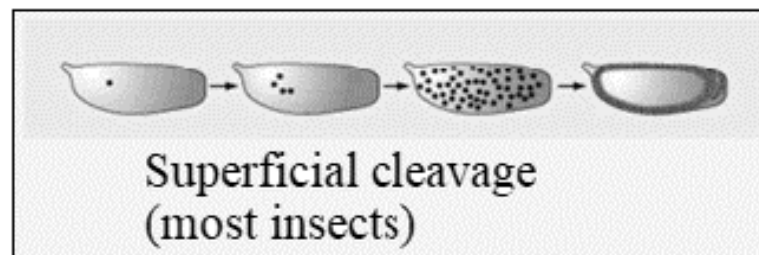
Fig 3.12: Superficial Cleavage

Influence of yolk: The yolk is always an integral part of the egg. Its quantity varies significantly from microlecithal to telolecithal egg. Cleavage occurs in the active cytoplasm. Yolk behaves passively during cleavage. Yolk has a significant influence on cleavage by regulating the process of mitosis by extending beyond the dense area of the egg and moving the germ layer to its final position.

The influence of yolk can be summed by:

1. The yolk is responsible for moving the zygotic nucleus from the geometrical center of the egg to the less yolky cytoplasm. This results in unequal-sized blastomeres.

2. The yolk is responsible to retard the process of mitosis. Thus, its amount and distribution affect the process of cleavage.

3.6 METABOLIC CHANGES DURING CLEAVAGE

Chemical change during cleavage: A significant change occurs during the process of cleavage. They are namely:

1. **Increased Nuclear Material:** A steady increase in the genetic material (predominantly DNA) has been seen. The cytoplasm of the egg contains mitochondria and yolk platelets aid in an increase in nuclear material by acting as a source. A large amount of energy is required to facilitate the movement of genetic material towards the pole. These ATP molecules are made in ooplasm and mitochondria through glycolysis and aerobic oxidation of yolk, glycogen, and other energy-yielding molecules. A continuous supply of deoxyribonucleotides, ribonucleotides, purines, pyrimidines, amino acids, and ribose is needed for the synthesis of DNA and RNA.

2. **RNA Synthesis:** mRNA (messenger RNA) and tRNA (Transport RNA) are synthesized in large numbers during cleavage.

3. **Synthesis of Protein:** There has been a steady increase in protein synthesis during the entire process of cleavage.

3.7 MORULATION AND BLASTULATION IN FROG, CHICK AND RABBIT

Cleavage splits the fertilized egg into smaller cells called blastomeres. These blastomere increases in the typical double sequence of 2, 4, 8, 16 so on. Cleavage forms the layers where each layer is loosely joined together by a stacking gel. This heap of cohering, sticky blastomeres is known as Morula. This has been named as its resemblance to mulberry (Morula means mulberry in Latin). The arrangement of blastomere varies among animals. For example: In a megalecithal egg, a planoconvex-like mass of blastomere is formed. The morula stage is followed by the next phase of development called a blastula. Cleavage led to an increase in the number of blastomeres. This blastomere undergoes a rearrangement which results in arranging themselves into a single cell thick

epithelium called blastoderm. A fluid-filled space or cavity called blastocoel appears between the blastomeres. This hollow, spherical and nonepithelial thick embryonic stage is called a blastula. This process of creating a blastula is known as blastulation.

Morulation in Frog: Cleavage is the early stage of embryogenesis. The cleavage leads the ovum cell partitioning from a single cell to a 32 cell using orders of 2-4-8-16 and finally 32. Cleavage in frogs produces unequal microsomes with a smaller one (micromere) and the larger one (megamere). The cleavage after 32 cells becomes quite difficult to follow. Micromeres divide more rapidly as compared with megamere. This is because micromere has less or lacks yolk. The zygote starts to appear like a mulberry-shaped solid ball of the cell. This mulberry-shaped ball of a cell is known as a morula.
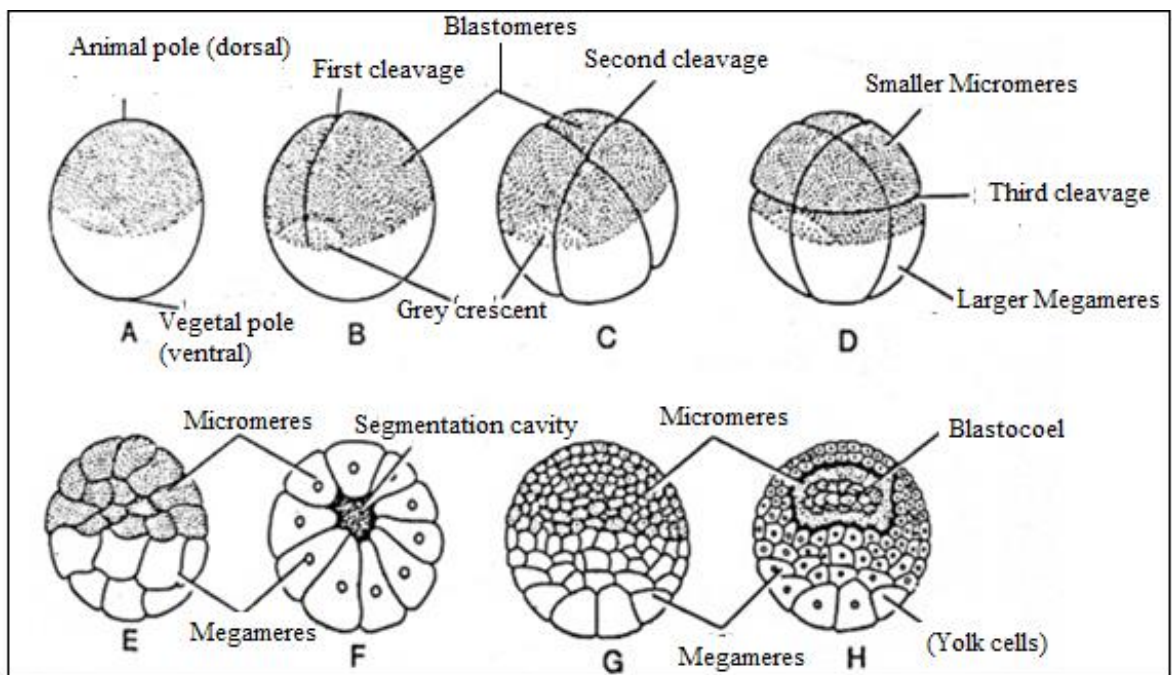
Fig 3.13: Morulation in frog

Morulation in Chick: Cleavage starts to happen after 3 hours of fertilization. In birds due to the availability of enormous yolk, the cleavage cannot happen in one furrow. This led to the rise of small-sized cells called mesomeres. Cleavage in birds is initially restricted to blastodisc with yolk remaining unaffected. The first cleavage is restricted to the area around the center of the blastodisc. It is superficial with no blastomere formed. The second cleavage just happens at the right angle to the first cleavage. 3rd Cleavage is formed just parallel to the first and is vertical. Thus eight blastomeres are formed with no signs of a boundary. 4th Cleavage results in the formation of eight

central blastomeres and eight peripheral blastomeres. It is after the 4th Cleavage a clear demarcation of a cell is seen. The eight central blastomeres get completely separated from the yolk. After the 4th cleavage division becomes irregular. The central blastomeres and peripheral blastomeres start to divide rapidly. The cell of peripheral blastomere is added with central blastomeres resulting in increased volume. These cells start to arrange themselves resulting in the formation of a cavity called the blastocoel. The cleavage in birds is partial, teloblastic, or meroblastic.
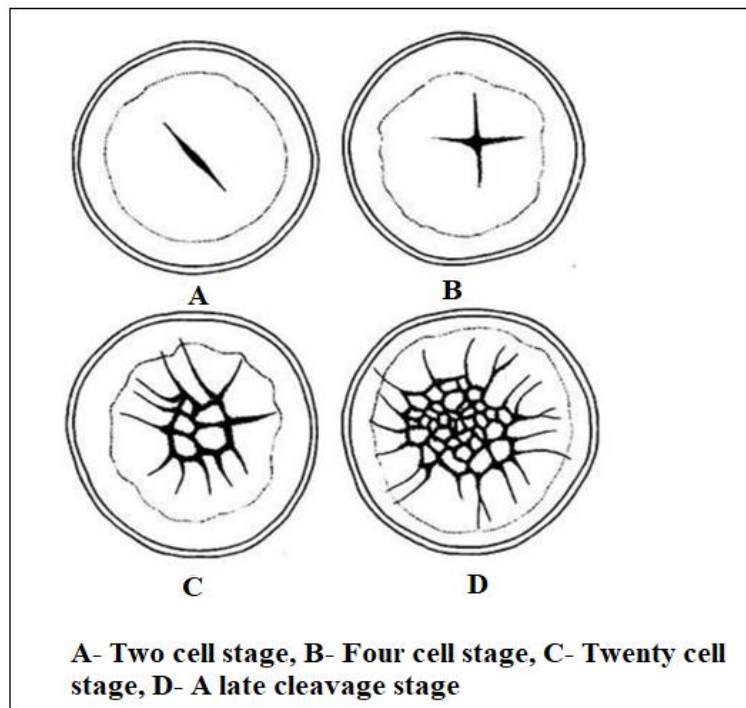
Fig. 3.14: Morulation in chick

Morulation in Rabbit: Cleavage in mammals tends to be holoblastic but unequal. The first cleavage divides the cell vertically into two slightly unequal blastomeres. The second cleavage is at the right angle to the first, dividing vertically resulting in four blastomeres. The third cleavage is also at the right angle to the second with cleavage occurring in four blastomeres separately. Thus, a total of 8 blastomeres are created by the end of the third cleavage. At this rate of division, the 16-cell stage is reached. This 16-cell stage is known as morula. Fully formed morula has an outer or superficial layer of cells called the trophoderm or trophoblast. This morula stage passes through the oviduct to enter the uterus. Later this morula makes an attachment with the mother uterus and absorbs liquid fluid.
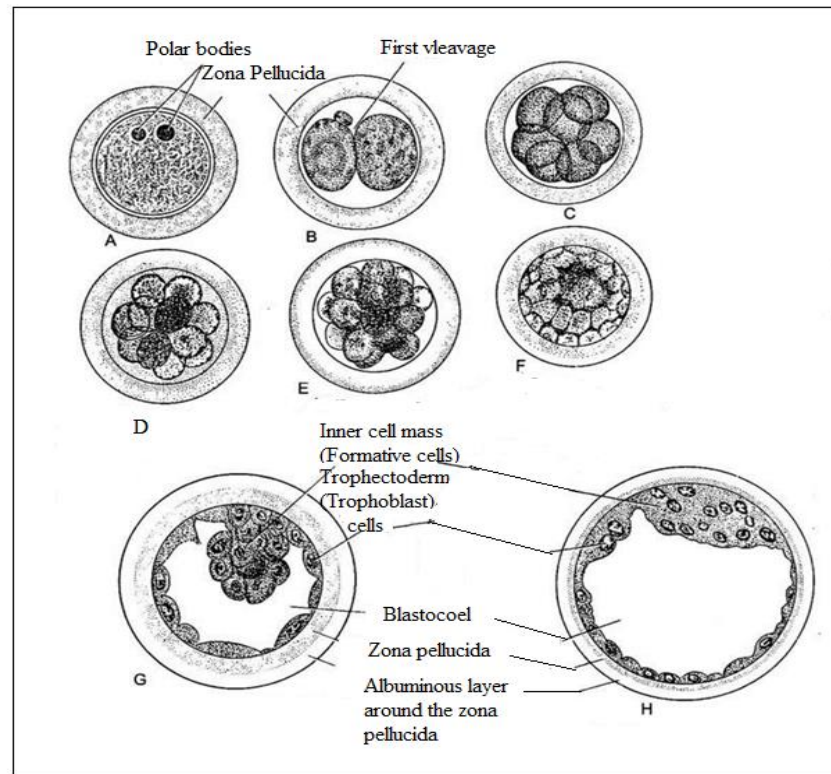
Fig. 3.15: Morulation in rabbit

Blastulation in Frog: Blastulation stages start after morulation when one cell has grown into 32 cells (blastomere). These blastomeres start to arrange at the periphery and a small fluid cavity or space starts to form within the embryo. This cavity is known as a blastocoel or segmentation cavity. The whole embryo formed is called a blastula. The process of formation of blastula is known as blastulation. After the formation of the blastula, the process of formation of body parts starts with a specific area marked within the cell. These are:

- Presumptive ectoderm: The region of the animal pole of the blastula.
- Presumptive Notochord: An area near the vegetal pole.
- Presumptive Mesoderm: An area close to the notochord.
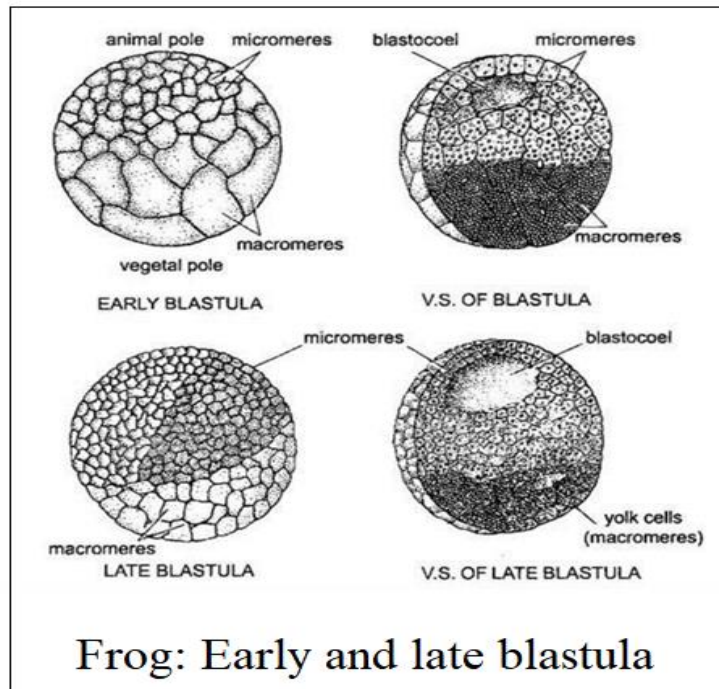
Fig. 3.16: Blastulation in frog

Blastulation in Chick: The morulation stage is of short duration. The cell undergoes further division resulting in the creation of several layers with their complete boundaries. Cell present near the periphery is not free from yolk known as marginal cells. This region is known as the zone of the junction.

The area in the center of the blastoderm is free from yolk with four to five layers of the cell, these cells undergoes arrangement. Space is created between blastoderm and yolk. This created space is known as blastocoel. This region is called area pellucida and is transparent. Area pellucida form the core of the embryo. This region is in contact with a region known as area opaca. Area opaca is responsible for the extra-embryonic structure.

Area opaca is opaque and white. They are differentiated into three more or less distinct zones. In birds, embryo blastomeres grow on the surface of a large yolk sphere. This blastomere forms an outer ring with no well-defined boundary.

An inner layer within the embryo is in close contact with the yolk. Thus two types of cells are found one with large yolk laden and another with small or yolk-free blastomeres. Yolk-laden blastomeres accumulate the under the surface of the blastoderm with yolk free at the surface. This led to the formation of two layers namely the upper layer and lower layer. These layers are called epiblast and the lower layer hypoblast respectively. A thin cleft appears between the epiblast and hypoblast. This cleft is known as blastocoels.
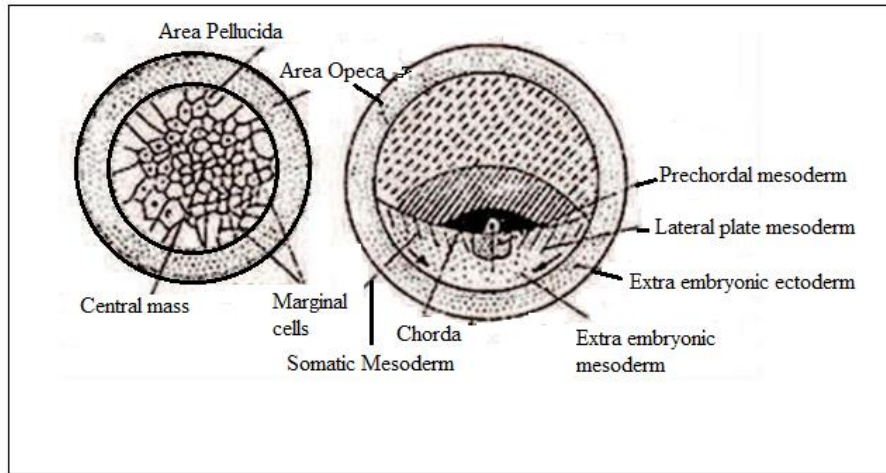
Fig. 3.17: Blastulation in chick

Blastulation in Rabbit: A fluid-filled cavity appears within the morula called the blastocoel appears. This is then known as a blastocyst or blastula. This embryo grows in size with the liquid food getting collected in the cavity, and separating the outer layer of the small trophoblast cell from the inner cell mass. This embryo is now known as a blastocyst. Inner cell mass grows inside Blastocyst and becomes a knob-like thickening at one pole. This knob is known as an embryonal knob because all parts of the embryo will be derived from it. Later this blastocyst becomes attached to the uterus of the mother and the villi absorb food from the villi.
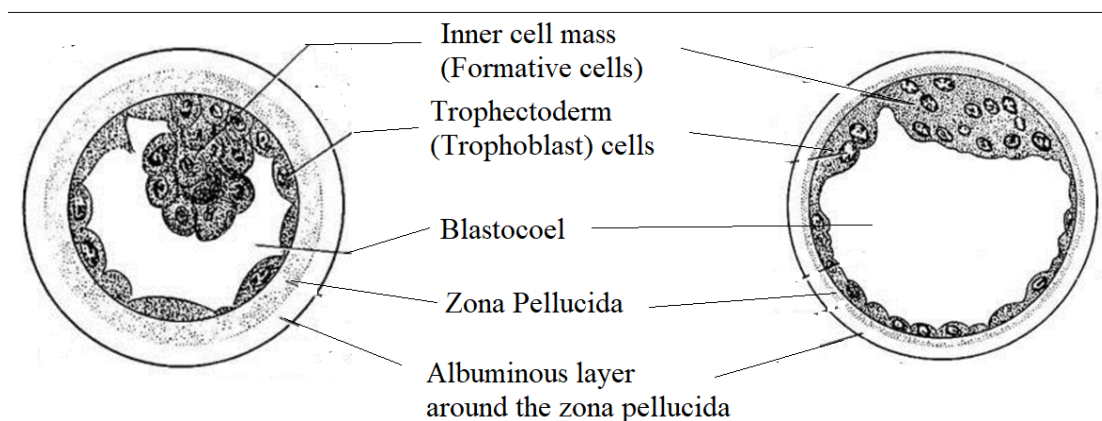
Fig. 3.18: Blastulation in rabbit

3.8 TYPES OF BLASTULAE

There are six types of blastulae found in the animal kingdom with differencing on various factors like size of the egg, amount and distribution pattern of yolk, and rate of cleavage.

1. **Coeloblastula:** This blastula is hollow and the blastocoel is surrounded by a single-layer cell. Examples: Echinoderms, *Amphioxus*, and frog.

2. **Stereoblastula:** This blastula is solid and has no blastocoel. Examples: Annelids, Molluscs, Nemerteans, and some species of planarians.

3. **Discoblastula:** This blastula is a multilayered flat disc at the animal pole separated by narrow segmentation from the yolk. They are found in eggs with a large and developed yolk. Examples: Reptiles, Birds, Prototherians, and Fishes.

4. **Blastocyst:** This blastula has a regular cleavage and a small cavity inside each cell. Two types of cells are found namely the outer layer of epithelial having nutritive cells and the inner mass of the formative cell. Example: Mammals.

5. **Superficial blastula or peri blastula:** This blastula has blastocoel which is filled with yolk and surrounded by a peripheral layer of cell Example. Insects.

6. **Amphiblastula:** This blastula is made up of two different types of structurally different blastomeres. Example: Amphibian
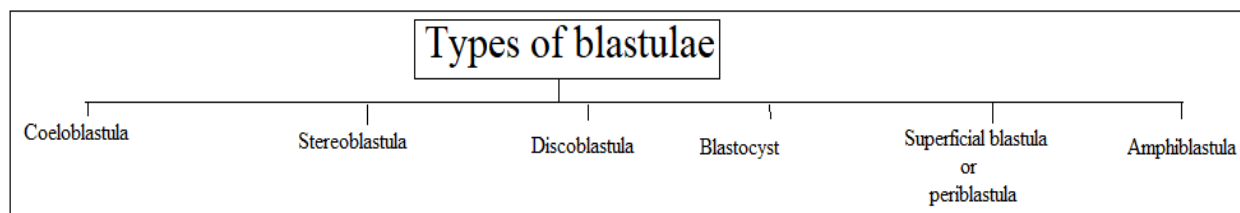
Fig. 3.19: Types of blastulae

3.9 MAJOR EVENTS OF GASTRULATION AND FATE MAPS

Gastrulation is a process of formation of germ layers. This formation involves complex cell movements that rearrange those cells within themselves. This is one of the most important phases of embryonic development.

Gastrulation involves the following major events:

1. **Morphogenic Movement (Movement of blastular cells or blastomeres):** Blastomeres move in a different direction to form multiple patterns to develop germ layers and start the process of developing multicellular organisms.

2. **The rate of cell division (Cleavage) is slowed down:** Before reaching the stage of Gastrulation, Cleavage has produced a sufficient number of cells, so the emphasis lay on how to arrange those cells so that they can be used as per the requirement.

3. **Types of Metabolism changes and oxidation rate increases:** Gastrulation emphasized developing germ layers and the start of developing different parts of the animal. This requires a different type of protein synthesis. To facilitate the different proteins that are been secreted and oxidation rate increases.

4. **Nuclei control embryonic cell activities:** Gastrulation starts the process of differentiation where different parts of the animals are formed based on the genetic material. The cell nucleus stores the genetic code and the arrangement of the blastomeres are been done on the basics of the genetic code. Thus, the Nuclei enforce the genetic code in managing the process of gastrulation.

5. **Chemo-Differentiation is started:** Gastrulation initiates the process of chemo-differentiation where the blastomeres are subjected to a different set of chemicals and proteins to mold the blastomere's behavior. This molding of blastomeres results in the development of different parts of the multicellular animals.

Major Events of Fate Map: Fate map is the process of mapping which part of the blastula will develop into which organs in the embryo. It varies from animal to animal.

Fate map in frog: In frog the area demarcated for the different part are already been demarcated at the end of cleavage.

1. Macromeres of vegetal pole will become endoderm.
2. Micromeres of the animal pole will become ectoderm.
3. Prechordal both sides will become mesoderm.
4. Micromeres present near notochord and mesoderm becomes neurectoderm.

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.
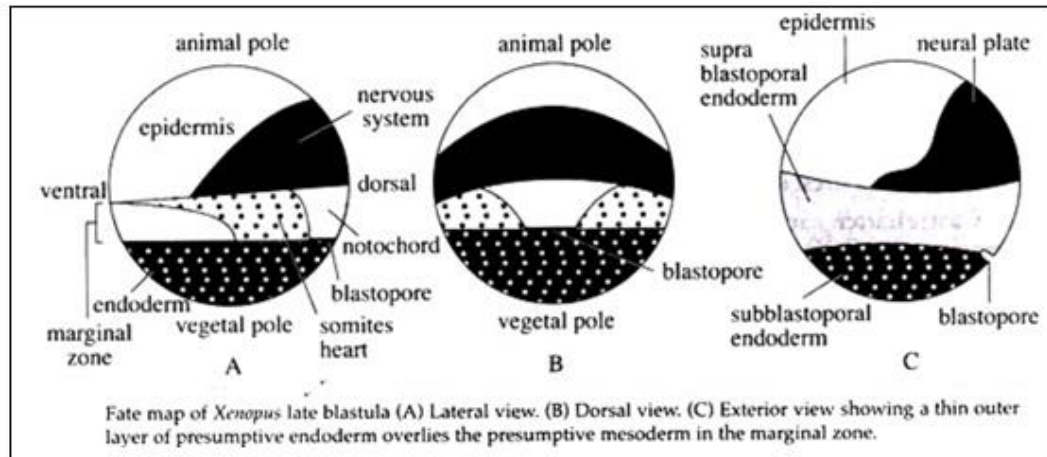
Fig. 3.20: Fate Map of frog

Fate map in chicks: In chicks after the formation of hypoblast, the area marked for which organs can be detailed:

1. The hypoblast area forms the endoderm.
2. Epiblast forms the various organ of the chick.
3. Area opaca forms the extra-embryonic membrane and blood vessels.
4. Area pellucida forms epidermal ectoderm, neurectoderm, prechordal, notochordal and mesoderm cells.

Fate map of chick embryo.
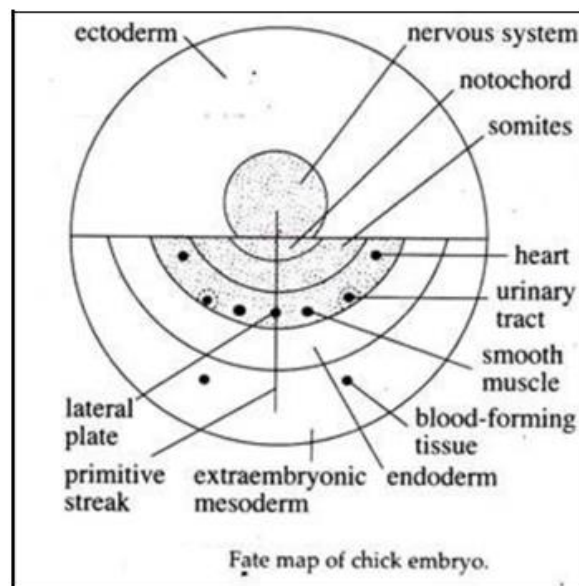
Fig. 3.21: Fate Map of Chicks

Fate map in mammals: Fate Map in Mammals is marked after the blastocyst stage.

1. The Innermost layer forms the endoderm.
2. The embryonic disc forms the ectoderm and mesoderm.

3. Anterior germ disc forms the epidermal ectoderm.

4. Behind the crescent, the area forms the notochord.

5. Behind the notochordal area forms a prechordal plate.

6. The trophoblast forms the ectoderm layer of the chorion.

3.10 MORPHOGENETIC MOVEMENTS IN FROG, CHICK AND RABBIT

Morphogenetic movements occur during gastrulation:

These movements are classified according to the number of cells migrating together.

Movement by individual cell:

i.   **Migration** – It is a movement where an individual cell moves over to the other cells.

ii. **Ingression**– It is the movement of individual cells from epithelium into a cavity.

Groups of cells move by: Types of Morphogenetic movement based on the direction. They are classified as epiboly and emboly.

Epiboly - In this movement, a group of cells arranges themselves to form an outside cell layer to cover the yolk. They do it by thinning the layers. Their movement is over ectoderm.
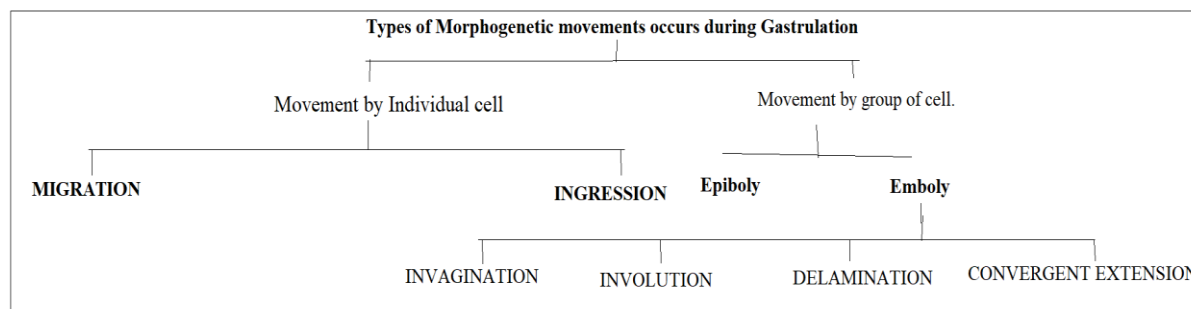
Fig. 3.22: Diagram of classification

Emboly: Their movement is over Endoderm and mesoderm. They are of 4 Types

i.   **Invagination**– It is a movement where the cell moves inward.

ii. **Involution** - It is an inward movement of a group of cells or epithelial sheets around a point or an edge. This is done to form an underlying layer.

iii. **Delamination** - In this process cells splits themselves into two different cell layers namely epiblast (outer layer) and hypoblast (inner layer).

iv. **Convergent Extension**– In this process, two or more cell rows move together intending to elongate the structures in one dimension while shortening them in another direction.
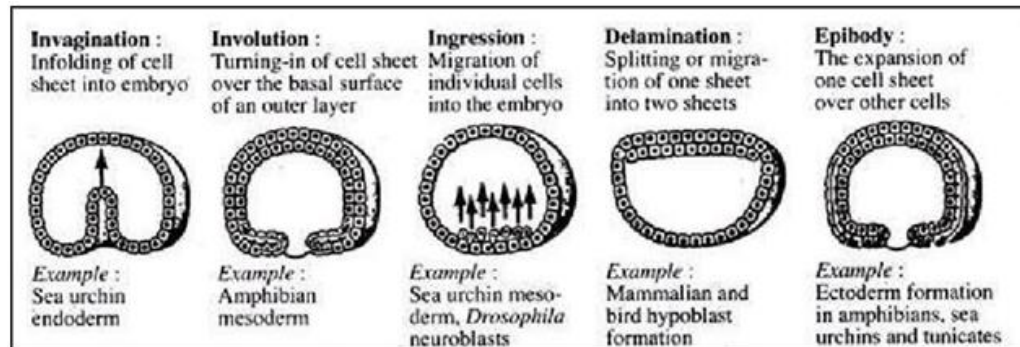
Fig.3.23: Morphogenetic movements

3.11   SIGNIFICANCE   OF   GASTRULATION   AND EXOGASTRULATION

Gastrulation: The process in which a blastula or a single layer of the cell is converted into multiple layers of cells called gastrula is known as gastrulation.

Significance:

- Gastrulation starts the process of a multicellular organism. It sets the process of developing different parts of the body and brings the morphology changes in the embryo.
- Morphological changes bring an increased metabolic activity of the cells.
- Gastrulation brings the three primary germ layers i.e. ectoderm, mesoderm, and endoderm. This starts the process of developing skin.
- Gastrulation shows the influence of paternal chromosomes.
- The Blastocoel cavity is transformed into archenteron.

Exogastrulation: The abnormal gastrula in which the mesoderm, notochord, and endoderm are made to evaginate to the outside. This is just the opposite of the normal process where inward movement takes place. In this process, the embryo never develops. It is done experimentally to the induced disturbance in the embryo.

Significance:

1. Exogastrulation helps to understand the factors which affect normal gastrulation.

2. Exogastrulation helps to understand how the cells behave and divide and form structure after being subjected to certain chemicals and proteins.

3. The gained knowledge can be used in research and development so that certain embryonically can be modified as per the needs.

3.12 SUMMARY

All multicellular organisms have evolved from a single cell which is a result of the fertilization of sex gametes. These gametes' fusion starts to divide themselves into several mitotic divisions. These mitotic divisions led to an increase in the number of cells from 2 to 16. This process of division is known as cleavage. These cleavages have been different depending on the size of the egg, amount and distribution pattern of yolk, rate of cleavage, etc.

The cleavage brings a significant change in catabolic and anabolic activities of the newly created cells called mesomeres. All cleavage follows four basics law namely Sach's Law, Hertwige Law, Plugger Law, and Balfour Law. Cleavage leads to the stage of morula where each cleavage cell arranges itself over the cell of its previous cleavage loosely joined together by a stacking gel. Morula is a 16-celled stage arranged in the shape of a mulberry. The morula stage is short-lived.

The cell division process continues and starts to take on the next phase of development called a blastula. Cleavage led to an increase in the number of blastomeres. This blastomere undergoes a rearrangement which results in arranging themselves into a single cell thick epithelium called blastoderm. A fluid-filled space or cavity called blastocoel appears between the blastomeres. This hollow, spherical and nonepithelial thick embryonic stage is called a blastula. This process of creating a blastula is known as blastulation where a single layer of blastoderm is created.

Gastrulation follows blastulation where a single layer of blastoderm is changed into three germ layers i.e. mesoderm, ectoderm, and endoderm. This transformation requires Morphogenic movement (movement of blastulae cells or blastomeres), rate of cell division (cleavage) to be slowed, metabolism changes and oxidation rate increases, and nuclei control the embryonic cell activities. These morphological changes can be inwards or outwards known as epiboly and emboly respectively. A lot of research has been done to understand the behavior of the morphological movement of blastulae cells. It is done to induce a disturbance in the embryo. This inducement of making an embryo work differently from the normal embryo is known as exo-gastrulation. This exo-gastrulation has assumed a significant role in understanding and developing an embryo as per the requirement.

3.13 TERMINAL QUESTIOS AND ANSWERS

3.13.1 MULTIPLE CHOICE QUESTIONS:

1. Cleavage produces a hollow sphere of cell called _____.

a. Morula

b.Blastomere

c.Blastula

d. Mesomere

2. Nematodes, annelids, molluscs and ascidian follows_____ cleavage.

a. Determinate

b.Indeterminate

c. Both a and b

d. None of them

3. In _____ cleavage blastula can be divided into two identical halves.

a. Biradial cleavage

b. Radial cleavage

c. Bilateral cleavage

d. Spiral cleavage

4. Cleavage is divided into _____ types based on yolk distribution in an egg.

a. 1

b. 2

c. 3

d. 4

5. This process of creating a blastula is known as:

a. Gastrulation

b. Cleavage

c. Blastulation

d. Morulation

6. _____ blastula is made up of two different types of structurally different blastomeres.

a. Coeloblastula

b. Stereoblastula

c. Discoblastula

d.Amphiblastula

7. In _____ step of development nuclei controls the embryonic cell activities.

a. Gastrulation

b. Morulation

c. Blastulation

d.Cleavage

8. In _____ morphological movement cells splits themselves into two different cell layers namely epiblast (outer layer) and hypoblast (inner layer).

a. Invagination

b. Involution

c.Delamination

d. Convergent Extension

9. In this process the embryo never develops. It is done experimentally to induce a disturbance in the embryo. Who am I?

a. Cleavage

b. Gastrulation

c. Exo-Gastrulation

d. Blastulation

10. In _____ process blastocoel cavity is transformed into archenteron.

a. Cleavage

b. Gastrulation

c. Exo-Gastrulation

d. Blastulation

Answers: 1(c), 2(a), 3(b), 4(b), 5(c), 6(d), 7(a), 8(c), 9(c), 10(b)

3.13.2 VERY SHORT QUESTION

1. Discuss the Significance of Gastrulation and Exo-Gastrulation.

2. How Epiboly is different from Emboly.

3. How Exo-Gastrulation will help in understanding how the embryo develops.

4. Name the various Law which governs Cleavage.

4. Name the various morphogenetic techniques used in the animal kingdom.

5. Write the various characteristics of cleavage.

6. Explain the morulation in the frog.

7. Name the stage where the roles of genetic material first come during the process of development of the multicellular animal.

8. How invagination is different from involution.

3.13.3 LONG QUESTIONS

1. Explain how determinate and indeterminate cleavages are different from each other. Write the name of animals that exhibits deterministic and indeterminate cleavage.

2. Explain the influence of yolk on cleavage.

3. What type of metabolic changes happen during cleavage?

4. Explain the significance of morulation and blastulation.

5. Explain the various type of cleavage. How they are different from each other?

6. Discuss the various types of blastulae.

7. Illustrate the significance of gastrulation. Explain the various events that happen in gastrulation.

8. Illustrate the significance of the fate map. How fate map of frog is different from mammals?

9. Explain the various morphogenetic movements that happen during gastrulation.

10. Explain the process of blastulation in frogs and rabbits.

11.

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3.15 GLOSSARY

Archenteron: Cavity in an embryo during the gastrulation stage which forms the digestive tract.

Blastocoel: The fluid-filled cavity of the blastula.

Blastomere: cell which is the result after the division of a fertilized egg

Blastula: a 32-celled structure of an embryo that is hollow and is surrounded by an inner-filled fluid called the blastocoel.

Cleavage: Process of mitotic cell division of fertilized egg.

Ectoderm: The outermost layer of the germ layer

Emboly: The process of movement of gastrula during gastrulation where cells move inwards to form archenteron.

Endoderm: The innermost layer of the germ layer

Epiboly: The process of movement of gastrula during gastrulation where cells move outwards to cover up the yolk and the remaining cell form the ectoderm.

ExoGastrulation: The process in which an embryo is induced with some disturbance to see the behavior of the gastrula.

Gastrulation: Stage of embryo development where three germ layers are formed and the process of development of body parts and organs starts.

Holoblastic: Cell which undergoes a complete cleavage.

Meroblastic: Cell which undergoes partial cleavage only.

Mesoderm: The middle layer of the germ layer

Morula: A 32-celled structure resembling mulberry which further makes blastula.

Trophoblast: the membrane of cells that forms the wall of a blastocyst during early pregnancy, providing nutrients to the embryo and later developing into part of the placenta

Yolk: The Yellow and the main food of the egg

UNIT 4: EARLY DEVELOPMENT

CONTENTS

4.1 OBJECTIVES

After studying this module, you shall be able to learn and understand:

1. The tissue, cellular, and molecular basis for neural induction and neural tube formation.

2. Explain how neuronal precursors are generated in the CNS.

3. The early changes in neural tube shape and the formation of the primary brain vesicles.

4. How two important signaling molecules, sonic hedgehog (Shh) and bone morphogenic protein (BMP-4), regulate the expression of regional distinctions in the nervous system.

5. Where and how the neural crest forms, the origin of the migratory pathways that lead crest-derived cells to stop specific targets.

6. Secondary Neurulation

4.2 INTRODUCTION

Neurulation is a process in which the neural plate bends up and later fuses to form the hollow tube that will eventually differentiate into the brain and the spinal cord of the central nervous system. In humans, it begins in the 3rd week after fertilization and requires that the top layers of the embryonic germ disc elevate as folds and fuse in the midline. Neurulation is the embryological process that forms the precursors of the central nervous system and occurs after gastrulation. It has established the three primary cell layers of the embryo: ectoderm, mesoderm, and endoderm. In humans, the majority of this system is formed via primary neurulation, in which the central portion of the ectoderm—originally appearing as a flat sheet of cells—folds upwards and inwards, sealing off to form a hollow neural tube. As development proceeds, the anterior portion of the neural tube will give rise to the brain, with the rest forming the spinal cord. The epidermis, the central and peripheral nervous systems, and some non-neuronal cells of the head and heart are derived from ectoderm (Fig. 4.1). During the third week of gestation, a portion of the dorsal ectoderm is specified to become neural ectoderm. This region of the embryo is called the neural plate. The process by which the neural plate forms a neural tube is called neurulation.

4.3 NEURULATION AND ECTODERM ORIGIN AND FATE OF NEURAL CREST CELLS

The original ectoderm can be divided into three sets of cells: (i) the internally positioned neural plate, (ii) the externally positioned future epidermis of the skin, (iii) and the neural crest cells that connect the neural plate and epidermis.

At the time the neural plate becomes specified, an interaction between the surface ectoderm (SE) and neural plate (NP) creates an intermediate structure, known as the neural crest (Fig. 4.2).
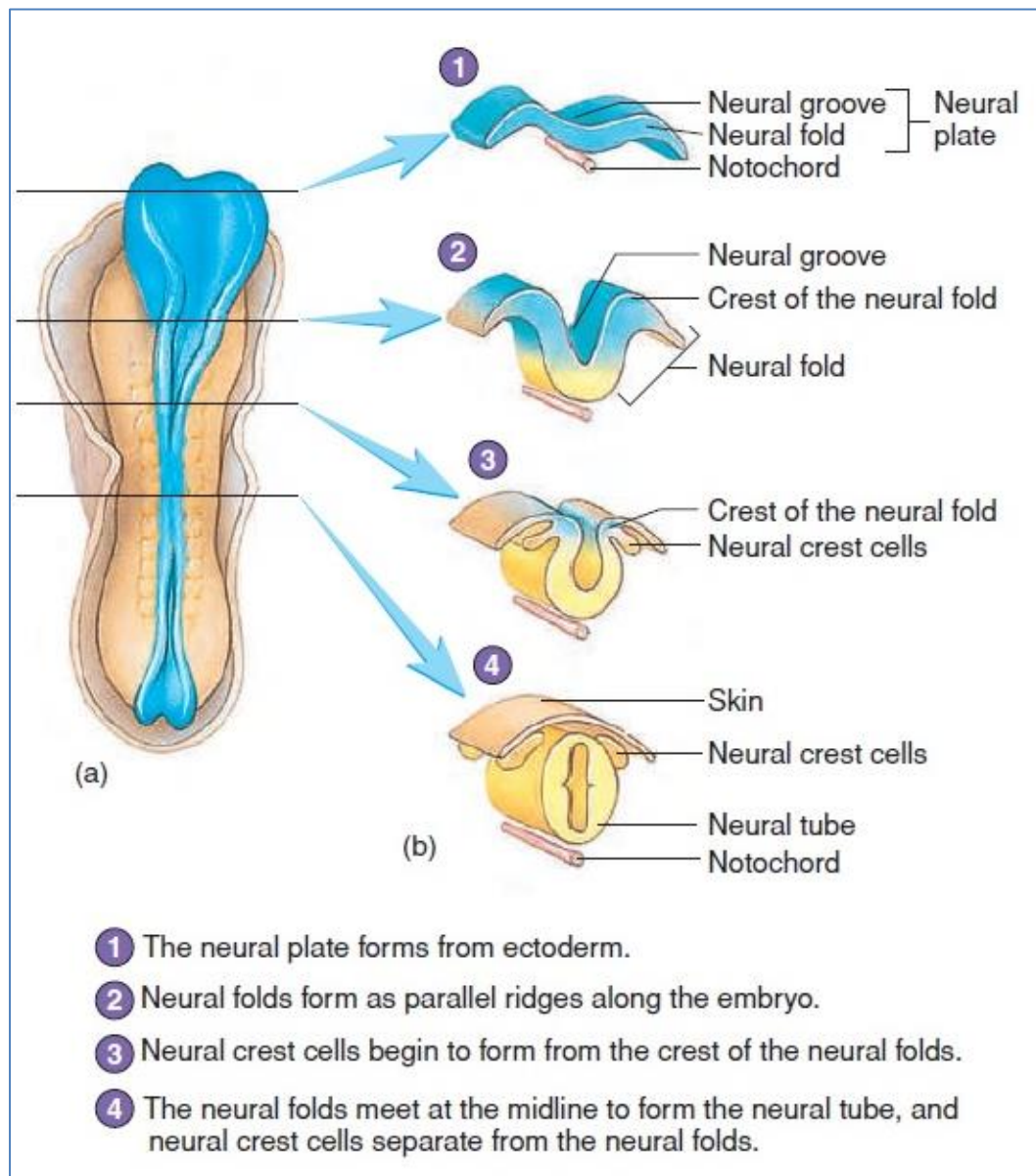
Fig.4.1 Cross sections through the forming neural tube (Human Embryology & Developmental Biology, 2nd edition, Carlson, B. M.)

The neural plate folds in stages to form the neural tube and the Scanning electron micrographs of chick embryosis are depicted in Fig. 4.2. During neural tube closure, when the dorsal tips of the

neural folds converge, these cells delaminate from the dorsal neural tube and migrate from their original site in the neuraxis, via specific routes, to colonize peripheral targets.

At the junction of the neural and surface ectoderm, there is another population of cells, which is called the neural crest. As the neural folds begin to appear, neural crest cells (NCCs) can be seen in their tips through the expression of characteristic markers, like the Pax7 transcription factor. As development proceeds and the neural folds are fused, NCCs are seen either in the topmost portion of the neural tube or migrating along this structure's sides towards the lower regions of the embryo. Crest-derived cells are capable of differentiating into an astonishing number of different and diversified cell types and tissues including Schwann cells or glial cells of the sensory, sympathetic, parasympathetic, and enteric nervous systems, cells of the adrenal medulla, pigment cells in the epidermis, and connective tissue components of the head (Fig. 4.3 and 4.4) yet they express only those phenol types that are appropriate for the organ to which they have migrated.
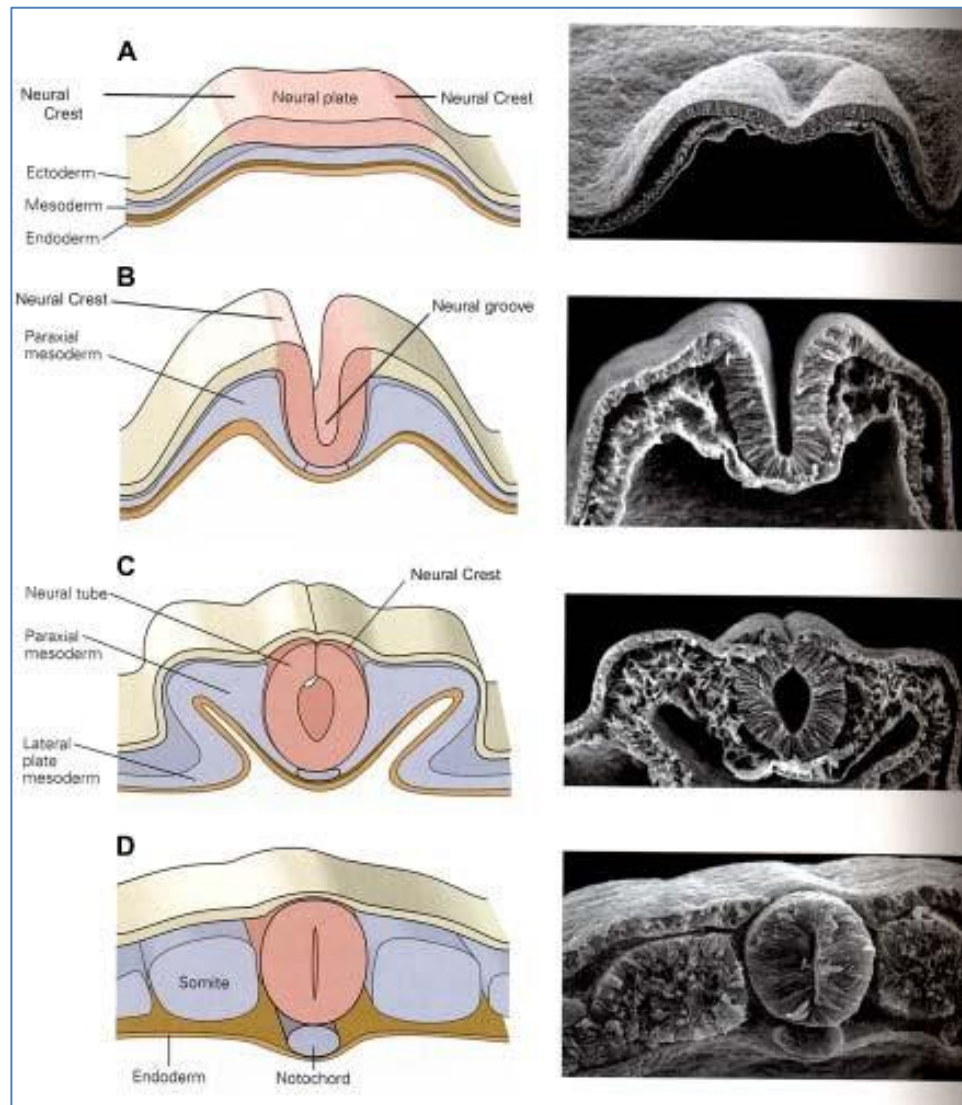
Fig.4.2: The neural plate folds in stages to form the neural tube. (Scanning electron micrographs of chick embryos are provided by G. Schoen wolf.) A. Position of the neural plate about then on neural ectoderm, the mesoderm, and the endoderm. B. Foldingof the neural plate to form the neural groove. C. Dorsal closure of the neural folds to form the neural tube and neural crest. D. Maturation of the neural tube and its position n relative to the axial mesodermal structure, notochord, and somites (derived from the paraxial mesoderm). (Adapted from Jessell & Sanes, Principles of Neuroscience 4thedition, 2002, E. Kandeleditor)
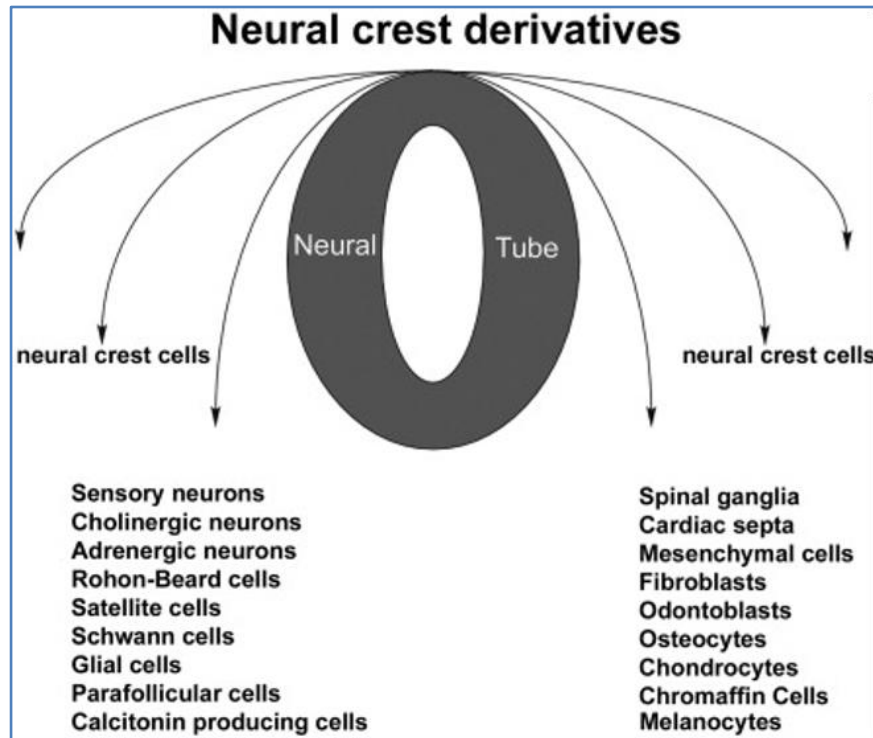
Fig. 4.3: Some derivatives of the neural crest (After Jacobson, 1991)

4.3.1. NEURULATION

Neurulation is a process in which the neural plate bends up and later fuses to form the hollow tube that will eventually differentiate into the brain and the spinal cord of the central nervous system. In humans, it begins in the 3rd week after fertilization and requires that the top layers of the embryonic germ disc elevate as folds and fuse in the midline. Neurulation is the embryological process that forms the precursors of the central nervous system and occurs after gastrulation.

4.3.2. PRIMARY NEURULATION

This term refers to the formation of the neural tube from the neural plate, situated between the anterior and posterior neuropores (fig.4.6).

1. Neural induction formation of the neural plate: Neural induction is the first step whereby the uncommitted or naïve ectoderm becomes committed to the neural lineage. During gastrulation, signals from the node or its derivative, the notochord, induce commitment. Classical studies led to the notion that inducing substances, secreted by the underlying prechordal plate and the cranial portion of the notochordal plate, were responsible for ectodermal commitment to a neuronal lineage by the overlying epiblast cells. There is now good evidence that neural induction' actually involves suppression of duction of an epidermal fate rather than induction of a neural fate so that

the default state of the naïve ectoderm is neural, not epidermal as suggested by older studies. In amphibians, molecules (e. g. noggin, chordin, follistatin) that inhibit the expression of bone morphogenetic protein 4(BMP-) appear to block epidermal expression. Although the suppression signal is generated by Hensen's node in birds, suppression of BMP-4maynotbe the only requirement for neural induction in mammals. The original ectoderm can be divided into three sets of cells: (i) the internally positioned neural plate,(ii) the externally positioned future epidermis of the skin, (iii) and the neural crest cells that connect the neural plate and epidermis. Lateral folding or bending of the neural plate results in elevation of two walls, the neural folds, flanking a ventral midline floor plate (composed of non-neuronal cells) of the neural groove.
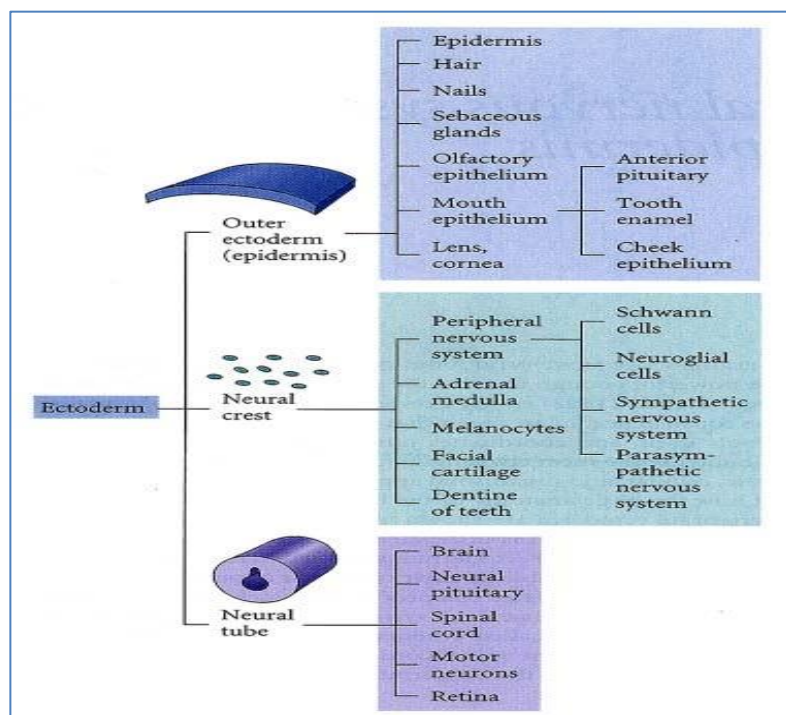
Fig. 4.4: Major derivatives of the ectodermal germ layer.The ectoderm is divided into three major domains the surface ectoderm (primarily epidermis), the neural tube (brain and spinal cord), and the neural crest (peripheral neurons, pigment, facial cartilage). (Gilbert, DevelopmentalBiology, 6th edition)

The principal early morphological response of the embryonic ectoderm to neural specification is an increase in the height of the cells destined to become components of the nervous system. These transformed cells, now known as neuroepithelial cells or neuroectoderm, are evident as a thickened neural plate visible on the medial dorsal surface of the early embryo.

2. Shaping of the Neural Plate: At the time of its formation, the neural plate is shaped like a spade being relatively wide mediolaterally and short rostro caudally (Fig. 4.5). The caudal wings of the spade flank the primitive node. During shaping, the nascent neural plate becomes narrower and longer. Although the processes of neurulation and gastrulation can be uncoupled experimentally, full craniocaudal formation and extension require the normal cellular movement of gastrulation.

3. Formation of the neural tube: It occurs when the two dorsolateral apical surfaces of the neural folds meet, fuse at the dorsal midline, and separate from the overlying ectoderm. Forces generated by the surface epithelium as it expands towards the dorsal midline cause elevation of the neural folds and ultimately, closure of the neural tube. The bends in the medial portion of each neural fold maintain the structure of the tube so that the lumen remains patent as the neural folds converge.
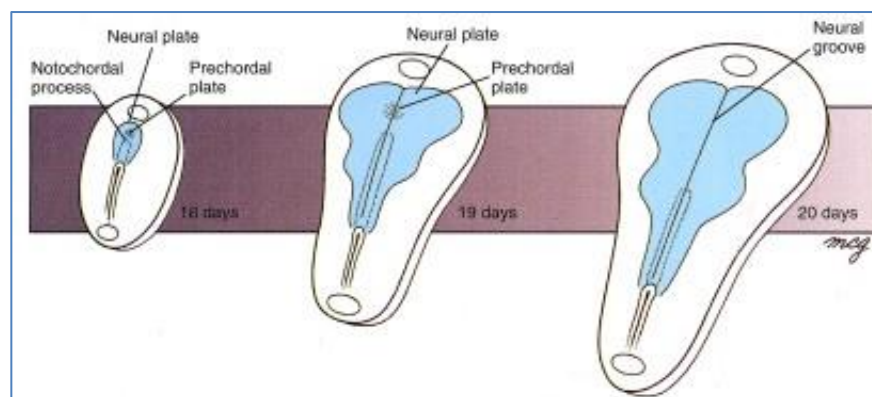
Fig. 4-5: A schematic sequence showing how the neural plate grows and changes proportions between da18 and day 20. The primitive streak shortens only slightly, but it occupies a progressively smaller proportion of the length of the embryonic discs the neural plate and embryo grow (Larsen, 3rd edition)

The molecular signals for primary neurulation in human embryos (Fig.4.6) remain largely unknown but several candidate genes that perturb neurulation when mutated have now been identified. Sonic hedgehog (Shh) is an important signaling center. Not only does it induce elevation of neural folds but also the formation of the neural groove and floor plate. In the dorsal portions of the future neural tube, Wnt6, secreted by the epidermal ectoderm adjacent to the neural plate and BMPs induce slug in the future neural crest cells. The BMPs also appear to maintain the dorsal expression of Pax transcription factors. Shh signaling from the floor plate, suppresses the expression of dorsal Pax genes in the ventral half of the neural tube where motor neurons devlop. Closure of the neural tube begins almost midway along the craniocaudal extent of the nervous system of the 21-22 day human embryo (Fig. 4.6 A, B). Over the next couple of days, closure extends both cephalically and caudally in a manner resembling the closing of a double-headed zipper. The unclosed cephalic and caudal parts of the neural tube are called the anterior (cranial) and posterior (caudal) neuropores.

The neuropores will ultimately close (24 days gestation for the cranial neuropore and 26days for the caudal) so that the future central nervous system (CNS) is organized in a way that resembles an irregular cylinder sealed at both ends. Neural tube defects occur when various parts of the neural tube fail to close. An open posterior neuropore result causes spina bifida (Fig. 4.6 E), the severity of which depends on the length and position of the open segment. Anencephaly (Figure 4.7 D) is a lethal condition in which the anterior neuropore fails to close. The forebrain remains in contact with the amniotic fluid and subsequently degenerates.
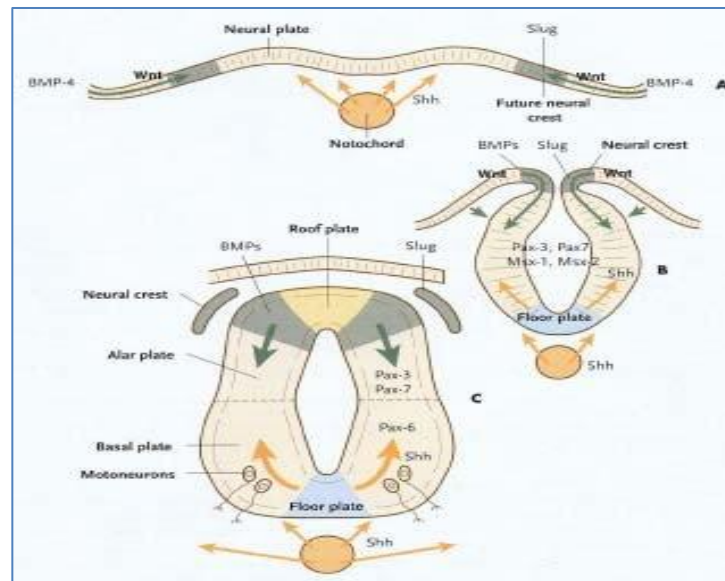
Fig.4.6: Dorsal and ventral signaling in the early central nervous system. A. Signals from sonic hedgehog (Shh) (orange arrows) in the no-to chord induce the floor plate. B. In the dorsal part of the future neural tube, Wnt from the ectoderm adjacent to the neural tube induces slug in the future neural crest and maintains Pax-3 and Pax-7 expression dorsally. Ventrally, the sonic hedgehog, now produced by the floor plate, induces motoneurons. C. Sonic hedgehog, produced by the floor plate, suppresses the expression of dorsal Pax genes (Pax-3 and Pax-7) in the ventral half of the neural tube. (Carlson, HumanEmbryology&DevelopmentalBiology, 2ⁿᵈ edition).
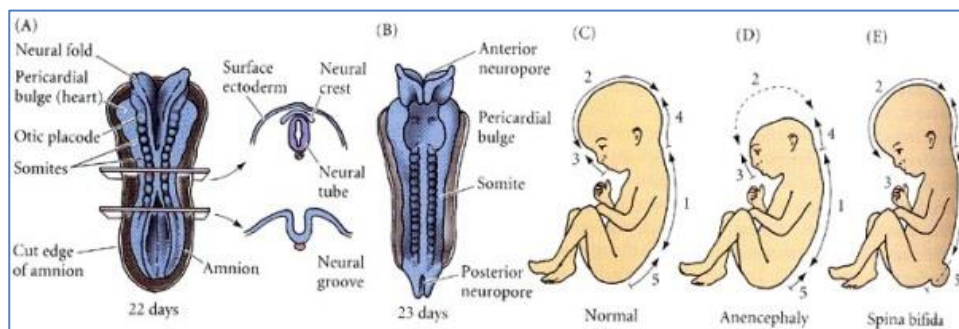
Fig.4.7: Neurulation in the human embryo: (A) Dorsal and transverse sections of a 22 day human embryo initiating neurulation. Both anterior and posterior neuropores are open to the amniotic fluid. (B)Dorsal view of the neurulating human embryo a day later. The anterior neuropore region is closing while the posterior neuropore remains open. (C) Regions of neural tube closure postulated by genetic evidence (superimposed on newborn body). D) Anencephalyis caused by the failure of neural plate fusion in region 2. (E)Spina bifida is caused by the failure of region 5 to fuse (or of the posterior neuropore to close). (C-Eafter VanAllen et al.1993.)(Gilbert, DevelopmentalBiology, 6ᵗʰ edition)

4. The neural tube forms the primordia of the central nervous system: Even before the neuropores have closed the future brain and spinal cord are recognizable and the brain becomes subdivided into a forebrain (prosencephalon), midbrain (mesencephalon), and hindbrain (rhombencephalon) (Fig. 4.8 and 4.9). The increased volume of the early brain is the result of an increase in cavity size, not tissue growth. In the chick embryo, brain volume expands 30 fold between days 3 and 5 of development. This rapid expansion is thought to be caused by pressure from fluid exerted against the walls of the neural tube after the surrounding dorsal tissues push in to temporarily constrict the neural tube in the region between the presumptive brain and spinal cord (Fig. 4.8). The concluded region reopens after the initial rapid enlargement of the brain vesicles. Another prominent force in shaping the early nervous system is the overall bending of the cephalic end of the embryo into a C-shape.
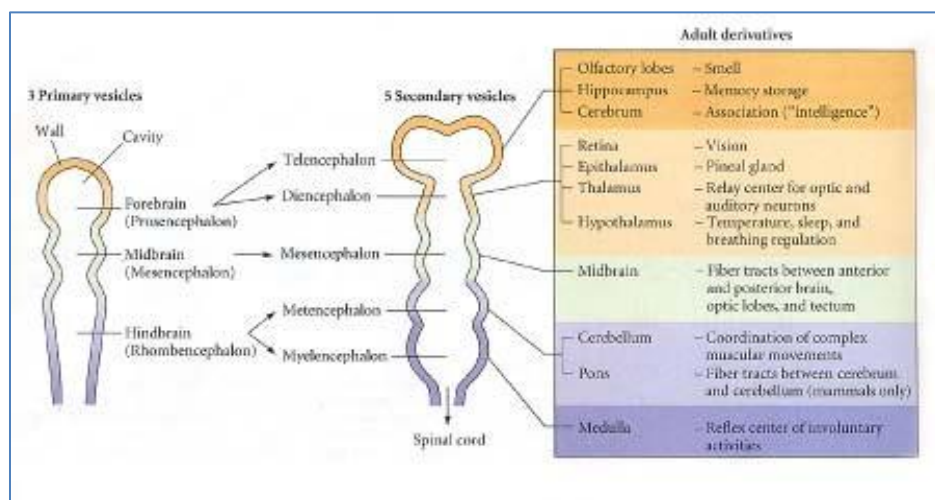
Fig. 4.8: Early human brain development. The three primary brain vesicles are subdivided as development continues. At the right is a list of the adult derivatives formed by the walls and cavities of the brain. (After Moore and Persaud1993)(Developmental Biology, 6th edition, S.Gilbert)

Soon the brain almost doubles back on itself at the cephalic flexure. At the beginning of the fifth week, a second cervical flexure appears at the boundary between the hindbrain and the spinal cord. By the end of the fifth week, the prosencephalon becomes further subdivided into a telencephalon and a more caudal diencephalon with prominent optic vesicles extending from its lateral walls. The rhombencephalon divides into the metencephalon and more caudally, the yelencephalon. These five primary brain vesicles, plus the spinal cord, comprise the early fundamental organization of the CNS.

The original neural tube is lined by a ventricular zone, composed of a single layer of rapidly dividing neural stem cells, called the neuroepithelium (sometimes known as a germinal epithelium). All the cells of the neuroepithelium extend to the luminal surface but their nuclei are at different heights thereby giving the structure a pseudostratified appearance. DNA synthesis (S phase) occurs while the nucleus is positioned at the outside edge of the zone. As the cell cycle proceeds, the nucleus migrates within the cell cytoplasm toward the lumen. Mitosis occurs at the luminal side of the ventricular zone and the two daughter cells then continue to cycle. A cell that has undergone its last mitotic division and is derived from a stem cell that divides parallel to the ventricular surface. The daughter cell adjacent to the lumen remains connected to the ventricular surface, continuing in the cell cycle, while the post-mitotic daughter migrates out of the germinal epithelium.

5. Neuronal survival depends upon target related trophic signals: It should be noted that not all neuroblasts survive. Of the huge number generated, nearly half are destined to undergo apoptosis and die (Fig. 4.8). Only those neurons that make structural and functional synaptic connections with specific targets are not eliminated. Both in CNS and PNS, neuronal survival and neuronal cell death are under the tight developmental control of gene products secreted by target structures. These trophic factors are required to sustain growth and survival.

6. The Neural Crest: At the time the neural plate becomes specified, an interaction between the surface ectoderm (SE) and neural plate (NP) creates an intermediate structure, known as the neural crest (Fig. 4.9). During neural tube closure, when the dorsal tips of the neural folds converge, these cells delaminate from the dorsal neural tube and migrate from their original sites in the neuraxis, via specific routes, to colonize peripheral targets. Crest-derived cells are capable of differentiating into an astonishing number of different and diversified cell types and tissues including Schwann cells or glial cells of the sensory, sympathetic, parasympathetic, and enteric nervous systems, cells of the adrenal medulla, pigment cells in the epidermis, and connective tissue components of the head, yet they express only those phenotypes that are appropriate for the organ to which they have migrated.

7. Origin of neural crest migration pathways that lead crest-derived cell to target organs: Studies utilizing avian chimeric embryos (Le Douarin and colleagues) have provided a great deal of information regarding the specificity of individual neural crest migration pathways, as well as the development potential and restriction of crest-derived phenotypes. Neural crest migration

routes originate from specific sites along the cranial-caudal axis (neuraxis) of the dorsal neural tube. The migration pathways lead the dividing crest-derived cells to specific end targets where they stop dividing and differentiate into target-related phenotypes. Thus, the site in the neuraxis from which a crest cell originates determines the target it will reach (Fig.4.9). Heterotopic (ectopic) transplantation of crest cells into a migration pathway that they normally do not traverse, leads them to a new target where, depending on their developmental potential, they may express a new phenotype that is appropriate for the target they have colonized. Research conducted on mammalian embryos suggests that except for relatively minor structural details, information learned from birds can be directly applied to mammalian development.

 The crest-derived cells that reach a target at the end of the migration pathway are different than those that entered it. As they migrate, they encounter extracellular signaling molecules, e.g. growth factors and trophic factors, and components of the extracellular matrix, e. g. fibronectin, laminin, and collagen, which are conducive to their continued migration and proliferation. As they migrate, the crest-derived cells develop appropriate receptors that allow them to interact with these environmental cues by the time they reach their specific target, and their number has increased significantly.
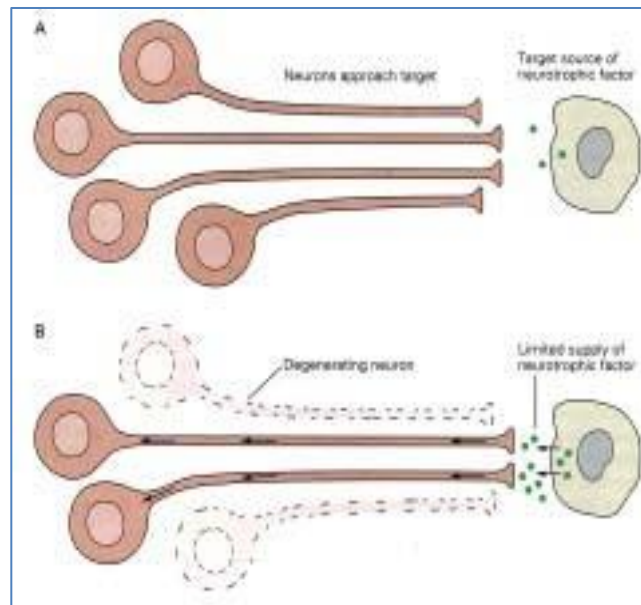
Fig. 4-9: The neurotrophic factor hypothesis. (Adapted from Reichardt andFarinas1997) A. Neuronsextendaxonstothevicinityof target cells. B.The target cells secrete limited amounts of neurotrophic factors.Theneurotrophic factors bind to specific cell surface receptors. Neurons that do not receive adequate amounts of neurotrophic factor die by apoptosis. (Jessell& Sanes, Principles of Neuroscience/2000, E. Kandeleditor).
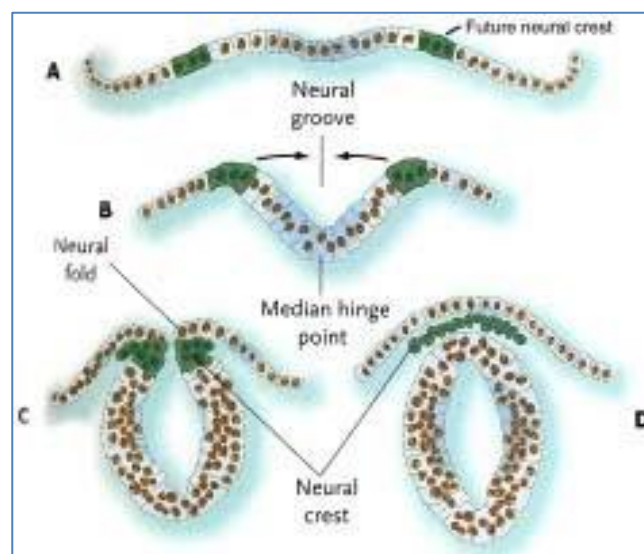
Fig.4.10: Cross sections through the forming neural tube. A. Neural plate. B. Neural fold, C. Neural folds appose and D. Neural tube complete (Human Embryology & Developmental Biology, 2nd edition, Carlson, B.M.)

8. The cranial neural crest: Crest-derived cells in the head region produce the craniofacial mesenchyme that differentiates into cartilage and bone, cranial neurons and glia, and connective tissues of the face. Other cells enter pathways traversing pharyngeal structures where they give rise to such diversified cells as those of the thymus, odontoblasts of the tooth primordia, and the bones of the middle ear and jaw (Fig. 4.11).
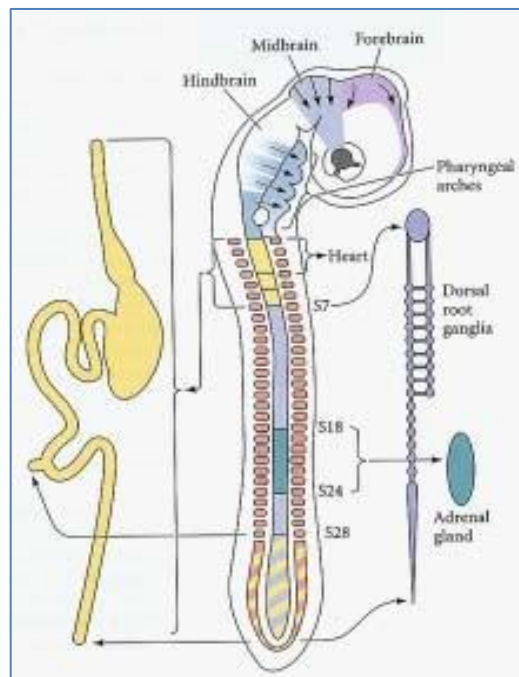
Fig.4.11: Regions of the neural crest.The cranial neural crest migrates into the branchial arches and the face to form the bone and cartilage of the face and neck. It also produces pigment and cranial nerves. The vagal neural crest (nearsomites1-7) and the sacral neural crest (posterior to somite28) form the intrinsic neurons of the gut. The cardiac neural crest cells arise from the neural crest near somites 1-3; they are critical in dividing the aorta and the pulmonary artery. Neural crest cells of the trunk (about somite 6 through the tail) make the sympathetic neurons, and a subset of these (at the level of somites18-24) form the medullary portion of the adrenal gland. (After Le Douarin 1982.) (Developmental Biology, 6thedition, S. Gilbert)

 9. Genetic potential, developmental restriction, differentiation: Developmental regulation of neural crest cell differentiation requires activation and expression of appropriate transcription factors and receptors. Some populations of neural crest-derived cells are pluripotent and although

they are capable of generating a remarkable number of differentiated cell types, their phenotypic repertoire is limited to the expression of those gene products that are appropriate for the target to which they have migrated. Heterotopic transplantation of these cells reveals their greater phenotypic capacity. Other crest-derived cells may constitute a more restricted population of stem cells. There are only a limited number of options in their genetic repertoire. Finally, some pre-migratory crest cells appear to be programmed for a specific developmental fate or if they are not committed before leaving the neuraxial crest, they are inhibited from further developmental expression during their migration.

4.3.3. SECONDARY NEURULATION

Secondary neurulation is a morphological process described since the second half of the 19th century; it accounts for the formation of the caudal spinal cord in mammals including humans. A similar process takes place in birds. This form of neurulation is caused by the growth of the tail bud region, the most caudal axial region of the embryo. Experimental work in different animal species leads to questioning dogmas widely disseminated in the medical literature. Thus, it is established that the tail bud is not a mass of undifferentiated pluripotent cells but is made up of a juxtaposition of territories whose fate is different. The lumens of the two tubes generated by the two modes of neurulation are continuous.
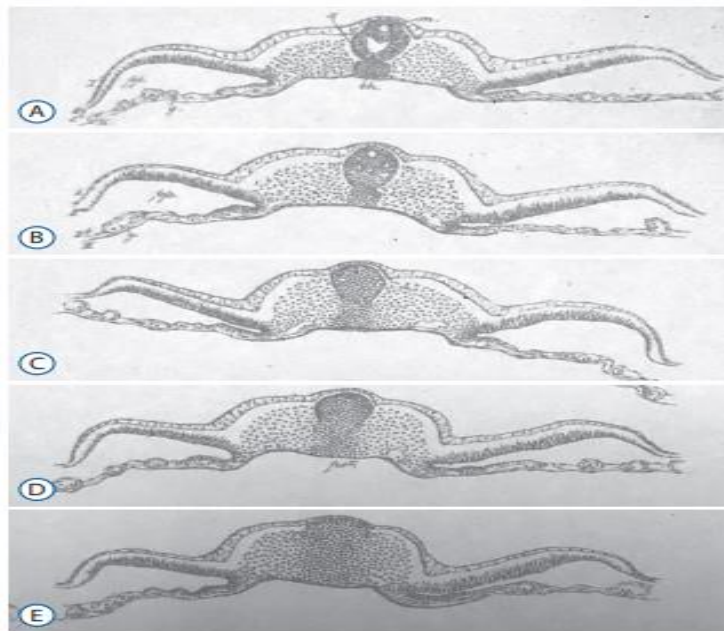
Fig. 4.12: Axial section of chick embryo from rostral (A) to caudal (E). This figure shows the first historical illustration of secondary neurulation in chick embryos. Multiple lumens can be evidenced in secondary neural tubes (A and B). C: The primordium of the spinal cord is formed as a solid cellular structure.D and E: Doros-ventral gradient of epithelialization (Adopted from Catala, 2021).

There seem to be multiple cavities in the human embryo, but discrepancies exist according to the authors. Finally, the tissues that generate the secondary neural tube are initially located in the most superficial layer of the embryo. These cells must undergo internalization to generate the secondary neurectoderm. A defect in internalization could lead to an open neural tube defect that contradicts the dogma that a secondary neurulation defect is closed by definition. Caudal to the posterior neuropore, the neural tube is formed by the process of secondary neurulation (Fig. 4.12).A rod-like condensation of mesenchymal cells forms beneath the dorsal ectoderm of the tailbud. Within the mesenchymal rod, a central canal forms by cavitation.This central canal becomes continuous with the one formed during primary neurulation and closure of the posterior neuropore. Because of the diminished development of the tail bud in humans, secondary neurulation is not a prominent process (Martin Catala, 2021).

PRIMARY VS SECONDARY NEURULATION

While primary neurulation forms most of the central nervous system in humans, a small area of the posterior spinal cord results from a distinct process called secondary neurulation. In this region, rather than having three distinct cell sheets, the embryo contains a mixture of loosely-packed cells covered by a thin layer of ectoderm. Some of these "loose" cells condense to generate a rod-like structure called the medullary or neural cord. This cord eventually hollows out, and merges with the more anterior primary neural tube, forming a continuous structure. Although secondary neurulation plays a relatively minor role in the formation of the human central nervous system, defects in this process can still have developmental consequences, such as certain types of spina bifida.

4.4 DEVELOPMENT OF MESODERM

The mesoderm generates all the organs between the ectodermal wall and endodermal tissues. During the third and fourth weeks of embryonic development the mesoderm is established as the 2nd germ layer. The mesodermal cells are organized into 4 regions: the axial mesoderm of the prechordal plate and notochord, paraxial mesoderm, intermediate mesoderm and lateral plate mesoderm. Each of these undergoes some form of segmentation. The most evident and complete segmentation occurs in the paraxial trunk mesoderm, where each segment becomes an entirely separate somite. Much of the paraxial and lateral plate mesoderm develops into mesenchyme, an embryonic connective tissue. The derivatives of mesenchyme are connective tissue proper,

cartilage, bone and blood. The cardiovascular and lymphatic systems are derived from mesoderm as well. Part of the paraxial mesoderm gives rise to all skeletal muscle cells. The intermediate mesoderm gives rise to most of the urogenital system. Part of the lateral plate mesoderm develops into the lining of the pericardial, pleural and peritoneal cavities.

Mesoderm Formation: Gastrulation is a series of cell movements that transforms the bilaminar germ disc (epiblast and hypoblast) into a 3 layered embryo (ectoderm, mesoderm, and endoderm, Fig. 4.13). Not only are the 3 germ layers established but cells also become committed to endodermal or mesodermal lineages during this process. The critical factors that determine the different fates of the mesodermal cell populations are: 1) the point of entrance of the epiblast cells into the primitive streak and 2) the direction of their subsequent migration. Depending on these 2 events, mesodermal cells can form tissues as varied as muscle, heart, kidney, or bone. After gastrulation the mesodermal sheet on either side of the notochord is a connected layer of undifferentiated mesenchymal cells (Fig. 4.13). During the third week, this undifferentiated mesoderm will begin to condense on both sides of the notochord to form the 1) paraxial, 2) Intermediate and 3) lateral plate mesoderm (Fig. 4.14). Starting on day 20 at what will be the base of the skull, the paraxial mesoderm (just lateral to the notochord; also called the segmental plate) begins to condense in a cranial to caudal direction (Fig. 4.15). These condensations will become the somites. Lateral to the paraxial mesoderm is the intermediate mesoderm. As the embryo begins to fold the intermediate mesoderm will lose its connection with the segmental plate and condense to form a solid mass of tissue running most of the length of the embryo. The intermediate mesoderm will give rise to the kidneys (two embryonic forms and the final adult form) and most of the urgenital tract, including gonadal tissue but excluding the primordial germ cells.
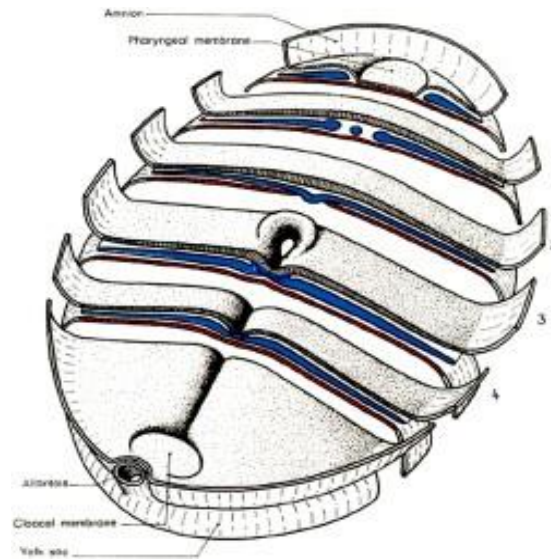
Fig. 4.13: Diagrammatic view with cross sections ofembryo during gastrulation
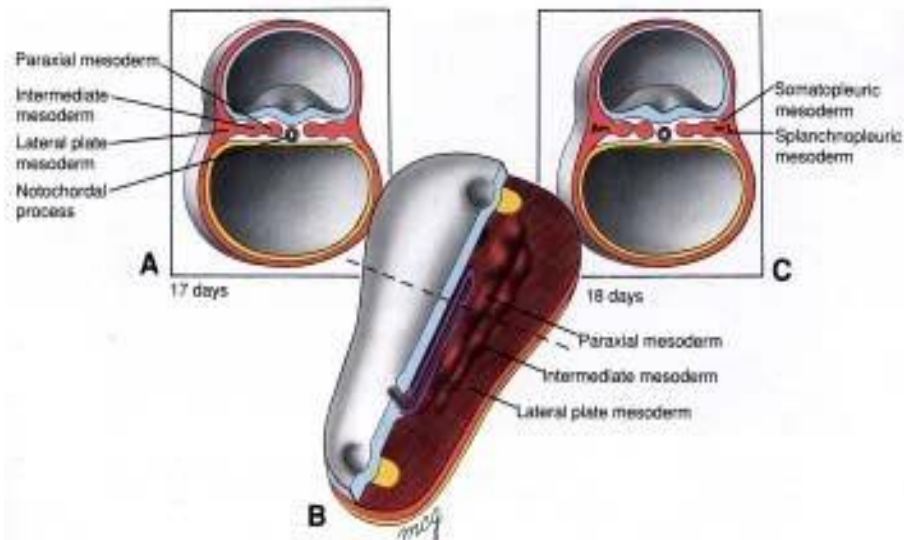
Fig. 4.14. Sections through a 17-day embryo showing the differentiation ofthe mesoderm on either side of the midline. (A) Early on day 17, the mesoderm has begun to differentiate into paraxial, intermediate, and lateral plate mesoderm. (B) Sagittal cutaway showing the rod-like condensations ofparaxial and intermediate mesoderm. The dotted line marks the plane of thetwo transverse sections. (C) Later on day 17, the lateral plate begins to vacuolate to form the rudiment of theintraembryonic coelom.

4.5 DEVELOPMENT OF ENDODERM

Animals have bodies of diverse shapes with internal collections of organs of unique morphology and function. Such sophisticated body architecture is elaborated during embryonic development,

whereby a fertilized egg undergoes a program of cell divisions, fate specification, and movements. One key process of embryogenesis is determination of the anteroposterior (AP), dorsoventral (DV), and left-right (LR) embryonic axes. Other aspects of embryogenesis are specification of the germ layers, endoderm, mesoderm, and ectoderm, as well as their subsequent patterning and diversification of cell fates along the embryonic axes. These processes occur very early during development when most embryos consist of a relatively small number of morphologically similar cells arranged in simple structures, such as cell balls or sheets, which can be flat or cup shaped. Gastrulation is a fundamental phase of animal embryogenesis during which germ layers are specified, rearranged, and shaped into a body plan with organ rudiments. The term gastrulation, derived from the Greek word *gaster*, denoting stomach or gut, is a fundamental process of animal embryogenesis that employs cellular rearrangements and movements to reposition and shape the germ layers, thus creating the internal organization as well as the external form of developing animals. Gastrulation is a complex series of cell movements that:

a. Rearranges cells, giving them new neighbors. These rearrangements put cells in a new environment, with the potential to receive new signals.

b. Results in the formation of the 3 germ layers that will form most of the subsequent embryo: Ectoderm**,** Endoderm and Mesoderm.

     The following general types of morphogenetic movements have been recognized:

a. Individual cells move by:

i. Migration -movement of individual cells over other cells or matrix.

ii. Ingression -movement of individual cells or small groups from an epithelium into a cavity.

b. Groups of cells move by:

i. Invagination -local inward buckling of an epithelium

ii. Involution -inward movement of a cell layer around a point or edge

iii. Epiboly -spread of an outside cell layer to envelop a yolk mass or deeper layer

iv. Delamination -splitting 1 cell sheet into 2 or more parallel sheets.

v. Convergent Extension -elongation of a cell layer in one dimension with shortening in another.

Epiboly: In the late blastula, the anterior half consists of micromeres which constitute the ectoderm while the posterior megameres constitute the endoderm. The germ ring forms the mesoderm. During epiboly the ectoderm overgrows backwards on the endoderm; ultimately the entire embryo (except for the small area called the yolk plug) is covered by the ectoderm. In other words the pigmented micromeres (animal half) grow over the megameres (vegetative half). The

reason for overgrowth is the rapid rate of division of micromeres.

Invagination: A small depression is formed in the region occupied by the grey crescent area. This depression grows inwards and forms the archenteron or gastrocoel or secondary body cavity. The outer opening of the gastrocoel is called the gastropole. As the gastrocoel increases in size the blastocoel gets reduced. Ultimately only a slit like semicircular cavity indicates the remnants of the blastocoel. The blastopore meanwhile becomes expanded and becomes ring shaped.

Involution: During this process the cells which have grown backward during epiboly now roll inside at the margin of the blastopore. The endoderm is the first to roll inside. The cells of the notochord and mesoderm which were formed outside now migrate over the lip of blastopore and become internal and arrange themselves on the roof, sides and the floor of the archenteron. The notochord cells are found on the roof along the midline. While the endoderm forms the anterior, lateral and ventral walls, the mesoderm forms wing like extensions in the archenteron.

Convergence: Convergence means the movement of cells towards a particular point. The presumptive cells of the notochord and mesoderm located on the surface of the blastula move towards the blastopore or primitive streak.

Infiltration: This involves the detachment of individual cells or groups of cells from the surface of the blastula and their falling into the blastocoels. In the blastocoels they arrange themselves as a single layer.

Divergence: It refers to the migration of involuted cells from the blastopore or primitive streak. In divergence the cells move in different directions from a sngle point. The involuted cells of notochord and mesoderm migrate and diverge from the blastopore and primitive streak to their future positions within the developing embryo.

Ingression: Ingression involves movement of individual or groups of cells from the external layer of blastula into the blastocoels. It is categorized into two types-unipolar and multipolar.
Unipolar ingression: in which individual cells migrate inwards at one end of a blastoderm.eg. Porifera and Coelenterata
Multipolar ingression: in which individual cells migrate inwards from all points of the blastocoels. Eg. *Echidna*

Delamination: The word delamination means mass separation of groups of cells from other cell groups. The separation of endodermal, mesodermal and notochordal cells from each other in teleost fishes is a good example for delamination. According to a widely accepted view the endoderm formation in birds takes place by delamination.

Germ Layer theory and derivatives of germ layers: In 1817, Pander described a trilaminar condition of the chick blastoderm. Later this trilayer concept was proved true for many types of embryos and this concept became an accepted embryological principle. Towards the end of 19th centuary the terms ectoderm, endoderm and mesoderm were introduced to refer to the outer, inner and middle layers of the embryo respectively. The adult organs do not arise directly from the cells derived by the cleavages of the zygote. The embryonic cells are at first arranged into layers called

germ layers from which various organs are formed. This concept is known as germ layer theory. Tissues and organs of animals arise from layers, or blocks, of embryonic cells called primary germ layers. Their development from a nondescript form in the early embryo to their form in late embryonic through adult stages is called differentiation. Ectoderm, gives rise to the outer body wall. Endoderm forms the inner lining of the digestive cavity. Mesoderm gives rise to tissues between ectoderm and endoderm. Undifferentiated mesoderm develops into muscles, blood and blood vessels, skeletal elements, and other connective tissues

Table 4.1: Derivatives of three germinal layers

Ectoderm	Mesoderm	Endoderm
Nervous tissue	Connective tissues, Dermis of the skin and Muscles	Gut tract lining
Epidermis of the skin	Circulatory system, Excretory structures Reproductive structures	Digestive glands
Sensory organs	Bones, tendons and ligaments	Respiratory tract lining
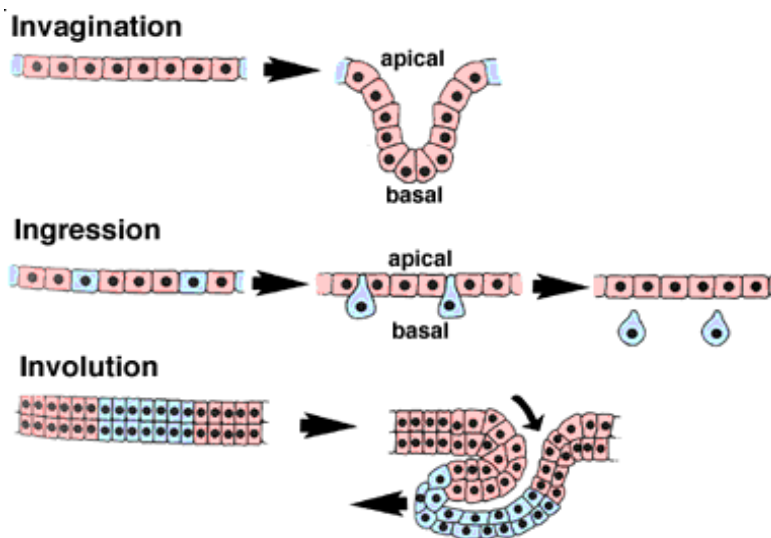
Fig. 4.15 a: Morphogenetic cellular movement during gatrulation
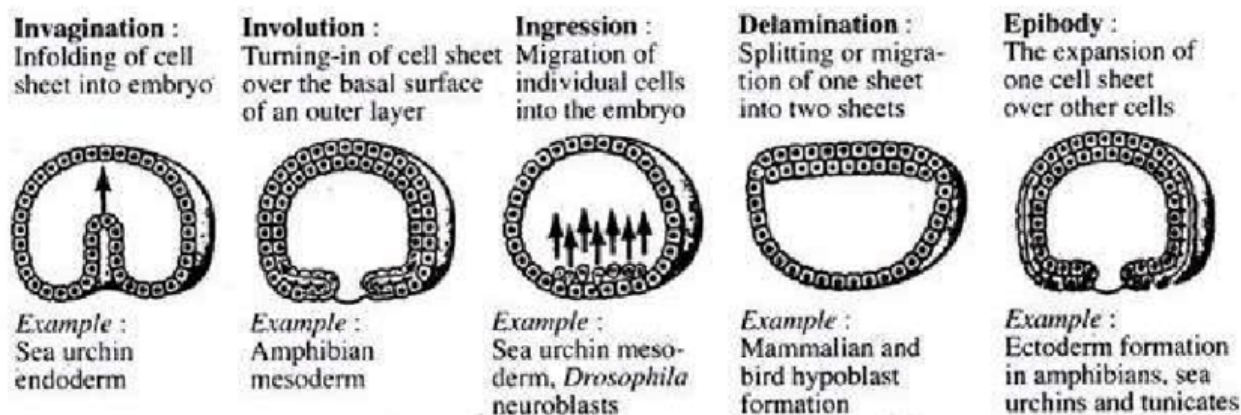
Fig. 4.15 b: Morphogenetic cellular movement during gatrulation

4.6 SUMMARY

Neurulation is a process in which the neural plate bends up and later fuses to form the hollow tube that will eventually differentiate into the brain and the spinal cord of the central nervous system. In humans, it begins in the 3rd week after fertilization and requires that the top layers of the embryonic germ disc elevate as folds and fuse in the midline. Neurulation is the embryological process that forms the precursors of the central nervous system and occurs after gastrulation. It has established the three primary cell layers of the embryo: ectoderm, mesoderm, and endoderm. In humans, the majority of this system is formed via primary neurulation, in which the central portion of the ectoderm—originally appearing as a flat sheet of cells—folds upwards and inwards, sealing off to form a hollow neural tube. As development proceeds, the anterior portion of the neural tube will

give rise to the brain, with the rest forming the spinal cord. The epidermis, the central and peripheral nervous systems, and some non-neuronal cells of the head and heart are derived from ectoderm. During the third week of gestation, a portion of the dorsal ectoderm is specified to become neural ectoderm. This region of the embryo is called the neural plate. The process by which the neural plate forms a neural tube is called neurulation. Neurulation is the embryological process that forms the precursors of the central nervous system and occurs after gastrulation.

Primary neurulation refers to the formation of the neural tube from the neural plate, situated between the anterior and posterior neuropores. The primary neurulation forms most of the central nervous system in humans, a small area of the posterior spinal cord results from a distinct process called secondary neurulation. In this region, rather than having three distinct cell sheets, the embryo contains a mixture of loosely-packed cells covered by a thin layer of ectoderm. Some of these "loose" cells condense to generate a rod-like structure called the medullary or neural cord. This cord eventually hollows out, and merges with the more anterior primary neural tube, forming a continuous structure. Although secondary neurulation plays a relatively minor role in the formation of the human central nervous system, defects in this process can still have developmental consequences, such as certain types of spina bifida.

The mesoderm generates all the organs between the ectodermal wall and endodermal tissues. During the third and fourth weeks of embryonic development the mesoderm is established as the 2nd germ layer. The mesodermal cells are organized into 4 regions: the axial mesoderm of the prechordal plate and notochord, paraxial mesoderm, intermediate mesoderm and lateral plate mesoderm. Each of these undergoes some form of segmentation. The most evident and complete segmentation occurs in the paraxial trunk mesoderm, where each segment becomes an entirely separate somite. Much of the paraxial and lateral plate mesoderm develops into mesenchyme, an embryonic connective tissue. The derivatives of mesenchyme are connective tissue proper, cartilage, bone and blood. The cardiovascular and lymphatic systems are derived from mesoderm as well. Part of the paraxial mesoderm gives rise to all skeletal muscle cells. The intermediate mesoderm gives rise to most of the urogenital system. Part of the lateral plate mesoderm develops into the lining of the pericardial, pleural and peritoneal cavities.

Animals have bodies of diverse shapes with internal collections of organs of unique morphology and function. Such sophisticated body architecture is elaborated during embryonic development, whereby a fertilized egg undergoes a program of cell divisions, fate specification, and movements. One key process of embryogenesis is determination of the anteroposterior (AP), dorsoventral (DV), and left-right (LR) embryonic axes. Other aspects of embryogenesis are specification of the germ layers, endoderm, mesoderm, and ectoderm, as well as their subsequent patterning and diversification of cell fates along the embryonic axes. These processes occur very early during development when most embryos consist of a relatively small number of morphologically similar cells arranged in simple structures, such as cell balls or sheets, which can be flat or cup shaped. Gastrulation is a fundamental phase of animal embryogenesis during which germ layers are specified, rearranged, and shaped into a body plan with organ rudiments. The term gastrulation, derived from the Greek word *gaster*, denoting stomach or gut, is a fundamental process of animal embryogenesis that employs cellular rearrangements and movements to reposition and shape the germ layers, thus creating the internal organization as well as the external form of developing animals.

4.7 TERMINAL QUESTIONS AND ANSWERS

Q.1 What is neurulation. Explain in detail.

Q.2 what are the steps in neurulation, describe in points and illustrate in diagrams.

Q.3 Write a short note on the importance of Neurulation.

Q.4 What is secondary neurulation?

Q.5 Explain how the neural tube formed.

Q.6 Explain the process of mesoderm formation.

Q.7 Write the short note on morphogenetic cellular movements.

4.8 REFERENCES

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UNIT-5 ORGANOGENESIS AND ORGANIZER CONCEPT

CONTENTS

5.1 OBJECTIVES

This chapter will assist in understanding:

1. The mechanism of chick brain, eye, and heart development.

2. The process of embryonic induction.

3. Primary organizers and their morphological differentiation.

4. Primary organizer's point of origin and inductive contact.

5. The inductive signal's nature (Possible mechanism of neural induction).

6. Concept of competence.

5.2 INTRODUCTION

The development of an egg into a fully grown chick appears to be a wonderful magical event. Despite being extremely complex, the process of creating a chick from an egg cannot be understood without a rational understanding of the embryology of the growing embryo. Before the chick hatches from the egg, it is incubated for three weeks.

Since some embryonic development has already taken place at the moment of egg laying, it is halted until favorable environmental circumstances are created for the incubation to resume. At first, all cells are identical, but throughout time, they begin to differentiate into distinct types, such as some becoming critical organs and others turning into wings or legs.

A pointed, thicker layer of cells known as a **"Primitive Streak"** that develops the embryo's longitudinal axis can be observed in the caudal, or tail, region when incubation begins (by 18 hours of incubation). The head and backbone of the chick are derived from it. Along with these structures, the precursor to the digestive tract, blood islands that subsequently give rise to the vascular or blood system, and the beginning of eye development all appear.

On the second day of incubation, the blood islands that originally emerged on the first day of incubation begin to connect to each another to build the vascular system, while the creation of the heart occurs simultaneously somewhere else. On the 44th hour of incubation, the circulatory system and heart are linked, and the heart starts to beat. An embryonic circulatory system for the embryo and a vitelline system supplying the egg are both created at this stage. The liver also develops. The endoderm begins to give rise to the interior lining epithelium of the respiratory,

immunological, and digestive systems on the same day. By the twelfth day, the digestive system is fully developed and the organs begin to visualize.

By the conclusion of the third day of incubation, the limb buds for the wings and legs are apparent. Additionally, the beak begins to form. Torsion and flexion continue for the entire fourth day of incubation, twisting the chick's entire body 90 degrees, causing it to lie down with its left side on the yolk. As the embryo's head and tail move toward one another, it assumes a "C" form. Mouth, tongue, and nasal pits develop from the respiratory system and alimentary canal. Even though it's outside the body, the heart is growing and can be seen beating by cracking open an egg. The tissue that eventually becomes the respiratory organs is undifferentiated and disordered at this stage. Other internal organs also continue to grow at the same time. As a result, by the end of the fourth day of incubation, the chick embryo has grown all of its vital organs.

The embryo develops quickly, and by the seventh day of incubation, the heart is entirely enclosed in the thoracic cavity. The wings and feet's digits are visible. The immune system starts taking shape as the spleen, thymus, and cloacal bursa began to appear on the tenth day. After the tenth day of incubation, feather and feather tracts are apparent, and the differentiation of the respiratory system is also complete. The beak also becomes harder. Syrinx, however, is not noticed until the 19th day. On the fourteenth day, when the claws are formed, the embryo starts to migrate towards the hatching position. On the twentieth day, when the beak pierces the air cell and pulmonary breathing starts, the embryo is in the hatching position.

After 21 days of incubation, the chick finally starts to break out from the shell by poking its beak through the air cell. By this point, the allantois, which had served as the chick's lungs, is beginning to dry out. At this point, the chick is using its lungs. As the chick continues to thrust its head outward, the egg teeth (sharp horny structures) on the upper beak and the muscles on the back of the neck cut the shell. The process continues as the chick shifts positions and continues to cut the shell until its head pops out of the cracked shell. Following that, it kicks itself free from the bottom of the shell. The bird becomes fatigued after such a lengthy workout, and it sleeps while its naval openings mend and it dries from the bottom. It gets strength once more and begins to move. Within a few days of the chick's hatching, the horny cap falls off the beak.

Due to the economic significance and expansion of the use of this animal model in studies like genetics, the study of chicken organogenesis based on germ layers is exceedingly complex and understudied. As a result, it is crucial to understand chicken embryology.

5.3 DEVELOPMENT OF ORGANS IN CHICKS

Development of brain, eye and heart: The telolecithal egg of the chick undergoes discoidal meroblastic cleavage after fertilization in the oviduct, during which the blastoderm is separated into a tissue that is 5–6 cell layers thick by equatorial and vertical cleavages. Tight junctions are typically used to connect these cells. The **subgerminal cavity** is a compartment located between the blastoderm and the yolk

(Fig.5.1). At this point, the deep cells in the blastoderm's center die and are shed, leaving an **area of pellucida** that is one cell thick. The majority of the embryo is made up of this portion of the blastoderm. The **area opaca** is made up of the outer ring of blastoderm cells that have not yet lost their deep cells. The **marginal zone** is a thin layer of cells that lies between the **area pellucida** and the **area opaca** (or marginal belt). During the early stages of chick development, certain marginal zone cells play a crucial role in determining cell destiny.
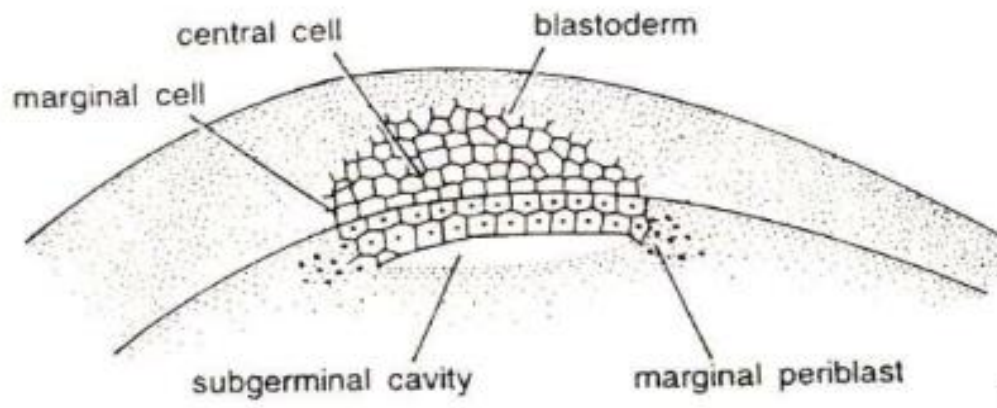
*Fig.5.1. Chick embryo in blastula stage (**source:** https://biologyease.com)*

When a hen lays an egg, it has about 20,000 cells. The majority of the cells in the **area of pellucida** form the epiblast at the surface, but some of the remaining cells have delaminated and moved separately into the submarginal cavity to create **polyinvagination islands** or **primary hypoblast**. After a short period, a layer of cells from the blastoderm's posterior boundary moves anteriorly to join the poly invagination islands, forming the **secondary hypoblast**. Epiblast and hypoblast, the two layers of the blastoderm, are fused at the edge of the **area opaca**, and the space between the layers creates a **blastocoel**. Thus, the bird embryo completely comes from the epiblast. The growing embryo receives no cells from the hypoblast. The hypoblast cells, on the other hand, are responsible for the formation of some of the exterior membranes, particularly the yolk sac and the stalk connecting the yolk mass to the endodermal digestive tube. The epiblastic cells develop

into the three germ layers of the embryo proper, as well as a sizeable portion of the extraembryonic membrane.

5.3.1. DEVELOPMENT OF BRAIN

The ectoderm is given instructions to build the nervous system and the epidermis, as is now widely accepted. A section of the dorsal ectoderm is designated as neural ectoderm, and its cells can be identified by their columnar shape. The **neural plate** is the name of this area of the embryo. **Neurulation** is the process by which this tissue develops into a neural tube, the beginning of the central nervous system. An embryo going through this process is referred to as a **neurula** (Fig.5.2).

Formation of the neural tube: There are primarily two methods for creating a neural tube. The cells that surround the neural plate instruct the neural plate cells to multiply, invaginate, and pinch off from the surface to form a hollow tube during **primary neurulation**. The neural tube develops during **secondary neurulation** from a solid cord of cells that enters the embryo and then cavitates to form a hollow tube. Different vertebrate classes utilize these processes of formation to different extents.
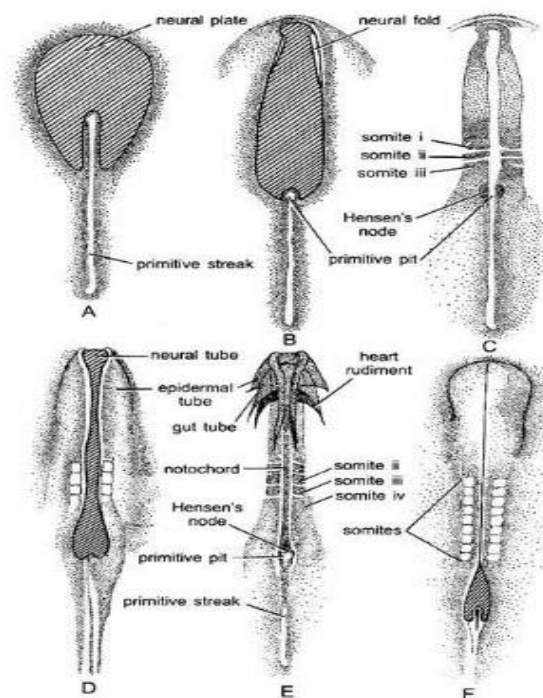
Fig.5.2: Neurulation in Chick. (A) Primitive streak bearing embryo, neural plate epidermis ; (B) Antero-posterior extension of neural plate formation of the neural folds and shortening of the primitive streak

(stage 7-8); (C)Internal anatomy of the embryo (stage 6-8); (D) Surface view of the embryo of the stage having 3-4 pairs of somites; (E) Internal anatomy of stage 8 embryo;(F) Surface view of the embryo of 9-10 pairs of somites. (source:https://www.notesonzoology.com/vertebrates/chick/development-of-chick-with-diagram-vertebrates-chordata-zoology/8645)

Primary neurulation: Fig. 5.3 shows the chick's major neurulation events. The original ectoderm is split into three groups of cells during primary neurulation: the neural tube, which will eventually give rise to the brain and spinal cord, the skin's epidermis, and the neural crest cells. The neural crest cells develop at the area that joins the neural tube and epidermis before migrating elsewhere. These cells produce the skin's pigment cells, peripheral neurons, and glia, as well as several other cell types. In mammals, birds, reptiles, and amphibians, the main neurulation mechanism seems to be comparable. A U-shaped neural groove develops in the center of the neural plate shortly after it forms, dividing the future right and left sides of the embryo. The neural plate's margins thicken and travel upward to produce the neural folds. The neural folds move toward the embryo's midline and eventually join to form the neural tube, which lies beneath the ectoderm above. The neural crest is formed from the cells at the dorsalmost end of the neural tube. Distinct parts of the body have slightly different processes for neurulation to take place. The inductive link between the pharyngeal endoderm, prechordal plate, and notochord and its covering ectoderm is reflected in how the head, trunk, and tail of the neural tube each form their respective regions. The formation of the neural plate, shaping of the neural plate, bending of the neural plate to form the neural groove, and closing of the neural groove to form the neural tube are the four distinct but spatially and temporally overlapping stages of primary neurulation that occur in the head and trunk regions.
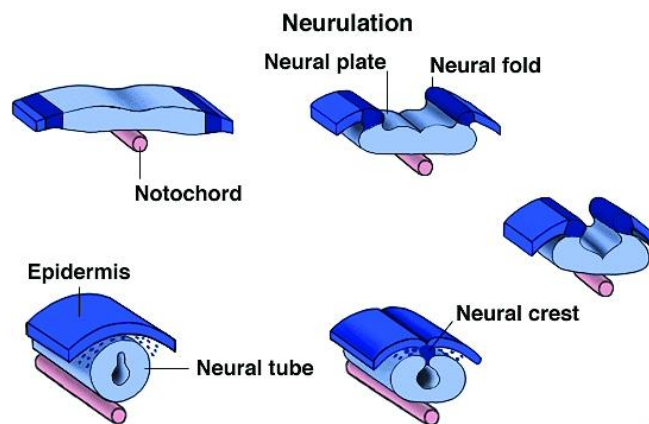
Fig.5.3.Neurulation in Chick development
(Source:https://biology.kenyon.edu/courses/biol114/Chap14/Chapter_14B.html)

Formation and shaping of the neural plate: When the pharyngeal endoderm in the head area and the underlying dorsal mesoderm signal the ectodermal cells above them to expand into columnar neural plate cells, neurulation occurs. The cells of the potential neural plate can be distinguished from the flatter pre-epidermal cells around them by their elongated shape. The neural plate contains up to 50% of the ectoderm. The inherent motions of the epidermal and neural plate areas mold the neural plate.

The neural plate enlarges along the anterior-posterior axis and contracts to produce a tube-like shape when bent later. The neural plate expands and contracts via convergent expansion in both amphibians and amniotes, intercalating several layers of cells into a few layers. The neural plate cells also divide preferentially in the rostral-caudal (beak-tail; anterior-posterior) orientation. Even if the affected tissues are isolated, these events will still take place. In an isolated neural plate, the cells converge and spread out to form a narrower plate, but they do not roll up into a neural tube. It will, however, develop into tiny neural folds in culture if the "boundary region"—which contains both presumed epidermis and neural plate tissue—is isolated (Fig.5.4).

Bending of the Neural Plate: Where the neural tube touches the surrounding tissues, hinge areas are formed, causing the neural plate to bend. In these areas, the presumed epidermal cells stick to the lateral neural plate margins and push them in the direction of the midline (Fig.5.4). The cells in the neural plate's midline are known as **medial hinge point** (MHP) cells in both birds and mammals. They come from Hensen's node's anterior midline and the area of the neural plate immediately in front of it. The MHP cells form a hinge that creates a furrow at the dorsal midline and anchors to the notochord beneath them. The MHP cells become shorter and more wedge-shaped when attached to the notochord. The MHP's side cells do not experience this alteration**.** Soon later, two more hinge regions develop furrows close to where the neural plate connects to the remaining ectoderm. The surface ectoderm of the neural folds serves as an anchor for these areas, which are known as the dorsolateral hinge points (DLHPs). These cells also grow taller and develop a wedge shape.

Changes in cell shape are closely related to cell wedging. Both microtubules and microfilaments are implicated in these modifications in the DLHPs. The neural plate is initially furrowed, and then it bends around these hinge areas. Each hinge serves as a center, controlling how the cells rotate around it. Extrinsic factors are also in action in the meanwhile. The surface ectoderm of the chick embryo, which thrusts in the direction of the embryo's midline, is another force that bends the neural plate (Figure 5.4.). It may also be essential for the neural tube to invade internally rather

than externally for the neural plate to adhere to the underlying mesoderm and the assumed epidermis to migrate. The neural folds are produced by the neural tube being furrowed and the presumed epidermis being pushed toward the center.
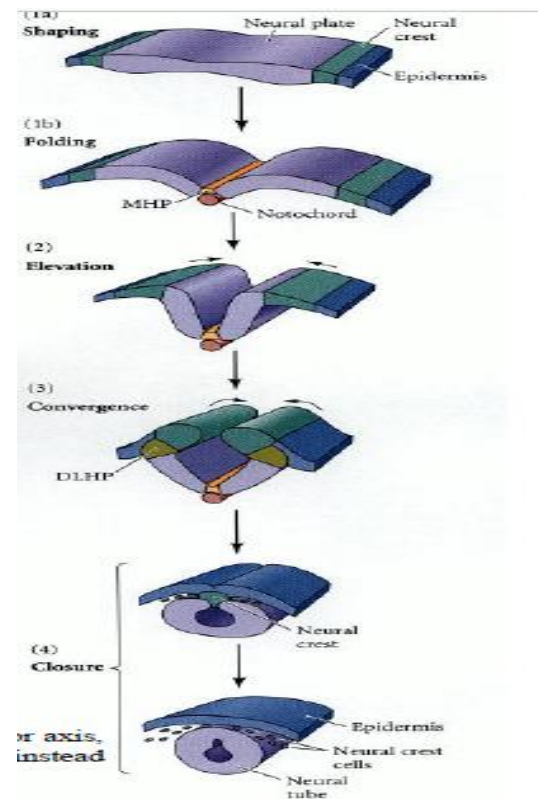
Fig.5.4. Formation and shaping of the neural plate
(Adapted from Developmental-Biology-7[th]ed-sf-Gilbert-pdf)

Closure of the neural tube: As the paired neural folds are brought together at the dorsal midline, the neural tube closes. The cells from the two folds combine when the folds cling to one another. The cells at this juncture give rise to neural crest cells in some species. The neural tube at the dorsal part of the bird does not close before the neural crest cells move from that area. However, in mammals, the cranial neural crest cells—which develop into the facial and neck structures—migrate as the neural folds rise, or before the neural tube closes. In contrast, the crest cells in the spinal cord region wait until the closure has taken place. Not all areas of the ectoderm experience the neural tube's closure at once. This is most evident in vertebrates with elongated body axes before neurulation, such as birds and mammals. While gastrulation is still occurring in the embryo's caudal (tail) area, neurulation is well underway in the cephalic (head) region. Changes in the shape of the tube leading to regionalization of the neural tube as well. The wall of the tube is broad and

thickest towards the cephalic end, where the brain will develop. The distinct brain compartments are defined by a succession of swells and constrictions in this area. The neural tube, however, continues to be a straightforward tube from the caudal to the head area that tapers out toward the tail. The anterior neuropore and the posterior neuropore are the two open ends of the neural tube. Mammals' neural tube closure begins at many locations along the anterior-posterior axis, in contrast to chicks, where neurulation begins at the level of the future midbrain and "zips up" in both directions.
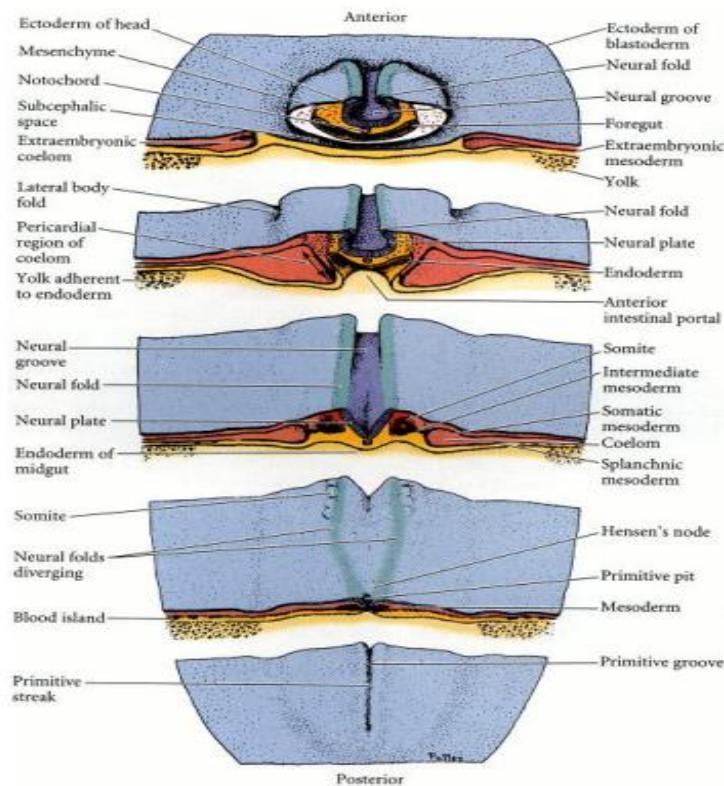
Fig.5.6. Stereogram of developing chick embryo (24 hr stage)
(Adapted from developmental-biology-7[th]ed-sf-gilbert-pdf)

The neural tube eventually separates from the surface ectoderm to form a closed cylinder. It is believed that the expression of several cell adhesion molecules mediates this separation. Although the neural tube-forming cells initially produce E-cadherin, they stop making this protein as the neural tube develops in favor of N-cadherin and NCAM (Fig.5.7). The surface ectoderm and neural tube tissues stop adhering to one another as a result.

Secondary neurulation: A medullary cord is created during secondary neurulation, and the cord is then hollowed out to form a neural tube**.** Secondary neurulation typically occurs in the neural tubes of the lumbar (abdominal) and tail vertebrae in frogs and chicks. It can be viewed as a continuation

of gastrulation in both situations. In the frog, the cells of the dorsal blastopore lip continue to develop ventrally rather than involuting into the embryo. Precursors for both the posteriormost component of the neural plate and the posterior portion of the notochord can be found in the region that is developing near the tip of the lip, known as the **chordoneural hinge.** The 1.2 mm diameter, somewhat spherical gastrula grows in this area, becoming a linear, and 9 mm long tadpole. The blastopore cells that line the neurenteric canal are directly descended from the dorsal blastopore lip, which is located at the tip of the tail.

The distal component of the neurenteric canal becomes the ependymal canal (i.e., the neural tube lumen), while the proximal portion unites with the anus (Fig.5.8).
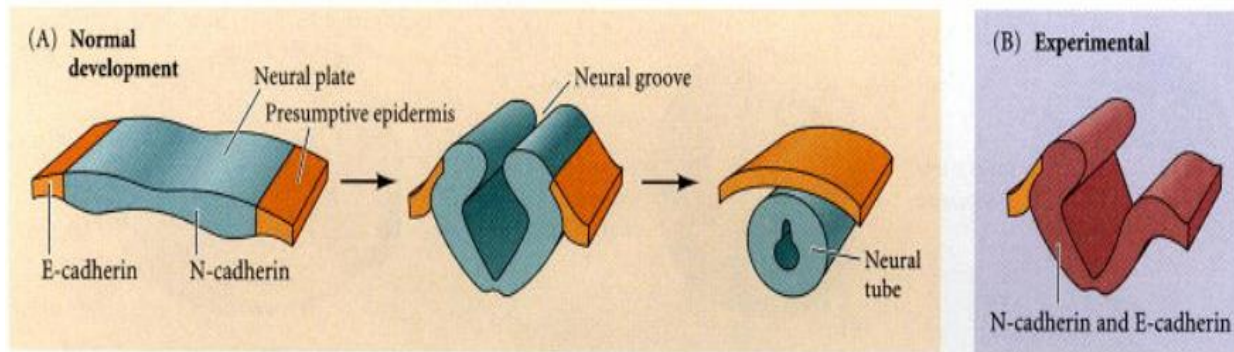
Fig.5.7: Showing neural tube cells producing N-cadherin and E-cadherin

(Adapted from developmental-biology-7thed-sf-gilbert-pdf)
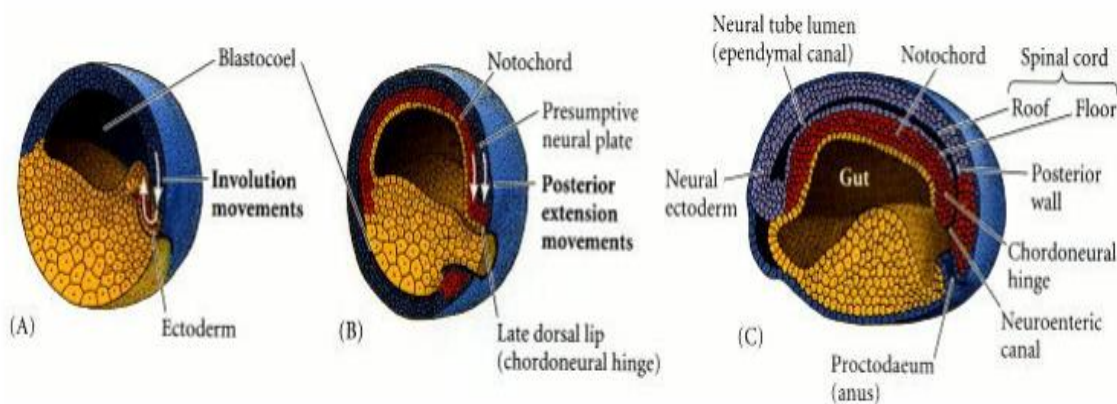
Fig.5.8: Secondary neurulation

(Adapted from developmental-biology-7thed-sf-gilbert-pdf)

Development of the neural tube: Three distinct processes simultaneously separate the neural tube into the different regions of the central nervous system. The neural tube and its lumen expand and contract to form the brain and spinal cord's chambers at the gross anatomical level. The diverse functional regions of the brain and spinal cord are formed at the tissue level by the rearrangement of the cell populations within the neural tube wall. The neuroepithelial cells themselves finally differentiate into the many types of nerve cells (neurons) and supporting cells (glia) present in the body at the cellular level.

The anterior-posterior axis: The neural tube of the early mammalian has a straight structure. The anterior part of the tube, however, is experiencing significant modifications even before the posterior portion of the tube has developed. The neural tube expands here, forming the forebrain (prosencephalon), midbrain (mesencephalon), and hindbrain (rhombencephalon).

The optic vesicles have spread laterally from each side of the growing forebrain by the time the posterior end of the neural tube closes. The front **telencephalon** and the more caudal **diencephalon** are divisions of the **prosencephalon**. The diencephalon eventually gives rise to the thalamic and hypothalamus brain areas that receive neuronal input from the retina, whereas the telencephalon eventually gives rise to the cerebral hemispheres. The cerebral aqueduct finally develops from the lumen of the **mesencephalon,** which does not further split. A posterior **myelencephalon** and a more anterior **metencephalon** separate from the rhombencephalon. The medulla oblongata, whose neurons produce the nerves that control respiratory, gastrointestinal, and cardiovascular motions, finally develops from the myelencephalon. The cerebellum, the area of the brain in charge of controlling posture, balance, and movement, develops from the metencephalon. The rhombencephalon forms a segmental pattern that identifies the locations from where particular nerves emerge. The rhombencephalon is divided into smaller compartments by recurring swellings known as rhombomeres. These rhombomeres serve as distinct developmental "territories" since cells can freely mingle within each one, but not with cells from other rhombomeres. Furthermore, the developmental fate of every rhombomere is unique. Every rhombus will develop ganglia, which are collections of neuronal cell bodies whose axons make a nerve. The chick, in which the first neurons occur in the even-numbered rhombomeres, r2, r4, and r6, has been the subject of the most thorough research on the development of the cranial nerves from the rhombomeres. The fifth (trigeminal) cranial nerve is made up of neurons from the r2 ganglia; the seventh (facial) and eighth (vestibuloacoustic) cranial

nerves are made up of neurons from r4, and the ninth (glossopharyngeal) cranial nerve is made up of neurons from r6.

The early embryonic brain balloons at an astonishing rate and scale, and it does so predominantly due to an increase in cavity size rather than tissue growth. The brain volume in the developing chick embryo increases thirty-fold between days three and five. This quick expansion is assumed to be brought on by the fluid inside the neural tube exerting positive fluid pressure against the walls of the tube. With this pressure, the surrounding dorsal tissues push in to compress the neural tube at the base of the brain as the neural folds close in the region between the presumed brain and the presumed spinal cord. The putative brain region and the prospective spinal cord are effectively divided by this blockage (Fig.5.10)
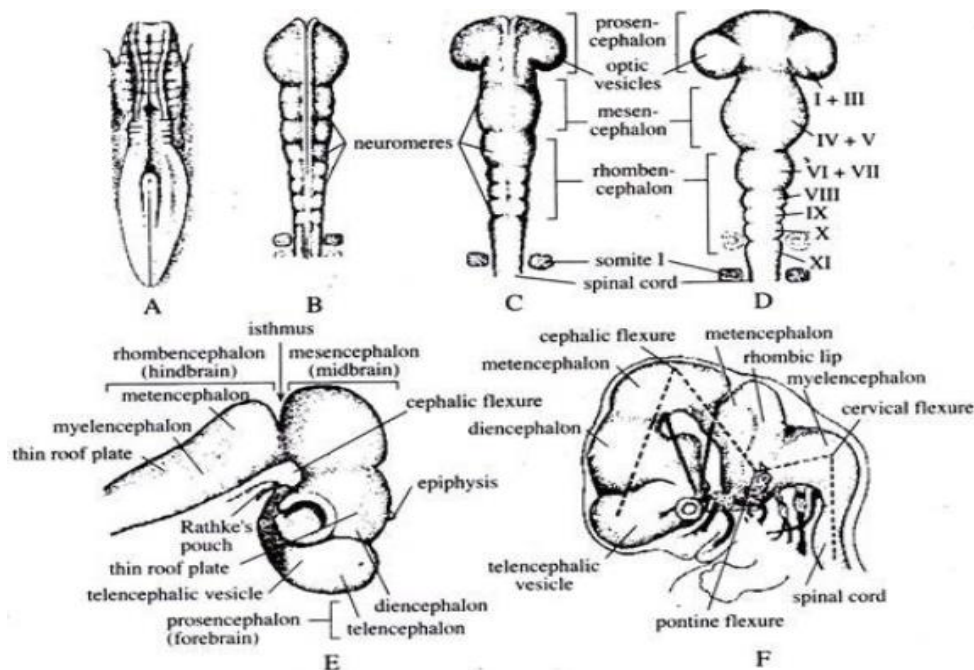
Fig.5.10.Early development of the brain in chick embryo (Source: https://www.notesonzoology.com/wp-content/uploads/2016/07/clip_image008-49.jpg)

The dorsal ventral axis: The dorsal-ventral axis determines the polarity of the neural tube. For example, in the spinal cord, the ventral region is home to the motor neurons, whereas the dorsal region is where the spinal neurons receive information from sensory neurons. Numerous interneurons in the middle act as communication hubs. Signals emanating from the neural tube's immediate environment cause it to become polarised. The epidermis imposes the dorsal pattern, while the notochord induces the ventral pattern.

Ventral patterning of the neural tube: It indicates that external tissues act as a mediator in the specification of the ventral neural tube. The Sonic hedgehog protein, which most likely originates

from the notochord, is one agent of ventral specification. Retinoic acid, which most likely originates from the nearby somites, is another agent that specifies the types of ventral neural cells. The protein sonic hedgehog creates a gradient, and differing concentrations of it lead to the emergence of various cell types. The medial hinge cells are induced by the secreted Sonic hedgehog to develop into the neural tube's floor plate. Sonic hedgehog, which produces a gradient with the highest concentration at the most ventral part of the neural tube, is likewise secreted by these floor plate cells. The cells near the floor plate that are exposed to high levels of Sonic hedgehog develop into ventral (V3) neurons, while the cells after them that are exposed to slightly lower levels of Sonic hedgehog develop into motor neurons. The V2 and V1 interneurons are the next two groups of cells, which receive progressively less of this protein. The various sonic hedgehog concentrations work by triggering the expression of various transcription factors in various populations of neurons. By activating the genes whose protein byproducts give the cell its identity, these transcription factors in turn activate the genes. It's also possible that sonic hedgehog suppresses the production of genes that make transcription factors for the dorsal neural tube. Otherwise, the neural tube would express these genes throughout.

Dorsal patterning of the neural tube: Proteins from the TGF-superfamily (Transforming Growth Factors), in particular the bone morphogenetic proteins 4 and 7 (BMP4 AND BMP7), dorsalin, and activin determine the dorsal destiny of the neural tube. The epidermis is where BMP4 and BMP7 were initially identified. The epidermis creates a secondary signaling center by stimulating BMP4 expression in the neural tube's roof plate cells, just as the notochord does with the floor plate cells on the ventral side of the neural tube. In neighboring cells, the roof plate's BMP4 protein triggers a cascade of TGF-superfamily protein. As a result, different cell types are exposed to various TGF-superfamily protein quantities at various times (Most dorsal exposed to more factors at higher quantities and sooner). Different types of transcription factors are induced by the TGF-superfamily proteins at different distances from the roof plate, which confers distinct identities on the cells.

Tissue architecture of the central nervous system: The brain's neurons are arranged into layers (called cortexes) and clusters (called nuclei), each of which has distinct connections and functions. A germinal neuroepithelium with one cell layer of thickness makes up the original neural tube. This is a layer of neural stem cells that divide quickly.

It is known as the neuron's birthday since this vertical division marks the last time the latter cell will divide. Birthdays of various glial and neuronal cell types occur at various times. The cells with the earliest birthdays travel over the shortest distances, according to labeling at various developmental stages. The more superficial parts of the cortex are formed by the migration of cells

with later birthdays through these layers. The places occupied by these neurons outside the germinal neuroepithelium determine the subsequent differentiation.

Spinal cord and medulla organization: The migrating cells create a second layer surrounding the initial neural tube as the cells close to the lumen keep dividing. As new cells from the germinal neuroepithelium are introduced to it, this layer thickens more and more. The germinal epithelium is now referred to as the ventricular zone (and, later, the ependyma), and this new layer is known as the mantle (or intermediate) zone. Mantle zone cells can develop into glia and neurons. The neurons link with one another and project axons away from the lumen, resulting in a marginal zone devoid of cells. Many of the axons in the marginal zone eventually develop myelin sheaths made of glial cells, which give them a whitish appearance. As a result, the axonal, peripheral layer is frequently referred to as the white matter, whereas the mantle zone, which contains the neuronal cell bodies, is frequently dubbed the grey matter. This fundamental three-zone arrangement of the ependymal, mantle and marginal layers is maintained throughout development in the spinal cord and medulla. A butterfly-shaped structure made of white matter gradually surrounds the grey matter (mantle), and both are then covered in connective tissue. The sulcus limitans, a longitudinal groove, separates the neural tube into dorsal and ventral halves as it develops. While the ventral region is responsible for numerous motor actions, the dorsal portion receives input from sensory neurons (Fig.5.11).
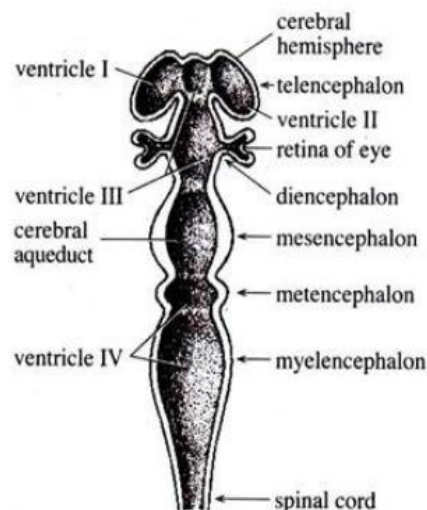
Fig.5.11.Showing basic five-part anatomy of Chick brain
(Source: https://www.notesonzoology.com/zoology/development-of-brain-in-chick/2697)

Cerebellar organization: The three-zone pattern changes in the brain as a result of cell migration, altered neuronal growth, and selective cell death. Some neural progenitors enter the marginal zone

of the cerebellum to create nuclei, which are collections of neurons. As a relay station between the outer layers of the cerebellum and other regions of the brain, each nucleus functions as a single functional unit. Some neural progenitors can also migrate away from the germinal epithelium in the cerebellum. Near the outermost limit of the neural tube, these precursor cells, known as neuroblasts, move to the cerebellum's growing outer surface and establish a new germinal zone called the external granule layer. Neuroblasts multiply at the one to two cells thick outer edge of the external granule layer. Postmitotic neuroblasts, which are the ancestors of the granule neurons that make up the majority of the cerebellar cortex, are found in the inner compartment of the external granule layer. The internal granule layer is created when these granule neurons migrate back into the growing cerebellar white matter. The cerebellum's original ependymal layer also produces a diverse range of neurons and glial cells, including the recognizable and large Purkinje neurons. In addition to being essential for the cerebellum's electrical system, granule neurons are supported by Purkinje neurons. Sonic hedgehog, which is secreted by the Purkinje cell, promotes the division of granule neuron progenitors in the external granule. Each Purkinje neuron has a massive dendritic arbor that extends like a tree above a cell body that resembles a bulb. More than any other neuron investigated, a typical Purkinje neuron can create up to 100,000 connections (synapses) with other neurons. Additionally, each Purkinje neuron releases a thin axon that joins to neurons in the deep cerebellar nuclei. For the cerebellum to operate properly, a spatial organization must be developed. The Purkinje cells, the sole output neurons of the cerebellar cortex, are eventually regulated by all electrical impulses. The right cells must differentiate at the right time and place for this to happen. The reciprocal recognition between glia and neuroblasts is at the heart of this intricate and interesting neural-glial relationship. Several proteins, including the adhesion protein astrotactin, help the neuron to stay attached to the glial cell.

Cerebral organization: The cerebral cortex also modifies the neural tube's three-zone layout. Two separate organizational systems exist in the cerebrum. It is initially arranged vertically into layers that communicate with one another, similar to the cerebellum. The second zone of neurons is created at the brain's outer surface by certain neuroblasts from the mantle zone migrating via glial processes through the white matter. The neocortex is the name given to this new layer of grey matter. The six layers of neuronal cell bodies that make up the neocortex ultimately stratify; the adult forms of these layers are not finished until middle childhood. Each layer of the neocortex is unique from the others in terms of its functional characteristics, the kinds of neurons it contains, and the connections that those neurons form. For instance, neurons in layer 6 give their principal output back to the thalamus, while neurons in layer 4 receive their major input from the thalamus.

Second, there are around 40 horizontally structured regions in the cerebral cortex that control a variety of physically and functionally diverse activities. For example, layer 6 neurons of the "visual cortex" project axons to the medial geniculate nucleus of the thalamus, while layer 6 neurons of the auditory cortex, which is located more anteriorly than the visual cortex, do the opposite. The cerebral cortex is not clonally defined in either its vertical or horizontal organization. The majority of the neuronal precursors produced in the ventricular (ependymal) zone migrate outward along glial processes to form the cortical plate at the outer surface of the brain just after final mitosis. The growing cortex arises from stem cell mixtures. The layer closest to the ventricle is made up of the neuronal precursors with the earliest "birthdays," as is the case throughout the rest of the brain. The cortex's outermost layers are formed by neurons that move farther apart later. An "inside-out" gradient of development is created by this procedure. Any of the cortical layers' neurons (and glial cells) can develop from a single stem cell in the ventricular layer.

5.3.2. DEVELOPMENT OF EYE

The neural tube interacts with a series of epidermal thickenings known as the cranial ectodermal placodes to form the principal sensory organs of the skull.

The mechanism of eye development: The nearby prospective head ectoderm interacts with the involuting endoderm and mesoderm at gastrulation to give the head ectoderm a predisposition toward lens formation. However, not every piece of the head ectoderm finally develops into a lens, and the lens and retina must have a precise spatial relationship. The optic vesicle is responsible for placing the lens in respect to the retina and triggering the head ectoderm's latent lens-forming potential. It emerges from the diencephalon and, upon coming into contact with the head ectoderm, causes the development of a lens placode, which later invaginates to create the lens. The two-walled optic cup that the optic vesicle transforms into has two layers differentiating in various directions. The cells of the outer layer eventually differentiate into the pigmented retina because they can create melanin pigment, one of the few tissues besides neural crest cells that can do so. Fast cell division gives rise to a variety of glia, ganglion cells, interneurons, and light-sensitive photoreceptor neurons being produced by the inner layer's cells. These cells make up the neuronal retina as a whole. Neurons are called retinal ganglion cells to have axons that carry electrical signals to the brain. At the base of the eye, their axons converge before proceeding down the optic stalk. The optic nerve is the term used for this stalk.

There is evidence that the anterior tip of the neural plate expresses a cluster of the transcription factors Six3, Pax6, and Rx1. The bilateral regions that make up the optic vesicles will later arise from this one domain. The Pax6 protein seems to be particularly crucial for the growth of the lens

and retina. It seems to be a feature shared by photoreceptive cells across all phyla. The eyes appear to be the most susceptible organs to Pax6 deficiency, even though the murine forebrain, hindbrain, and nasal placodes are all expressed.

The secretion of sonic hedgehog determines how the single eye field is divided into two bilateral fields. The single median eye field won't split if the sonic hedgehog gene is altered or if the protein's processing is prevented. A single eye in the middle of the face (often below the nose) is the consequence, known as cyclopia. The prechordal plate's sonic hedgehog protein divides the field by suppressing Pax6 expression in the center of the embryo.

Neural differentiation of the retina: The neural retina develops into a layered array of several neuronal types, similar to the cerebral and cerebellar cortices (Fig.5.12). The rod and cone photoreceptor cells, the ganglion cell bodies and the bipolar inter-neurons that relay electrical inputs from the rods and cones to the ganglion cells are all included in these layers.
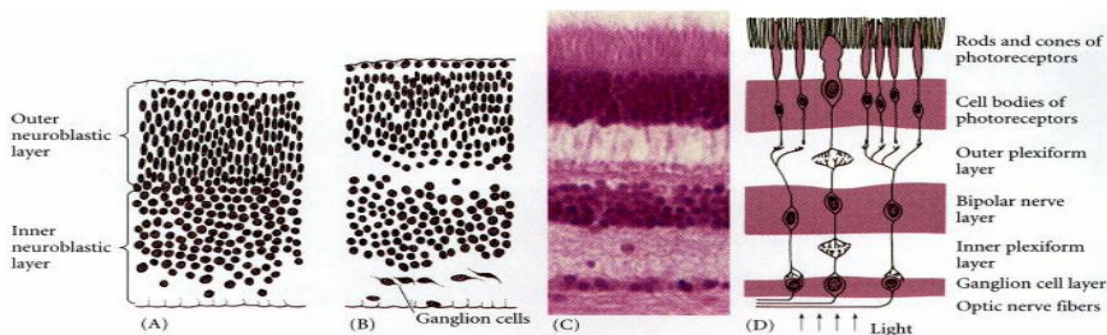
Fig.5.12 Showing Rods and Cones photoreceptor
(Adapted from developmental-biology-7thed-sf-gilbert-pdf)

Numerous horizontal neurons conduct electrical impulses in the plane of the retina, amacrine neurons (which lack long axons), and Müller glial cells preserve the integrity of the retina. The striated, laminar pattern of the neural retina is created during the earliest stages of retinal formation by cell division in a germinal layer, migration, and differential cell death of the resultant cells. At least three different types of neurons, or two different types of neurons and a glial cell, can develop from a single neuroblast precursor cell from the retinal germinal layer.

Differentiation of lens and cornea: The lens placode folds and touches the fresh ectoderm as it continues to develop into a lens. The transparent cornea is then formed by the lens vesicle by the induction of the ectoderm. Here, physical characteristics are crucial to the eye's growth. To ensure that the cornea has the proper curvature and can focus light onto the retina, intraocular fluid

pressure is required. A ring of scleral bones, which are most likely descended from the neural crest, serves as an inelastic constraint to maintain intraocular pressure. Changes in cell structure and shape as well as the production of translucent, lens-specific proteins known as crystallins are required for the differentiation of the lens tissue into a transparent membrane capable of directing light onto the retina (Fig.5.13). Under the influence of the neural retina, the cells at the inner section of the lens vesicle expand and eventually transform into lens fibers. These fibers during their process of development produce crystallins, which eventually fill the cell and lead to the nucleus extrusion. As they keep expanding, the crystallin-synthesizing fibers eventually cover the gap between the two layers of the lens vesicle. An ongoing germinal epithelium is made up of the anterior lens vesicle cells. These dividing cells travel toward the vesicle's equator, where they pass through the equatorial area and start to lengthen as well (Fig.5.13).
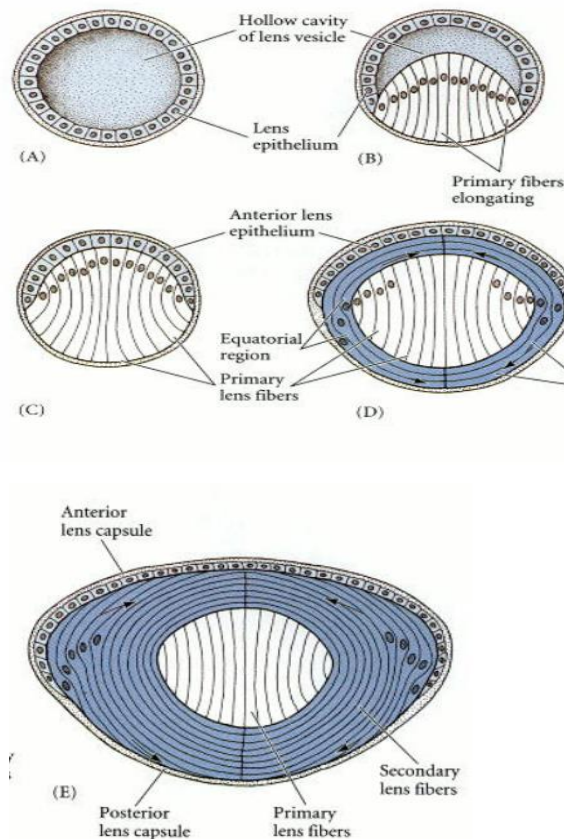
Fig.5.13: Showing differentiation of lens

(Adapted from developmental-biology-7[th]ed-sf-gilbert-pdf)

As a result, there are three distinct zones in the lens: an anterior zone of proliferating epithelial cells, an equatorial zone of cellular elongation, and a posterior and middle zone of fiber cells that

contain crystalline. Due to the ongoing laying down of fibers, this arrangement endures throughout the animal's lifetime. It takes the adult chicken two years to transition from an epithelial cell to a lens fiber. The iris, a pigmented and skeletal tissue, is located directly in front of the lens. The iris muscles regulate the pupil's size. Part of the iris is derived from the ectodermal layer, in contrast to the other muscles of the body, which are produced from the mesoderm. Specifically, the optic cup section that forms this area of the iris is continuous with the neural retina but does not produce photoreceptors.

5.3.3. DEVELOPMENT OF HEART

One of the lateral plate mesoderm's greatest accomplishments is the circulatory system. The circulatory system, which is made up of a heart, blood cells, and a complex network of blood vessels, nourishes the growing vertebrate embryo. The heart is the first functional organ and the circulatory system is the first functional component of the growing embryo. The splanchnic mesoderm sections on each side of the body interact with surrounding tissue to become specialized for heart development, giving rise to the vertebrate heart.

Specification of heart tissue and fusion of heart rudiments: The embryo in amniote vertebrates is a flattened disc, and the lateral plate mesoderm does not completely envelop the yolk sac. The early primitive streak, which starts slightly posterior to Hensen's node and extends approximately halfway down its length, is where the alleged heart cells also known as the cardiogenic mesoderm, originate. As they go through the streak, these cells divide to generate two clusters of mesodermal cells that are lateral to and at the same level as Hensen's node (Fig.5.14). These two clusters are the source of the cells that make up the endothelium lining of the heart, the cushion cells of the valves, the Purkinje conducting fibers, and the atrial and ventricular muscles.
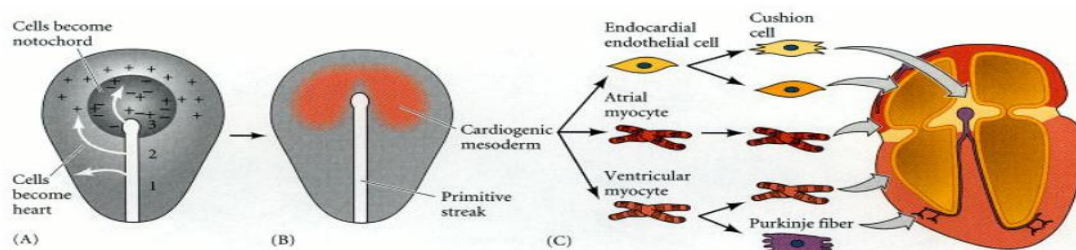
Fig.5.14: Showing heart development
(Adapted from developmental-biology-7thed-sf-gilbert-pdf)

The presumptive heart cells travel anteriorly between the ectoderm and endoderm toward the center of the embryo when the chick embryo is just 18–20 hours old, maintaining close touch with the endodermal surface. The migration of these cells stops when they get to the anterior gut tube's lateral walls. Foregut endoderm gives the movement direction. The cardiac mesoderm moves in the opposite direction when the heart region endoderm rotates about the rest of the embryo. An anterior-to-posterior concentration gradient of fibronectin is the endodermal component in charge of this migration.

Some cardiogenic cells are also designated by the endoderm and primitive streak to develop into heart muscles. The migrating mesodermal cells that will become the heart are induced to produce the Nkx2-5 transcription factor by Cerberus and an unidentified substance, presumably BMP2 in the anterior endoderm. Nkx2-5 is an essential protein that directs the mesoderm to develop into cardiac tissue and triggers the production of other transcription factors (especially members of the GATA and MEF2 families). These transcription factors stimulate the expression of genes that code for proteins particular to the heart muscle (such as cardiac actin, atrial natriuretic factor, and the alpha myosin heavy chains).

The heart's cells gradually become defined, with the ventricular cells doing so before the atrial cells. The two advancing heart-forming primordia each experience separate cell differentiation. The cells start to display N-cadherin on their apices and unite to form an epithelium as they move. The endocardium (lining of the heart along the blood vessels) is formed when a small population of these cells detach from the epithelium and downregulate N-cadherin, while the myocardium is made up of epithelial cells. The myocardium will develop into the heart's pumping muscles for the whole life of the organism.
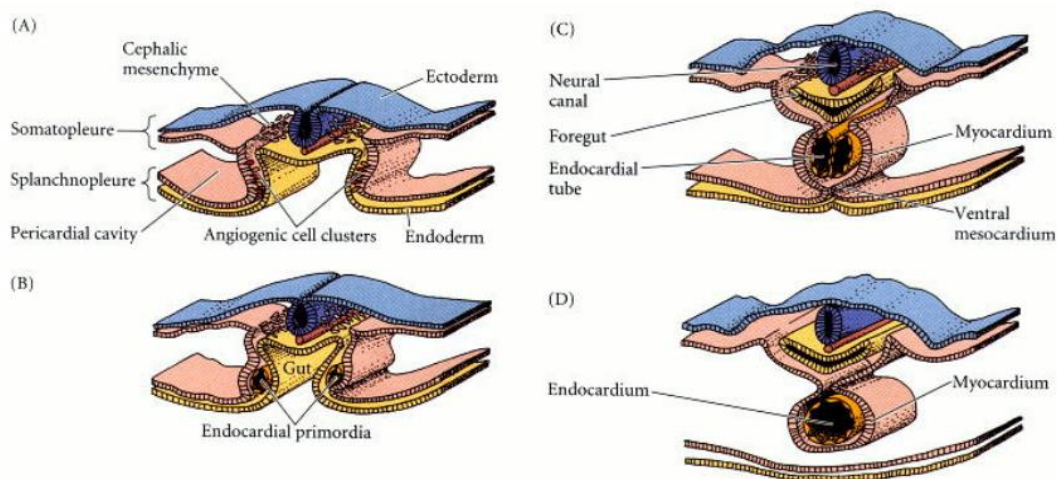
Fig.5.15: Shows the development of the heart

(Adapted from developmental-biology-7[th]ed-sf-gilbert-pdf)

Numerous heart valves are made by endocardial cells, which also govern the positioning of neural tissue in the heart and secrete the proteins that control myocardial growth. The splanchnic mesoderm folds inward during neurulation to create the foregut. The myocardium eventually becomes one tube after this movement pulls the two cardiac tubes together. A condition known as "cardio Bifida" causes a distinct heart to develop on each side of the body. For a brief period, the two endocardia share this same tube; however, they also fuse. The previously paired coelomic chambers now combine to create the body cavity that houses the heart. At about 29 hours of the development of a chick, this fusion takes place. The apertures of the vitelline veins in the heart are formed by the unfused posterior sections of the endocardium. These veins transport the yolk sac's nutrients into the sinus venosus. The blood then enters the atrial portion of the heart through a flap that resembles a valve. **Truncus arteriosus** contractions hasten blood flow into the aorta. The heart starts to beat while the paired primordia are still joining together. The sinus venosus acts as the contraction's pacemaker. A wave of muscular contraction propagates up the tubular heart from where contractions start. This allows the heart to begin pumping blood even before the intricate valve system is fully developed. Heart muscle cells can naturally contract on their own. By the fourth day, the ECG of a chick embryo is similar to that of an adult. In the embryo, these contractions are controlled by electrical inputs from the medulla oblongata via the vagus nerve.
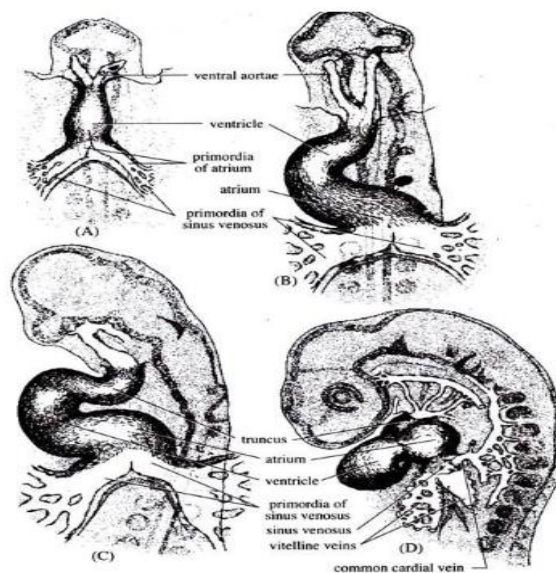
Fig.5.16. Looping and Formation of Herat Chambers in Chick Embryo

(Source: https://www.notesonzoology.com/zoology/development-of-heart-in-chick/2705)

Looping and formation of heart chambers: The heart is a two-chambered tube with an atrium and a ventricle in a three-day-old chick embryo. The unaided eye may observe the amazing cycle

of blood entering the lower chamber and being pumped out through the aorta in the chick embryo. The heart's looping changes the heart tube's original anterior-posterior polarity into the right-left polarity found in adults**.** As a result, the area of the heart tube that will eventually become the right ventricle is located ahead of the area that will eventually become the left ventricle. The left-right patterning proteins are required for this looping. Nkx2 5 controls the Hand1 and Hand2 transcription factors within the heart primordium. Although it appears that the Hand proteins are produced throughout the early heart tube, once looping begins, Hand1 is limited to the left ventricle and Hand2 to the right. Without these proteins, looping is disrupted, and the ventricles are unable to develop appropriately. The Pitx-2 transcription factor is essential for correct cardiac looping and is only activated on the left side of the lateral plate mesoderm. It may also control the expression of proteins like the extracellular matrix protein lectin to control the physical strain of the various heart components. The Xin gene may mediate the cytoskeletal alterations required for heart looping and is also activated by transcription factors Nkx2-5 and MEF2C. The various transcription factors that becomes limited to either the anterior or posterior region of the heart tube help to define the separation of the atrium from the ventricle**.** When cells from the myocardium release a substance (likely transforming growth factor-β3) that triggers cells from the neighboring endocardium to separate and enter the hyaluronate-rich cardiac jelly between the two layers, the tube is partitioned into a distinct atrium and ventricle (Fig.5.16 A-D)

5.4 EMBRYONIC INDUCTION

New shapes and forms are created during gastrulation, which involves irreversible movements also known as morphogenetic movements. During gastrulation, when distinct sections of the blastoderm are displaced and brought into new spatial connections to one another, the organization of the embryo as a whole appears to be defined to a great extent. Components of various origins can interact more readily because groups of cells that were far apart in the blastula come close together. The sliding of cells (presumptive mesoderm) into the interior and their positioning on the dorsal side of the archenteron (in the archenteric roof), in direct contact with the overlying ectoderm, are of major importance in development and subsequent differentiation, particularly in vertebrate development. Though ectoderm lacks the capacity for any form of progressive development during the beginning of gastrulation, it attains that ability only after invagination, when chordamesoderm is directly beneath it. The overlaying ectoderm divides into the neural plate by the effect of dorsal mesoderm and dorsal mesoderm eventually differentiates into the notochord, prechordal

mesoderm, and somites. Overlying ectoderm is developed into the skin via lateral mesoderm. **Embryonic induction** refers to the effect that some embryonic organs exert over groups of cells to direct their growth in a particular direction. These gastrulation movements are caused by the combined actions of the embryo's components rather than by the embryo as a whole. It must be assumed that there are inducing chemicals with specialized activity because the consequences of induction vary depending on the organ's rudiments. For instance, the neural plate from the same type of ectoderm is not differentiated by the lateral mesoderm, but the skin is. However, as some differentiations may be brought about by a combination of two or more inducing chemicals or the same inducing substance may have different effects on various tissues, the number of inducing substances need not be the same as the number of distinct types of tissues and organs. The gradient distribution of just two inducing substances, the neuralizing substance, and the mesodermalizing substance, along the length of the embryo, might regulate the regional organization of the complete vertebrate body. The mesodermalizing substance is concentrated at the posterior end and diminishes toward the anterior end whereas the neuralizing substance is concentrated at the anterior end and gradually declines toward the posterior end. The relative proportions of the two inducing substances at any given time during the embryo determine how differentiated the induced structures will be. When used alone, the neuralizing and mesodermalizing substances only produce nervous tissue (the future forebrain) and mesodermal structures respectively.

Although close closeness between the interacting pieces is necessary for induction, physical touch is not required. A diffusible chemical released by the activating cells is the inciting influence (**the inductor**). The mesoderm is induced by a big molecule, most likely a protein or nucleoprotein. Transplanting mammalian tissues into frog embryos or transplanting chick embryo tissue into rabbit embryos have both been used to induce the development of main organs in vertebrates of many different classes.

5.4.1. DETERMINATION OF THE PRIMARY (1°) ORGAN RUDIMENTS

Induction is responsible for the formation of several early organ structures in vertebrates, in addition to the division of the ectoderm into the neural plate and epidermis. The adjacent somites and nephrotomes, which appear to work in concert to cause the formation of limb rudiments from the lateral plate mesoderm, are induced by the notochord. Therefore, embryonic induction is the consequence of contact between an inducing tissue and a responsive tissue, which causes the responding tissue to shift the direction of its differentiation. This is most likely the only process responsible for cell differentiation and cell organization into tissues and organs in vertebrates. The

phenomenon is credited **to Spemann** in **1901** and **Lewis** in **1904**, who proved that in some species of *Rana,* the development of a lens from the ectoderm is influenced by the brain's underlying optic lobe. A heteroplastic transplanting between two species of newts from the genus *Triturus* was carried out by **Spemann (1921).** He transferred a portion of one early gastrula embryo's presumptive neural ectoderm into another embryo's prospective epidermis area. The tissue that was implanted transformed into the epidermis. On the other hand, a portion of the prospective epidermis that was implanted into the presumptive neural ectoderm grew into a component of the neural tube. Therefore, at the early gastrula stage, the fates of presumptive neural tissue or prospective epidermis are not fixed. If the donor embryo used is in a late stage of gastrulation as opposed to an early stage of gastrulation, completely different outcomes are seen. A fragment of a late gastrula's neural plate can be transplanted into another area of the embryo, where it will grow into nerve tissue. An epidermal patch forms inside the nervous system if a portion of the potential epidermis is transplanted into the area of the neural plate. The range of possibilities for the tissues' fate is gradually reduced throughout gastrulation.

In an experiment by **Spemann and Mangold (1924),** the dorsal lip of an unpigmented *Triturus cristatus* embryo was transplanted to the ventral region of a pigmented *Triturus taeniatus* gastrula. This caused the pigmented host cells to create a secondary axis (**Spemann, 1938**). The 1935 Nobel Prize in Medicine or Physiology went to this T 2 experiment. Even in this unsuitable environment, involution took place. Axis induction, when a secondary axis develops with a stomach, neural tube, notochord, and somites, also took place. It was seen due to pigment difference that a large portion of the secondary axis is made from host tissue. As a result, the graft had an impact on the neighboring cells' ability to develop into particular organs. This effect was called 'Embryonic induction. The component that is the source of the influence is known as the **inductor**. The secondary axis and the primary axis are always parallel. Therefore, secondary axis orientation is determined by the host. A parallel secondary axis is also revealed by transplant experiments using avian Henson's Nodes. This experiment demonstrated that the chordamesoderm, the underlying tissue, is what induces the neural system (presumptive notochord and somite mesoderm).
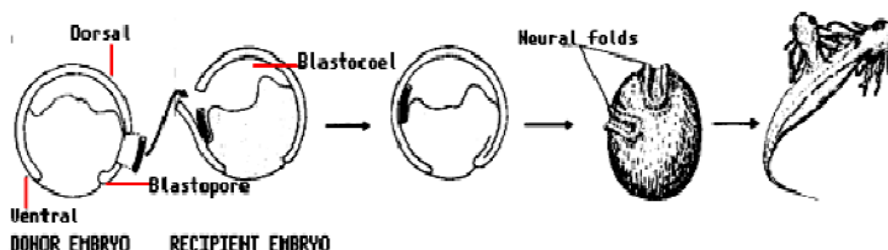
Fig.5.17: Embryonic Induction (https://www.biology-pages.info/S/Spemann.html)

Because it can start the development of a secondary embryo when transplanted, Spemann dubbed the dorsal lip of the blastopore the **Primary Organiser**. It is now also known as the Spemann organizer.
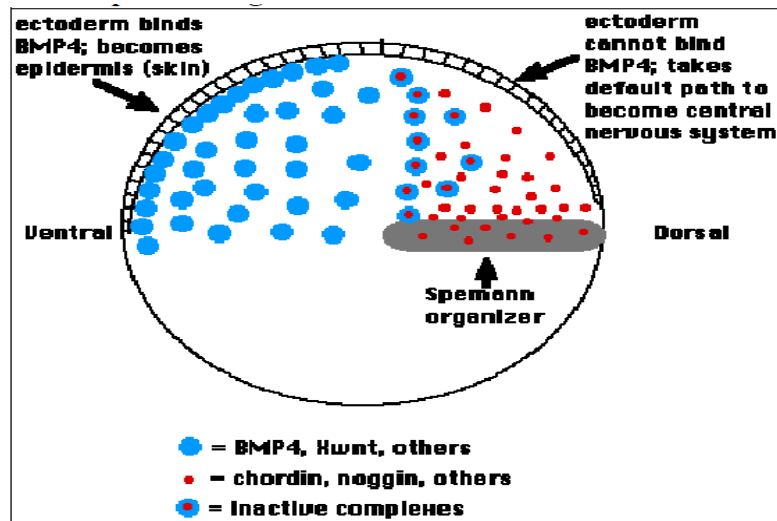
Fig.5.18: The Spemann Organizer (https://www.biology-pages.info/S/Spemann.html)

In urodele amphibians, the primary organizer was shown to exist. Since then, it has been discovered that the chordamesoderm, or archenteron roof, can induce the nervous system and sensory organs in the majority of vertebrate species. Due to the specificity of the inductors in each region, the numerous portions that are induced to develop do so in a systematic way.

1. Head organs are induced to develop by the head inductor, which is an anterior chordamesoderm segment.

2. The tail bud and trunk organs are induced to form by the chordamesoderm's posterior component, the trunk inductor.

3. The chordamesoderm crosses the blastopore's dorsal lip first at the anterior end and the posterior end passes over in the last. Consequently, an early gastrula stage embryo's dorsal lip functions as a head inductor and induces head organs to form.

5.4.2. MESODERM INDUCTION OCCURS BEFORE PRIMARY EMBRYONIC INDUCTION

It has been discovered that the mesoderm is induced first, then the neural induction. Animal, vegetative, and equatorial or marginal cells make up the three different types of cells. Their

locations on the blastula are indicated by these names. Nieuwkoop (1969) cultivated explants from these 3 areas separately or jointly and observed the following outcomes:

Explant	Tissue Formed
Animal cells	Forms epidermis
Marginal cells	Forms mesoderm
Vegetal cells	Forms endoderm
Animal cells+Vegetal cells	Forms mesoderm

Mesoderm appears to be induced by a variety of molecules from many types of embryonic or adult cells like cultured mammalian cells, guinea pig bone marrow, carp swim bladder, and chick embryos. They all share the property of containing growth factors. One cell type releases growth factors that affect other cell types in different ways. This characteristic is in line with what is anticipated of an inducer.

5.4.3. SECONDARY INDUCTION

As the organism develops, inductive interactions lead to the emergence of numerous more cell types. This is known as **secondary induction**. The two types of inductive interactions are seen, **as permissive and instructive**. The inducing tissue reportedly provides precise information to commit cells to a new developmental route in an instructive interaction. Cairns and Saunders (1954) demonstrated in the chick that the overlying ectoderm evolved under the origin of the mesoderm from various locations of the leg or wing into foreign regions. The growing wing ectoderm will create thigh feathers above the grafted mesoderm if a piece of thigh mesoderm is transplanted under the ectoderm of the wing. The reacting tissue must be relatively unknown for such an interaction to occur. The parts of the embryo can develop into other parts in experimental situations in addition to having a clear typical fate, known as potential significance. Prospective potency refers to a portion of an early embryo's capacity to develop into more than one type of tissue. **Determination** is the process by which potential possibilities are reduced and the fate of the embryonic tissues is fixed. A tissue is said to be determined once the determination has taken place in it. Its potential potency is now more restricted.

Responding cells in permissive interactions are already prepared and ready to differentiate; all they need is a signal from the inducing tissue to enable them to realize their full potential. The pancreas for example grows as a gastrointestinal expansion, although the mesoderm must participate in its

formation. **Rutter and his colleagues (1964)** discovered that the pancreas endoderm would differentiate when a different form of mesenchyme, from the salivary gland, was substituted. Using early, pureed embryos and somites, which typically develop muscle and cartilage cells, this endoderm differentiation was also seen (embryo extract). Therefore, it appears that everything is set up at the point where the pancreatic endoderm is visible, to the point where a relatively non-specific cue will finish the differentiating process. The neural tube is located inside the secondary axis and is principally induced in the ectoderm by the overlying mesoderm.

5.5   PRIMARY ORGANISER AND ITS MORPHOLOGICAL DIFFERENTIATION

An embryonic interaction, also known as an organizer, is the process by which one biological tissue transmits a chemical that influences another embryonic section to create a structure that would not otherwise be feasible. This type of embryonic tissue is known as an inductor, and the substances that an inductor secretes are known as evocators. The term "responsive tissue" refers to tissue that reacts to an evocator operating on it. The action taken by the inductor via the evocator is referred to as induction activity or organizer action. This induction process has a substantial impact on the protein synthesis mechanism of sensitive tissues, which causes cells that produce certain structures to become more active. In 1924, the organizing idea for Spemann's experiment was initially put forth. German embryologist Hans Spemann and his student Hilde Mangold conducted a transplanting experiment on *Triturus cristatus*, an Urodela belonging to the class Amphibia, in 1924.

Spemann grafted a portion from the dorsal lip region of the early gastrula of *Rana sp.* (donor embryo) to the lateral lip of the early gastrula of *Triturus cristatus* (host embryo). The transplanted piece's cells entered the gastrula and formed the notochord and somites. The dorsal lip of the blastopore in this embryo creates the neural groove, notochord, mesoderm, etc. Likewise, the transplanted tissue has an impact on the mesoderm, the neural groove, and the notochord. That is, the second set of the notochord, nerve cord, and mesoderm are generated in the same embryo. In this instance, chemical compounds produced by the donor tissue caused the host embryo to develop neural grooves, notochords, and other structures. Donor tissue and the constructed neural groove included pigments. They noticed that a larva with two heads had formed after gastrulation was finished. One head was produced naturally throughout development, and the other was stimulated by donor tissue. It was seen under the microscope that the host embryo's tissue served as a secondary set to build the notochord, renal tubules, gut, and other structures. Such secondary

structures would not have formed if the donor tissue had not been grafted. From this experiment, the researchers deduced that the donor's dorsal lip had a significant impact on the tissue, changing how the host tissue developed. Spemann referred to this process of influencing other tissues as induction, and the tissue that caused the other tissues to be affected was known as the **inductor** or **organizer**.

Primary organizer: Spemann continued his grafting studies using tissues from different zones of the gastrula and discovered that it was only the dorsal lip of the early gastrula that could create a complete embryo while all tissues from other regions failed to do so. He designated the dorsal lip as the **organizer** since it organizes the embryo's developmental process. He asserts that the dorsal lip stimulates the development of the neural tube, which in turn stimulates the development of the eyes. He referred to the dorsal lip, or chordamesoderm, as the major or the primary organizers because it is made of chordamesoderm and serves largely as an inducer.

Secondary, tertiary, and quaternary organizers: Primary organs start to form as gastrulation progresses as a result of the primary organizer's induction, and these early stages of organ development are referred to as organ rudiments. These organ rudiments may function as organizers on their own, in which case they are referred to as **secondary organizers**. Further development may be induced by tissues that are created as a result of secondary organizer activity. They are therefore referred to as **tertiary organizers**. The primary organizer is the starting point for these subsequent levels of organizer activity. The development of the eye in amphibians, chicks, etc., serves as vivid instances of how these organizers function sequentially. First, eye developing cells are formed within the forebrain as a result of the forebrain's induction action. Outside of the forebrain, these cells protrude as a vesicle known as optic vesicles. Through the lateral mesenchyme, this vesicle develops till it reaches the epidermis. The outer layer of the vesicle invaginates to form a double-layered optic cup as soon as it comes into touch with the epidermis. Sensory cells make up the optic cup's inner layer, whereas pigmented cells make up the outer layer. Together, they make up the retina. Between the optic cup and the epidermis, the chemical substances that the optic cup secretes cause the formation of the lens. The optic cup, therefore, serves as a secondary organizer. Lens and retina together induce to make the cornea, thus they together act as the tertiary organizer.

5.6 CONCEPT OF ORGANIZER

5.6.1. ORIGIN OF PRIMARY ORGANISER

In 1924, the organizing idea for Spemann's experiment was initially put forth. Spemann grafted a portion from the dorsal lip region of the early gastrula of *Rana sp.* (donor embryo) to the lateral lip of the early gastrula of *Triturus cristatus* (host embryo). The transplanted piece's cells entered the gastrula and formed the notochord and somites. The dorsal lip of the blastopore in this embryo creates the neural groove, notochord, mesoderm, etc. Likewise, the transplanted tissue has an impact on the mesoderm, the neural groove, and the notochord. That is, the second set of a notochord, nerve cord, and mesoderm are generated in the same embryo. In this instance, chemical compounds produced by the donor tissue caused the host embryo to develop neural grooves, notochords, and other structures. Colored pigments were present in both the donor tissue and the artificially made neural groove. They noticed that a larva with two heads had formed after gastrulation was finished. One head was produced naturally throughout development, and the other was stimulated by donor tissue. It was seen under the microscope that the host embryo's tissue served as a secondary set to build the notochord, renal tubules, gut, and other structures. Such secondary structures would not have formed if the donor tissue had not been grafted. From this experiment, the researchers deduced that the donor's dorsal lip had a significant impact on the tissue, changing how the host tissue developed. Spemann referred to this process of influencing other tissues as induction, and the tissue that caused the other tissues to be affected was known as the **inductor** or **organizer**.

5.6.2. INDUCTIVE INTERACTIONS:

Organs are intricate structures made up of several tissue kinds. For instance, light enters the vertebrate eye through the translucent corneal tissue and is focussed by the lens tissue (the diameter of which is regulated by muscle tissue), and then impinges on the neuronal retinal tissue. It is impossible to alter the exact arrangement of tissues in this organ without affecting how well it works. One group of cells influencing the behavior of an adjacent group of cells, leading them to modify their form, mitotic rate, or fate, is how such coordination in the development of organs is achieved. Proximate interaction, also known as induction, is the term used to describe this type of proximity interaction between two or more cells or tissues with diverse histories and qualities. Every inductive contact has at least two elements. The inducer, or tissue that generates the signal (or signals) that alter the other tissue's cellular behavior, is the first part of the system. The responder is the second element, the tissue being stimulated.

5.7 NATURE OF INDUCTIVE SIGNAL (POSSIBLE MECHANISM OF NEURAL INDUCTION)

Reciprocal and sequential inductive events: The reciprocal character of many inductive interactions is another aspect of induction. Once the lens has developed, further tissues may be induced. The optic vesicle itself is one of these reacting tissues. The inducer now changes into the induced. The optic vesicle transforms into the optic cup under the action of substances secreted by the lens, and the wall of the optic cup differentiates into two layers, the pigmented retina and the neuronal retina (Fig.5.19). Reciprocal inductions are interactions like this.

The lens is also causing the ectoderm above it to develop into the cornea at the same time. The ectoderm that forms the cornea has developed a special capacity to react to inductive impulses, in this case, the signals from the lens, just as the lens-forming ectoderm. The corneal ectodermal cells are induced by the lens to become columnar and secrete many layers of collagen. This collagen matrix allows neural crest mesenchymal cells to enter the region and secrete a collection of proteins, including the enzyme hyaluronidase, that advance the cornea's differentiation. The hormone thyroxine, a third signal, causes the tissue to become translucent and dry. As a result, there are several reasons for each induction as well as successive inductive occurrences.
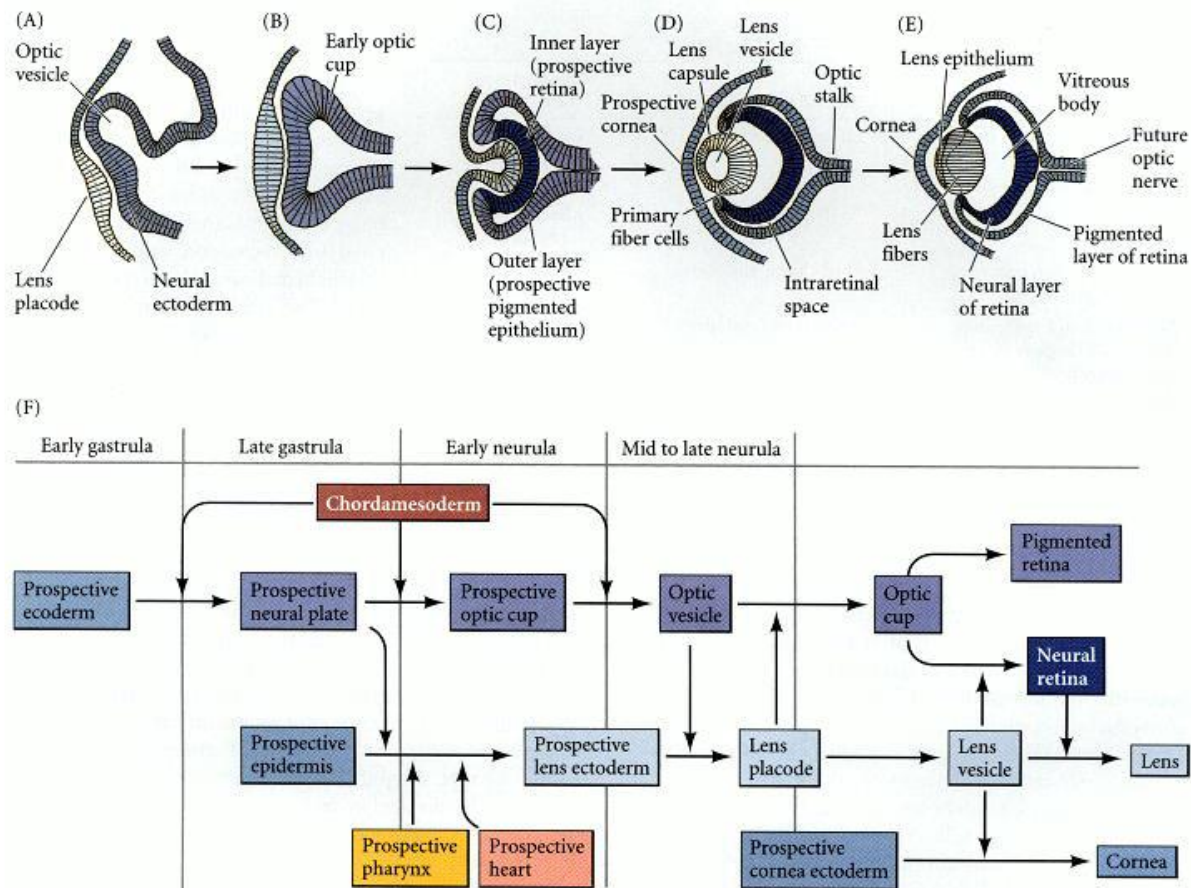
5.19. Neural Inductions and their interactions

(Adapted from developmental-biology-7thed-sf-gilbert-pdf)

Instructive and permissive interactions: There are two main categories of inductive contact:

(a) Instructive interaction: For new gene expression to begin in the responding cell, a signal from the inducing cell is required. The responding cell could not differentiate in that specific manner without the inciting cell. An instructive interaction, for instance, is when the optic vesicle is experimentally positioned below a new section of the head ectoderm and induces that region of the ectoderm to produce a lens. Three underlying principles define the majority of instructional interactions:

1. Responding tissue B develops in a specific way when tissue A is present.

2. Responding tissue B does not form in such a way in the absence of tissue A.

3. Tissue B does not develop in that way when tissue A is not there but tissue C is.

(b) Permissive interaction: In this kind of contact, the responding tissue already possesses all of the features that are intended to be produced; all that is required is a setting that permits the

production of these characteristics. For instance, the development of many tissues depends on the presence of a fibronectin- or laminin-containing solid substrate. The expression of what has been determined to be expressed is all that the fibronectin or laminin accomplishes; it does not change the sort of cell that will be formed.

Epithelial-mesenchymal interactions: The interactions of sheets of epithelial cells with nearby mesenchymal cells are some of the well-studied examples of induction. Such interactions are known as **Epithelial-mesenchymal interactions.** Connected cells give rise to sheets and tubes, which are the forms of epithelia that can be formed from any germ layer. Unconnected, loosely packed cells are referred to as mesenchyme. The neural crest or mesoderm is the source of mesenchymal cells. Epithelial-mesenchymal interactions are among the most significant natural processes since epithelium and the related mesenchyme make up every organ. Table 6.1 includes a list of some examples.

Table 5.1: Some epithelial- mesenchymal interactions

Organ	Epithelial component	Mesenchymal component
Cutaneous structures (hair, feathers, sweat glands, mammary glands)	Epidermis (ectoderm)	Dermis (mesoderm)
Limb	Epidermis (ectoderm)	Mesenchyme (mesoderm)
Gut organs (liver, pancreas, salivary glands)	Epithelium (endoderm)	Mesenchyme (mesoderm)
Pharyngeal and respiratory associated organs (lungs, thymus, thyroid)	Epithelium (endoderm)	Mesenchyme (mesoderm)
Kidney	Ureteric bud epithelim (mesoderm)	Mesenchyme (mesoderm)
Tooth	Jaw epithelium (ectoderm)	Mesenchyme (neural crest)

Regional specificity of induction: The spatial specificity of induction is the first characteristic of epithelial-mesenchymal interactions. The outer epidermis, an epithelial tissue formed from ectoderm, and the dermis, a mesenchymal tissue derived from mesoderm, are the two primary tissues that makeup skin. The condensed dermal mesenchyme reacts by secreting substances that cause the chick epidermis to form regionally specific cutaneous structures in response to the underlying dermal cells receiving a signal from the chick epidermis to form condensations likely by secreting sonic hedgehog and TGF- 2 proteins**.** The large wing feathers, the fine thigh feathers, or the scales and claws of the feet are examples of these structures. The embryonic epithelium and mesenchyme can be separated from one another and combined in various ways in laboratories.

Genetic specificity of induction: The genetic specificity of induction is the second characteristic of epithelial-mesenchymal interactions. While the mesenchyme may tell the epithelium which gene sets to activate, the epithelium's ability to follow these instructions depends on its genome. Through trials involving the transfer of tissues from one species to another, this characteristic was revealed. **Hans Spemann and Oscar Schotté (1932)** in an experiment, transplanted flank ectoderm from an early frog gastrula to the region of a newt gastrula that would later become elements of the mouth. Similarly, they implanted presumptive mouth areas of frog embryos with presumptive flank ectodermal tissue from a newt gastrula. The mouth region of salamander and frog larvae has a quite different anatomy. The frog tadpole produces mucus-secreting glands and suckers, but the salamander larva possesses club-shaped balancers beneath its mouth (Fig.5.20).
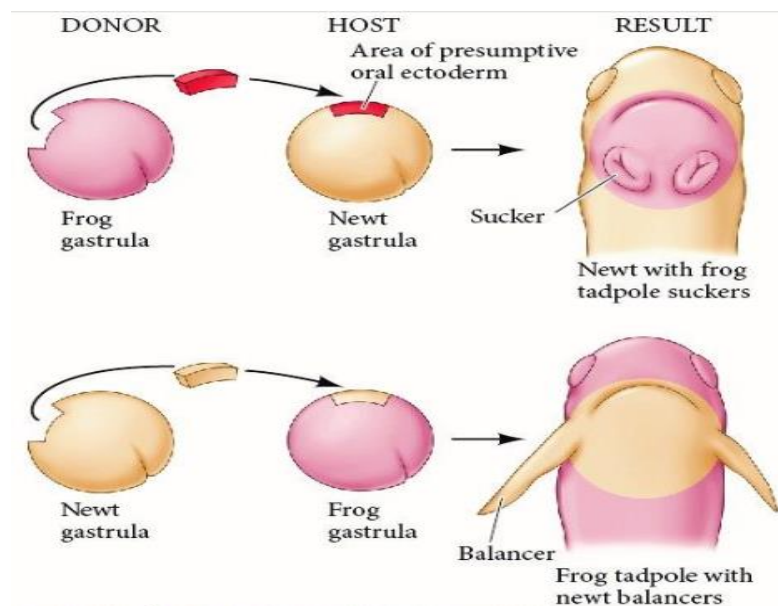
Fig.5.20.Genetic specificity of induction

(Source:https://learninglink.oup.com/access/content/barresi-12e-student-resources/barresi-12e-further-development-4-4-from-feathers-to-claws-and-frogs-to-newts-further-your-understanding-of-induction)

The salamander has a set of calcareous teeth in its jaw, whereas the frog tadpole also has a horny jaw without teeth. The transplants produced chimera larvae. The frog tadpoles possessed salamander teeth and balancers, and the salamander larvae had mouths like those of frogs. To put it another way, the ectoderm was told to create a mouth by the mesodermal cells, but instead, it created the only mouth that it "knew" how to create, regardless of how unsuitable it was. As a result, mesenchymal tissue signals can be transmitted across species boundaries. Frog signals are

recognized by salamanders, and mammalian inducers are recognized by chick tissue. But the epithelium reacts differently depending on the species. As a result, species specificity is typically regulated by the responsive epithelium, whereas organ type specificity (such as feather or claw) is typically regulated by the mesenchyme within a species. Changes in the way an organism responds to a certain inducer can result in significant evolutionary changes.

Paracrine Factors: Juxtacrine interactions occur when the cell membrane interacts with receptor proteins on a neighboring cell surface. The phenomenon is known as a **paracrine interaction**, and the diffusible proteins are known as **paracrine factors** or **growth and differentiation factors (GDFs)** when they can disperse across short distances to affect neighboring cells.Paracrine factors are secreted into the immediate areas around the cell that produces them, in contrast to endocrine factors (hormones), which travel through the blood to act. The embryo inherits a relatively small "tool kit," and builds the heart, kidneys, teeth, eyes, and other organs using many of the same proteins. Furthermore, the same proteins are used throughout the animal kingdom; the elements involved in developing the eye or heart of the Drosophila are quite similar to those responsible for developing mammalian organs. Based on their structural similarities, four main families can be formed from many of these paracrine components. These families include the TGF-superfamily, the Hedgehog family, the Wingless (Wnt) family, and the fibroblast growth factor (FGF) family.

The fibroblast growth factors: There are now more than a dozen structurally similar members of the **fibroblast growth factor (FGF)** family. FGF1 and FGF2 are also known as acidic and basic FGF, respectively, while FGF7 is also known as the keratinocyte growth factor. Vertebrates have more than a dozen different FGF genes that can produce hundreds of different protein isoforms by altering their RNA splicing or initiation codons in various tissues. The fibroblast growth factor receptors are a group of receptor tyrosine kinases that can be activated by FGFs (FGFRs). Proteins called receptor tyrosine kinases penetrate the cell membrane **(Fig.5.21).** The part of the protein that binds the paracrine factor is on the extracellular side. There is a latent tyrosine kinase on the intracellular side (i.e., a protein that can phosphorylate another protein by splitting ATP). The dormant kinase is activated and phosphorylates specific proteins in the receptive cell when the FGF receptor binds an FGF. The proteins can now carry out new tasks because they have been activated. FGFs have been linked to the development of mesoderm, axon extension, and angiogenesis (the production of blood vessels). Even while FGFs are frequently interchangeable, their different expression patterns offer them distinct roles. FGF8 is crucial for the development of the midbrain and limbs, whereas FGF2 is particularly significant in angiogenesis**.**
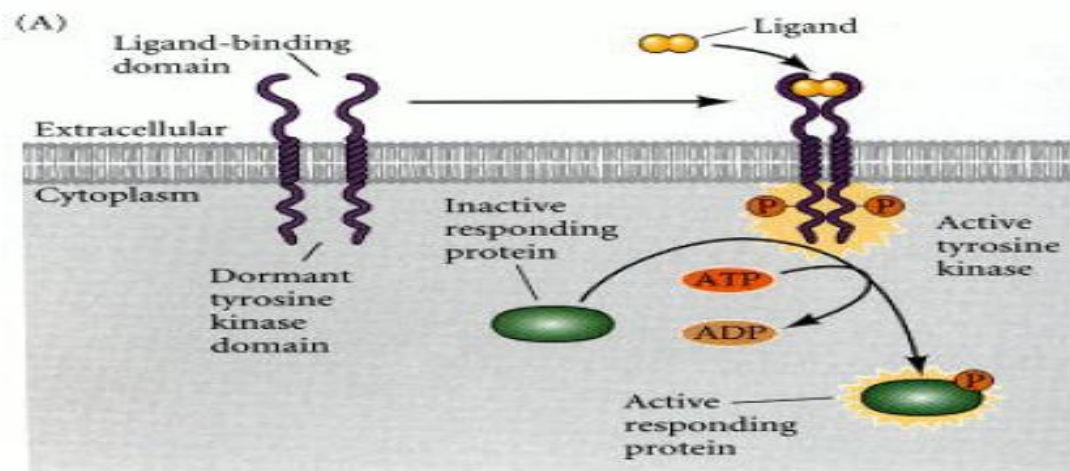
Fig.5.21.The receptor of tyrosine kinase
(Adapted from developmental-biology-7thed-sf-gilbert-pdf)

The Hedgehog Family: The Hedgehog proteins are a class of paracrine factors that the embryo frequently employs to stimulate specific cell types and establish tissue borders. The Drosophila hedgehog gene has at least three homologues in vertebrates: the sonic hedgehog (*shh*), desert hedgehog (*dhh*), and Indian hedgehog (*ihh*). The Sertoli cells of the testes express desert hedgehog, and mice homozygous for a null allele of *dhh* have poor spermatogenesis. Indian hedgehog plays a crucial role in postnatal bone formation and is expressed in the gut and cartilage. Of the three vertebrate homologues, Sonic Hedgehog is the most frequently encountered. Only the amino-terminal two-thirds of the molecule, which is produced by the notochord, is secreted. This peptide is in charge of shaping the neural tube such that sensory neurons develop from the dorsal neurons and motor neurons from the ventral neurons. The somites are patterned by Sonic Hedgehog so that the region closest to the notochord develops into the cartilage of the spine. Also, Sonic Hedgehog plays a role in the development of the left-right axis in chicks, the beginning of the anterior-posterior axis in limbs, the induction of regionally specialized digestive tube differentiation, and the induction of feather creation. Wnt and FGF proteins are two paracrine factors that frequently collaborate with a sonic hedgehog. Sonic hedgehog, FGF4, and other paracrine factors are concentrated in the area of the developing tooth where cell interactions are forming the teeth's cusps.

The Wnt family: A class of glycoproteins high in cysteine is known as the **Wnts**. Vertebrates contain at least 15 members of this family. Their name is a combination of the names of two

vertebrate homologs of the Drosophila segment polarity gene, integrated and wingless. While **Wnt1** appears to be active in driving the dorsal cells of the somites to become muscle, Sonic Hedgehog appears to be vital in patterning the ventral section of the somites (causing the cells to become cartilage). **Wnt** proteins are also used in numerous stages of the development of the urogenital system and are crucial in determining the polarity of insect and vertebrate limbs.

The TGF-β superfamily: There are over 30 structurally related members of the TGF-b superfamily, and they regulate some of the most important interactions in development. The proteins encoded by TGF-β superfamily genes are processed such that the carboxy-terminal region contains the mature peptide. These peptides are dimerized into homodimers (with themselves) or heterodimers (with other TGF-β peptides) and are secreted from the cell. The TGF-β superfamily includes the TGF-β family, the activin family, the bone morphogenetic proteins (BMPs), the Vg1 family, and other proteins, including glial-derived neurotrophic factor (necessary for kidney and enteric neuron differentiation) and Müllerian inhibitory factor (which is involved in mammalian sex determination). TGF-β family members TGF-β1, 2, 3, and 5 are important in regulating the formation of the extracellular matrix between cells and for regulating cell division (both positively and negatively). TGF-β1 increases the amount of extracellular matrix epithelial cells make (both by stimulating collagen and fibronectin synthesis and by inhibiting matrix degradation). TGF-βs may be critical in controlling where and when epithelia can branch to form the ducts of kidneys, lungs, and salivary glands. The effects of the individual TGF-β family members are difficult to sort out because members of the TGF-β family appear to function similarly and can compensate for losses of the others when expressed together. Moreover, targeted deletions of the Tgf-β1 gene in mice are difficult to interpret, since the mother can supply this factor through the placenta and milk.

The members of the **bone morphogenetic proteins (BMP)** family were originally discovered by their ability to induce bone formation; hence, they are the bone morphogenetic proteins. Bone formation, however, is only one of their many functions, and they have been found to regulate cell division, apoptosis (programmed cell death), cell migration, and differentiation. BMPs can be distinguished from other members of the TGF-β superfamily by their having seven, rather than nine, conserved cysteines in the mature polypeptide. The BMPs include proteins such as Nodal (responsible for left-right axis formation) and BMP4 (important in neural tube polarity, eye development, and cell death). The *Drosophila* decapentaplegic protein is homologous to the vertebrate BMP4, and human BMP4 can replace the *Drosophila* homologue, rescuing those flies deficient in **decapentaplegic protein (Dpp).**

Other paracrine factors: The majority of the paracrine factors belong to one of the four groups indicated above, while some have few or no close relatives. Although they do not belong to the families indicated above, factors including epidermal growth factor, hepatocyte growth factor, neurotrophins, and stem cell factor all play significant roles in development. Erythropoietin, cytokines, and interleukins are a few other components that are almost entirely involved in the development of blood cells. For instance, activin can trigger several sets of genes at various doses and can diffuse throughout a wide range of cell diameters. However, it's likely that the Vg1, BMP4, and Nodal proteins only affect their immediate surroundings. These factors may cause these neighbors to express more nearby short-range factors, which could start a chain reaction of paracrine inductions. There is autocrine regulation in addition to endocrine, paracrine, and juxtacrine regulation. When the same cells that secrete paracrine substances also react to them, autocrine regulation takes place. In this instance, a molecule that the cell has its receptor for is created by the cell. Placental cytotrophoblast cells produce and secrete platelet-derived growth factor, which has a receptor on its membrane. Although autocrine regulation is uncommon, it can be observed in these cells. The outcome is that tissue proliferates explosively.

5.8 COMPETENCE

Not all tissues are capable of responding to the inducer's signal. For instance, the *Xenopus laevis* optic vesicle, or presumed retina, can cause the formation of lens tissue when it is positioned beneath the head ectoderm in an ectopic location, or somewhere other than where it normally develops. This appears to be a unique ability of the optic vesicle, making it an inducer. The optic vesicle will, however, prevent the flank or abdomen of the same organism's ectoderm from responding if it is positioned beneath it. Only the head ectoderm is capable of creating a lens in response to signals from the optic vesicle. **Competence** is the capacity to react to a particular inductive signal. Competence is an actively gained condition rather than a passive state. For instance, the Pax6 protein appears to be crucial in enabling the ectoderm to react to the inductive signal from the optic vesicle in the developing chick and human eye. Pax6 expression is only present in the head ectoderm, which can develop lenses in response to the optic vesicle, and not in other parts of the surface ectoderm.
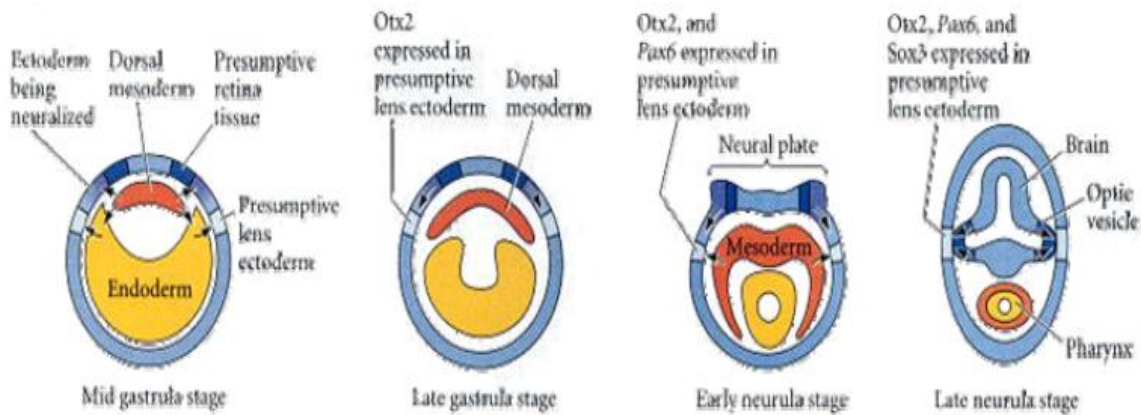
Fig.5.22. Showing the role of Pax6 as a competence factor
(Adapted from developmental-biology-7thed-sf-gilbert-pdf)

It is not required by the tissue that induces it. Although it is believed that the anterior portions of the neural plate generate Pax6 expression, it is unknown how this occurs in the embryo's anterior ectoderm. Ectodermal tissue can be given the ability to respond to the optic vesicle inducer by being incubated close to anterior neural plate tissue. Therefore, the lens cannot be caused by a single factor. The pharyngeal endoderm and heart-forming mesoderm, which support the lens-forming ectoderm throughout the early- and mid-gastrula stages, maybe the initial inducers, according to studies on amphibians. The following signals, such as one that encourages Pax6 production in the anterior ectoderm, may be produced by the anterior neural plate. Thus, although the anterior ectoderm has already been induced by at least two different stimuli, the optic vesicle appears to be the inducer.

5.9 SUMMARY

The process of creating a chick from an egg cannot be understood without a rational understanding of the embryology of the growing embryo. Before the chick hatches from the egg, it is incubated for three weeks. At this stage, all cells are identical, but throughout the time they begin to differentiate into distinct types. By the third day of incubation, the limb buds for the wings and legs are apparent. Even though it is not contained within the body, the heart is still getting bigger. The immune system starts taking shape as the spleen, thymus, and cloacal bursa began to appear. When a hen lays an egg, it has about 20,000 cells. During chick development, certain marginal zone cells play a crucial role in determining cell destiny. Epiblast and hypoblast, the two layers of the blastoderm, are fused at the edge of the area opaca. Neurulation is the process by which this tissue develops into a neural tube. An embryo going through this process is referred to as a neurula.

Different vertebrate classes utilize these processes of formation to different extents. A U-shaped neural groove develops in the center of the neural plate shortly after it forms, dividing the right and left sides of the embryo. The neural plate contains up to 50% of the ectoderm. It expands and contracts via convergent expansion in both amphibians and amniotes. In an isolated neural plate, the cells converge and spread out to form a narrower plate, but they do not roll up into a neural tube.

The neural folds are produced by the neural tube being furrowed and the presumed epidermis being pushed toward the center. The cells at this juncture give rise to neural crest cells in some species. This is most evident in vertebrates with elongated body axes, such as birds and mammals.

Neural tubes are hollowed out during secondary neurulation in frogs and chicks. In the frog, cells of the dorsal blastopore lip continue to develop ventrally rather than involuting into the embryo. The distal component of the neurenteric canal becomes the ependymal canal (neural tube lumen). The early embryonic brain balloons at an astonishing rate and scale. The brain volume in the developing chick embryo increases thirty-fold between days three and five.

The rhombencephalon forms a segmental pattern that identifies the locations from where particular nerves emerge. Every rhombus will develop ganglia, which are collections of neuronal cell bodies. The dorsal-ventral axis determines the polarity of the neural tube. The Sonic hedgehog protein, which most likely originates from the notochord, is one agent of ventral specification. Retinoic acid and TGF-superfamily determine the dorsal destiny.

The brain's neurons are arranged into layers (called cortexes) and clusters (called nuclei), each of which has distinct connections and functions. Different types of transcription factors are induced by the TGF-superfamily proteins at different distances from the roof plate, which confers distinct identities on the cells. Migrating cells create a second layer surrounding the initial neural tube as the cells close to the lumen keep dividing. As new cells from the germinal neuroepithelium are introduced to it, this layer thickens more and more. A butterfly-shaped structure made of white matter gradually surrounds the grey matter (mantle). The cerebellum is a relay station between the outer layers of the brain and other regions. Some neural progenitors migrate from the germinal epithelium to create nuclei. The internal granule layer is created when these granule neurons migrate back into the white matter. Neurons are initially arranged vertically into layers that communicate with one another, similar to the cerebellum. Adult forms of these layers are not finished until middle childhood.

Neurons in layer 6 give their principal output back to the thalamus, while those in layer 4 do the opposite. Sonic hedgehog determines how the single eye field is divided into two bilateral fields. The Pax6 protein seems to be particularly crucial for the growth of the lens and retina. The eyes appear to be the most susceptible organs to Pax6 deficiency, even though the forebrain, hindbrain, and nasal placodes are all expressed. Changes in cell structure and shape are required for the differentiation of the lens tissue into a transparent membrane capable of directing light onto the retina.

It takes the adult chicken two years to transition from an epithelial cell to a lens fiber. The iris, a pigmented and skeletal tissue, is located directly in front of the photoreceptors. The embryo in amniote vertebrates is a flattened disc, and the lateral plate mesoderm does not completely envelop the yolk sac. The early primitive streak, which starts slightly posterior to Hensen's node and extends approximately halfway down its length, is where the alleged heart cells originate. The heart is a two-chambered tube with an atrium and a ventricle in a three-day-old chick embryo. A condition known as "cardio bifida" causes a distinct heart to develop on each side of the body. By the fourth day, the ECG of a chick embryo is similar to that of an adult.

A pitx-2 transcription factor is essential for correct cardiac looping and is only activated on the left side of the lateral plate mesoderm. The Xin gene may mediate the cytoskeletal alterations required for heart looping and is also activated by transcription factors Nkx2-5 and MEF2C. Gastrulation movements are caused by the combined actions of the embryo's components rather than by the embryo as a whole. It must be assumed that there are inducing chemicals with specialized activity. Transplanting mammalian tissues into frog embryos or chick embryo tissue into rabbit embryos can induce the development of main organs in vertebrates. Induction is most likely the only process responsible for cell differentiation and cell organization into tissues and organs in vertebrates. The phenomena are credited to Spemann in 1901 and Lewis in 1904, who proved that in some species of Rana, the development of a lens from the ectoderm is influenced by the brain's underlying optic lobe. In urodele amphibians, the primary organizer, or archenteron roof, can induce the nervous system and sensory organs in the majority of vertebrate species. Spemann dubbed the dorsal lip of the blastopore the Primary Organiser because it can start the development of a secondary embryo when transplanted. Mesoderm appears to be induced by a variety of molecules from many types of embryonic or adult cells like cultured mammalian cells, guinea pig bone marrow, carp swim bladder, and chick embryos.

Cairns and Saunders (1954) demonstrated that the overlying ectoderm evolved following the origin of the mesoderm from various locations of the leg or wing. An embryonic interaction, also known

as an organizer, is the process by which one biological tissue transmits a chemical that influences another embryonic section to create a structure that would not otherwise be feasible. Responding cells are already prepared and ready to differentiate; all they need is a signal from the inducing tissue to enable them to realize their full potential. Donor tissue caused the host embryo to develop neural grooves, notochords, and other structures. They noticed that a larva with two heads had formed after gastrulation - one naturally occurring and the other stimulated by the transplanted tissue. In 1924, Max Spemann grafted a portion of the dorsal lip region of the early gastrula of *Rana sp.* (donor embryo) to create a complete embryo. He asserts that this region's development stimulates the development of the neural tube, which leads to the formation of the eyes. They noticed that a larva with two heads had formed after gastrulation was finished. One head was produced naturally throughout development, and the other was stimulated by donor tissue.

The lens-forming ectoderm that forms the cornea has developed a special capacity to react to inductive impulses. Once the lens has developed, further tissues - such as the optic vesicle - may be induced. There are several reasons for each induction as well as successive inductive occurrences. The interactions of sheets of cells with nearby mesenchymal cells are some of the most well-studied examples of induction. Connected cells give rise to sheets and tubes, which are the forms of epithelia that can be formed from any germ layer. Unconnected, loosely packed cells are referred to as mesenchyme. The mouth region of salamander and frog larvae has quite a different anatomy. The ectoderm was told to create a mouth by the mesodermal cells, but instead, it created the only mouth that it "knew" how to create. As a result, mesenchymal signals can be transmitted across species boundaries. The elements involved in developing the eye or heart of the *Drosophila* are quite similar to those responsible for developing mammalian organs. There are now more than a dozen structurally similar members of the fibroblast growth factor (FGF) family.

FGFs have been linked to the development of mesoderm, axon extension, and angiogenesis. Sonic hedgehog is a class of glycoproteins high in cysteine. This peptide is in charge of shaping the neural tube such that sensory neurons develop from the dorsal neurons and motor neurons from the ventral neurons. Sonic hedgehog, FGF4, and other paracrine factors are concentrated in the area of the developing tooth where cell interactions are forming the teeth's cusps. The members of the bone morphogenetic proteins (BMP) family were originally discovered by their ability to induce bone formation. Bone formation is only one of their many functions, and they have been found to regulate cell division, apoptosis (programmed cell death), cell migration, and differentiation. Some paracrine factors are almost entirely involved in the development of blood cells. Activin, for instance, can trigger several sets of genes at various doses and can diffuse throughout a wide range

of cell diameters. Autocrine regulation takes place when the same cells that secrete these substances also react to them. Ectodermal tissue can be given the ability to respond to the optic vesicle by being incubated close to anterior neural plate tissue. Pax6 protein appears to be crucial in enabling the ectoderm to react to the inductive signal from the optic vesicle.

5.10 TERMINAL QUESTION AND ANSWERS

5.10.1. MULTIPLE CHOICE QUESTIONS

1. How many days does it take for a chicken egg to hatch?

a. 19 Days

b. 28 Days

c. 24 Days

d. 21 Days

2. What is the part of the beak of the chick called that it uses to break out of the shell?

a. Comb

b. Pig

c. Beak

d. Egg tooth

3. Which of the tissue develops from the ectoderm of an embryo?

a. Nervous tissue

b. Muscular tissue

c. Connective tissue

d. None of the above

4. In the chick development of wing feathers, thigh feathers and claws depends on epithelial specificity conferred by induction from mesenchymal components from different sources of dermins which could be attributed to?

a. Autocrine interaction

b. Regional specificity of interaction

c. Regional activation by hormones

d. Inactivation of genetic interaction

5. Bones of vertebrates are derived from embryonic:

a. Ectoderm

b. Epiderm

c. Mesoderm

d. Endoderm

6. What would happen as a result of a transplantation experiment in a chick embryo where the leg mesenchyme is placed directly beneath the wing apical ectodermal ridge (AER)?

a. Distal hind limb structures develop at the end of the limb.

b. A complete hind limb will form in the region where the forelimb should be

c. The forelimb would form normally

d. Neither a forelimb nor a hind limb would form since the cells are already determined

7. The ability of cells to respond to a specific inductive signal is called

a. Regional specificity of induction

b. Competence

c. Juxtracrine signaling

d. Instructive interaction

Answers: 5.10.1: 1.d, 2.d, 3.a, 4.b, 5.b, 6.a, 7.b

5.10.2. LONG ANSWER TYPE QUESTIONS

Q.1. Write in detail about the Primary organizer and its morphological differentiation.

Q.2.What is embryonic induction? What are its various types?

Q.3. Write about various inductive interactions and factors affecting them.

Q. 4. Describe primary and secondary neurulation.

Q.5. Elucidate the mechanism of neural plate formation and its shaping.

Q.6. Explain the process of the development of a neural tube.

Q.7. How the tissues are organized in Central Nervous System? Explain.

Q.8. Write in detail about the development of the heart.

UNIT 6: REGENERATION AND METAPLASIA

CONTENTS

6.1 OBJECTIVES

After studying this unit, you will be able to:

1. Understand the phenomenon of regeneration in animals
2. Types of regeneration
3. Distinguish between physiological regeneration and reparative regeneration
4. Explain the terms autotomy and metaplasia.

6.2 INTRODUCTION

Regeneration can be defined as the natural ability of the organisms to replace worn-out parts, repair or renew damaged or lost parts of the body, or reconstitute the whole body from a small fragment during the post-embryonic life of an organism.

Regeneration thus is also a developmental process that involves growth, morphogenesis, and differentiation.

Many organisms show the ability or power of regeneration. The power to regenerate the lost parts of the body is found throughout the animal kingdom, having a wide diversity in its potency.   Regeneration is a fundamental phenomenon of life. It is dispensable for the survival of the organism. Richard L. Gross has described it aptly as "if there is no regeneration there could be no life, if everything is regenerated there would be no death, all organisms exist between these two extremes." Among animals, the power of regeneration was first discovered in Hydra by Abraham Trembley.

If the tail of a house lizard is cut, the missing part develops again from the remaining part of the tail. In some cases, regeneration is so advanced that an entire multicellular body is reconstructed from a small fragment of tissue.

Our body spontaneously loses cells from the surface of the skin and is replaced by newly formed cells, this is due to regeneration. Regeneration can be defined as the natural ability of organisms to replace worn-out parts, repair or renew damaged or lost parts of the body, or reconstitute the whole body from a small fragment during the post-embryonic life of an organism. Regeneration is thus also a developmental process that involves growth, morphogenesis, and differentiation.

Types of Regeneration:

In physiological Regeneration, there is a constant loss of many types of cells due to wear and tear caused by day-to-day activities. The replacement of these cells is known as physiological regeneration Eg: The replacement of Red Blood cells (R.B.Cs). The worn-out R.B.Cs is deposited in the spleen and new R.B.Cs is regularly produced from the bone marrow cells. To note further the life span of R.B.C's is only 120days and this process of regeneration is continuous.

Replacement of Epidermal Cells of the Skin: The cells from the outer layers of the epidermis are regularly peeled off by wear and tear. These are constantly being replaced by new cells, added by the malpighian layer of the skin.

2. Reparative regeneration: This kind of regeneration, as the name suggests, involves the repair of a wound or replacement of a body part removed intentionally or due to injury.

This type of regeneration may include restoration of parts of an organ or an organ as in the regeneration of the eye and lens in amphibians or parts of the whole organism as in limbs of urodeles, or it may be the regeneration of an entire organism from a pan detached from the parent body as you will see in hydra.

The power of this type of regeneration is not found uniformly in all animals. Some have great powers of such regeneration; in others, it is limited to varying degrees and in yet others, it is not found at all.

Example: Regeneration of limbs in *Salamanders*, regeneration of lost tail in lizard, healing of the wound, replacement of damaged cells etc.

Autotomy: In some animals like starfish (*Asterias*), some part of the body is broken off on being threatened by a predator. This phenomenon of self-mutilation of the body is called autotomy

Example: Crabs break off their leg on approaching the enemy, Holothurians throw off their internal viscera, Starfish breaks off an arm etc.

Regenerative capacity in animal group: The capacity of regeneration varies in its extent in various animal groups. Regenerative capacity is very high among protozoans, sponges, and coelenterates.

Invertebrates: Regeneration was first discovered in hydra by Trembley (1740). Even 1/1000th part of the body regenerates into new organisms. In Phylum Porifera (sponges), the whole body can be reconstructed from isolated body cells. The cells rearrange and reorganize to form a double-layered sponge body wall.

Phylum Coelenterates (*Hydra* and *Planaria*) are the best examples of regeneration. Small fragments of the body can give rise to a whole animal. In *Hydra* polyp may be cut into two or more parts and each part will grow into a new fully developed part. The anterior cut of the body regenerates the part of the posterior end with an adhesive end and foot while the posterior cut end reconstitutes the mouth and tentacles. (See fig 6.1)
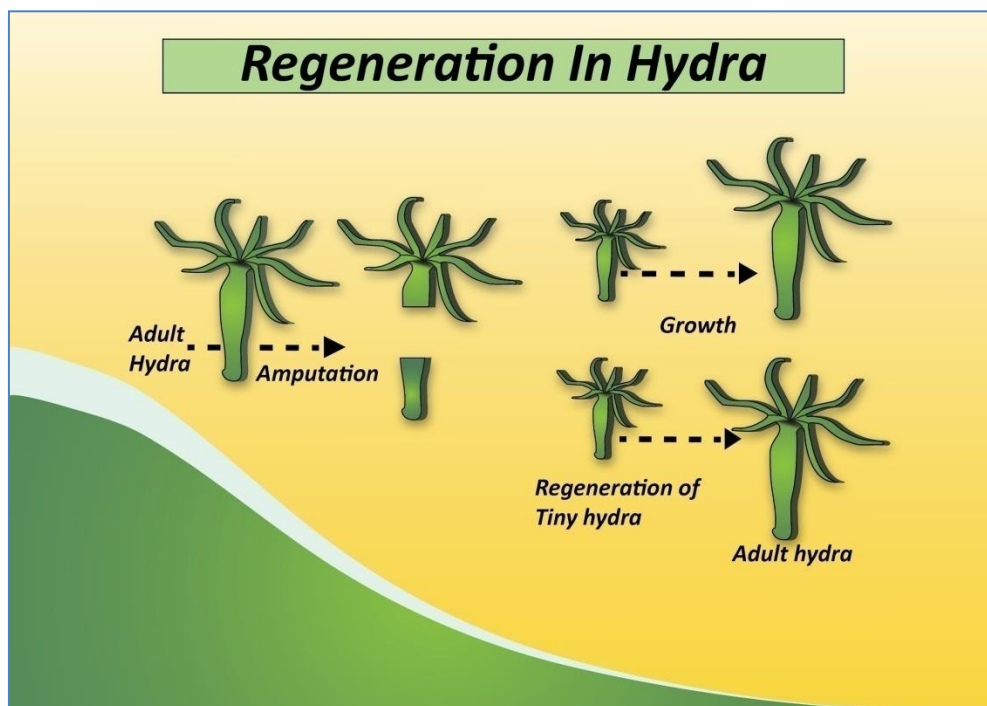
Fig 6.1: Regeneration in Hydra (Online sources)

When a *Planaria* is cut into many pieces, each part regenerates into a whole individual. The power of regeneration in *Planaria* is maximum near the anterior end while minimum near the posterior end. It is cut across lengthwise and each part of the body can regenerate the missing

half. If a planarian can be cut from the middle part of the body through the pharynx, it can regenerate a new one. (See fig 6.2).
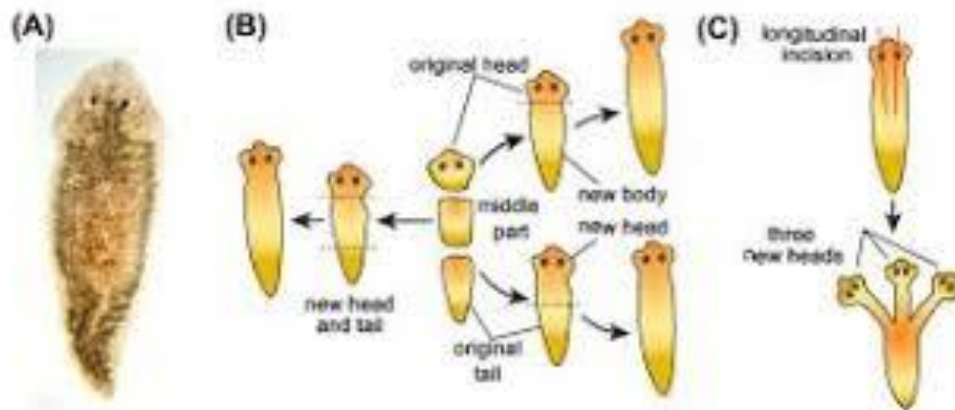
Fig 6.2: Regeneration in Planaria (Online source)

Some annelids like earthworms can regenerate some segments removed from the anterior and posterior ends of the body. Some molluscs can regenerate only the eyes and heads while squids can also regenerate their arms. Many arthropods (e.g., spiders, crustaceans, insect larvae, etc) can regenerate limbs only. Regeneration is faster in the young than in the adults. A regenerated part may not always be similar to the part lost. This type of regeneration is called heteromorphosis. Echinoderms (like starfish, brittle star, and sea Lilly) exhibit autotomy. They can regenerate arms and parts of the body.

Vertebrates:

Fishes: Lamprey can regenerate its lost tail. Some fishes can regenerate parts of their fins.

Amphibians: The regeneration power is well marked in urodele amphibians like salamanders, newts, and their axolotl larvae. They can regenerate limbs, tails, external gills, jaws, and parts of the eye like the lens and retina. Tail and limb regeneration is found in the larval stages of frogs and toads.

Reptiles: Lizards exhibit autotomy. When threatened, the lizard detaches its tail near the base to confuse its predator and later regenerates a new tail. The new tail differs from the old one in its shape, absence of vertebrae, and the kind of scales covering it.

Birds: Regeneration is restricted to parts of the beak.

Mammals: Regeneration is restricted to tissues only. External parts are not regenerated. Skin and skeletal tissues possess great power of regeneration. The liver has the maximum capacity for regeneration. If one kidney is damaged or removed, the other enlarges to compensate for the lost kidney. This is called compensatory hypertrophy.

Types of Regeneration based on Cellular Mechanism:

 Based on cellular mechanisms regeneration can be of two types:

1. Morphallaxis: In this type, regeneration occurs mainly by the remodeling of existing tissues and the re-establishment of boundaries, thus involving very little new growth. As a result, the regenerated individual is much smaller initially. It subsequently increases its size and becomes normal after feeding. This type of regeneration is known as morphallaxis or morphallactic regeneration. Example: Regeneration of hydra from a small fragment of its body. (See fig 6.3)
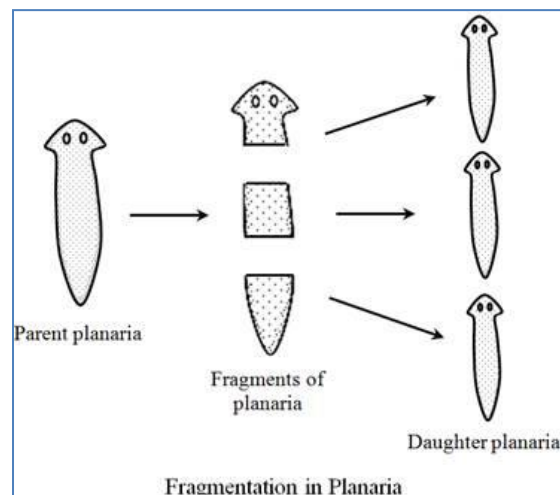
Fig 6.3: Fragmentation in Planaria (Online source)

2. Epimorphosis: In this type, regeneration involves the dedifferentiation of adult structures to form an undifferentiated mass of cells. They are highly proliferating and accumulate under the epidermis, which has already expanded. Within two days, the bulge transforms into a conical hump. This lump of dedifferentiated cells along with the epidermal covering is called

regeneration bud or regeneration blastema. The dedifferential cells continue to proliferate and finally redifferentiate to form a rudiment of the limb. The rudiment eventually transforms into a limb. This type of regeneration is known as epimorphosis or epimorphic regeneration.

 Example: Limb regeneration in amphibians.

3. Heteromorphosis or heteromorphic regeneration: When a different organ develops from the one that has been removed, the phenomenon is called heteromorphosis. Eg: In shrimp Palinurus, the eye is regenerated, if it is removed from the eye stalk. But if the eye is removed along with optic ganglion, instead of the eye an antenna-like organ is regenerated. This type of regeneration is exhibited by lower animals.

 4. Super regeneration: The development of a superfluous number of organs or parts of the body (eg. Heads, tail limbs) as a result of regeneration is known as super regeneration. When a deep incision is made on the head end of planaria or earthworm, additional heads will develop. Incisions in the middle part cause the development of both heads and tails.

5. Wolffian regeneration: It is a special kind of regeneration found in urodeles and anurans. In Newt, *Triturus*, if the lens of the eye is removed, a new lens is formed from the uninjured iris. The original lens is developed from epidermal ectoderm but the regenerating lens, formed from the iris is neuroectodermal in origin. Thus regeneration of a part of an organ from tissue other than its original embryonic tissue is called Wolffians regeneration, named after the discoverer Wolf (1935).
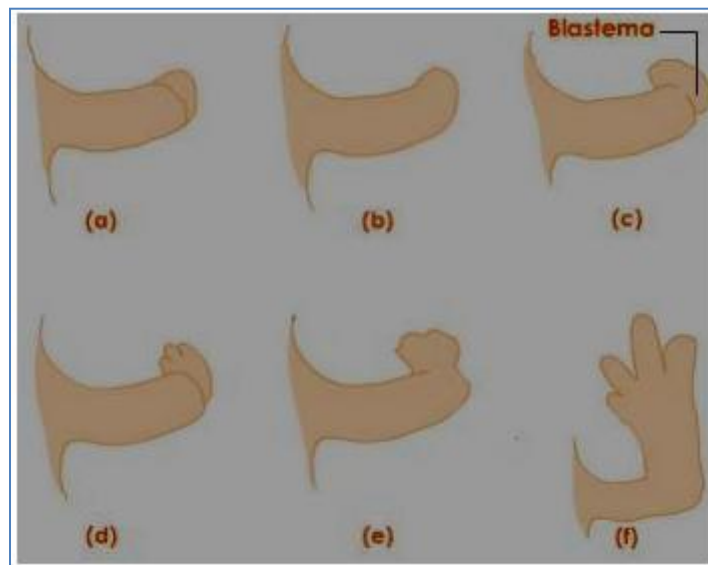
Fig 6.4: Regeneration of a Limb of a Newt (Online source)

Mechanism of Regeneration: Regeneration is a complex process that involves histological and physiological events.

Regeneration of a Limb of a Newt: The mechanism of regeneration in salamander involves the following stages.

- **Wound healing:** The epidermal cells from the edges of the wound migrate and spread over the exposed surface. This is known as wound healing.

- **Blastema formation:** A few days later, undifferentiated cells accumulate inside the epidermis, resulting in a bulge. This is known as regeneration bud or blastema.

- **Redifferentiation and morphogenesis:** The blastema develops rudiments of the lost organ, like the digits which grow into new digits.

- **Growth:** The regenerated limb increases and attains the size of a normal limb.

In planarians and Hydra, there are undifferentiated cells called neoblasts which multiply and then migrate from the deeper parts of the body to the cut surface.

6.3 DISTRIBUTION OF REGENERATIVE ABILITY

Although regeneration is found throughout the animal kingdom, the ability to regenerate lost parts differs greatly in various groups of animals.

Regeneration in micro-organisms and Protozoans: The single cell marine alga called Mermaid's Cap (Acetabularia), which has a 50-mm length and its body part as small as 1/100th of the total size, is capable of regenerating new individuals.

Regeneration in invertebrates:

(1) The process of regeneration is quite pronounced in sponges. The whole organism can be reconstituted even from a few undifferentiated archaeocytes.

(2) Regeneration ability is very high in coelenterates. Trembley discovered the regeneration in Hydra polyp in 1740 by cutting it into two or more parts; as a result, each part reconstitutes itself into new complete individuals of miniature size.

(3) Except planarians, Platyhelminthes do not regenerate to any great extent. When planarians cut across or length-wise, each part of the body will regenerate the missing half.

(4) Nemerteans have a great regenerating ability and a complete worm can be formed even starting from a very small fragment.

(5) Regenerating ability is very low in nematodes in which only closure of the superficial wound is possible by a high degree of cell differentiation up to a fixed limit of some cells.

 (6) In Annelida, if any oligochaete is cut into two halves, the posterior one regenerates the anterior end with the mouth and the anterior half regenerates the new posterior end.

(7) Regeneration is relatively poor in Mollusca. In gastropods, eye stalks with eyes as well as parts of the head or the foot may be regenerated.

(8) Regeneration is limited to the renewal of the lost part in Arthropoda. In most crustaceans, limbs may regenerate at any stage of development, while in insects limb regeneration occurs only in larval stages and regenerated limb often does not reach normal limb size.

(9) Among echinoderms, the starfishes, brittle stars, and sea lilies can regenerate arms and parts of the disc.

Regeneration in vertebrates: Regeneration power is restricted to fins in fishes. But in amphibia, regenerative power is highly specialized and most spectacular in urodele amphibians (newts, salamanders) in larval as well as adult stages. Tails, external gills, upper and lower jaws, lens, and retina of the eye can regenerate.

In newts and salamanders, repair starts in limbs by spreading the epidermis over the wound followed by the closing of the wound. When the tails of reptiles like lizards are broken off, a new tail regenerates which differs from the original one because of simplified vertebral columns and scales of a different type. In birds, only the beak can be regenerated. In mammals, except few marsupials, the regeneration power of limbs is very poor.

6.4 POLARITY IN REGENERATION

Generally, some animals show a clear example of polarity in Regeneration. The best example is *Hydra* (Coelenterate) which has a base for attachment, a long body, and a set of tentacles at the anterior end around the mouth. If we cut a *Hydra* into two parts, the anterior end will form the

tentacles and the lower end will form the base for attachment. Thus, there is a distinct polarity in the hydroids body. The upper end of each piece forms tentacles, while the lower end forms the base. This polarity is similar to the animal-vegetal polarity in the sea urchin eggs and the anterior-posterior polarity in the amphibian limbs. However, the polarity of the regenerating part is not fixed like that of the polarity in embryonic systems.

The polarity of the hydra can easily be changed by treating it into two cut ends of a polarized segment with oxygen. If we cut pieces in a chamber, dividing it into two, having different oxygen concentrations, the polarity is completely reversed, the posterior end which would normally develop into a base, forms the tentacles. Thus, it is evident that the organization within that is liable to change and be reversed by the application of different concentrations of oxygen at two ends.

Factors affecting the regeneration: As already stated, regeneration is the ability of an organism to regain lost parts. The regenerative ability of an organism is mainly by its developmental stages. There are many external and internal factors controlling regeneration. Some of them are discussed here:

Temperature: This is the main controlling factor. Temp. Directly affect the rate of regeneration. If the temperature is too low, the regeneration process also goes very slowly. The increase of temperature to a certain limit accelerates the regeneration process. But it should not be very much high as it is lethal to all the regenerative processes, for example, Planaria larva: this does not regenerate at 3^0C but the regenerative activities are most active at 29^0C. Although a temperature of 32^0C appears to be lethal for these types of animals.

Oxygen (O₂): This is also a very important factor for regeneration power among animals. The amount of O_2 supply affects the rate of regeneration. It increases the rate of regeneration.

Food: Food does not show any important effect on regenerative ability. Fasting animals regenerate at the expense of their stored food. However, the maximum quantity of food accelerates regeneration to a certain extent.

Chemicals: Chemicals, especially Beryllium showed a great reaction as a controlling agent. Traumatization with a needle is not sufficient to boost regeneration in the adult frog. Here, the application of the hypertonic salt solution to the wound after amputation gives encouraging results. While Beryllum salts applied to the amputated surface suppresses regeneration. It binds the substance released from the damaged cells at the wound that cause regeneration.

6.5 MECHANISM OF REGENERATION OF AMPHIBIAN LIMB AND LENS

Regeneration of amputated amphibian limbs is perhaps the most dramatic example of reparative growth among vertebrates. In anuran amphibians (frogs and toads), regeneration is restricted to the developing larval limb, but limb regeneration occurs throughout life in many, if not most, urodele species (newts and salamanders). Unlike fin and tail regeneration, the process in limbs includes reproduction, not only of a complex musculature and vasculature but also a skeleton of articulated endochondral bones with the original anterior/posterior patterning of the autopods (hands or feet). Regeneration occurs via several overlapping phases, including wound closure, dedifferentiation, cell proliferation and migration, growth, patterning, and differentiation. Understanding this process involves sorting out how the events elicited by the trauma of amputation set the stage for and integrate with the morphogenetic events and growth that allow the replacement structures of the limb to form.

Salamander limb regenerates in the following stages:

(1) Wound healing: After amputation of limb, the epidermal cells surrounding the wound migrate and spread over the exposed surface. This stops the bleeding. This is known as wound healing. This layer of epidermis proliferates to form apical ectodermal cap.

(2) Blastema formation: After some days cells below the epidermis start dedifferentiation. These dedifferentiated cells accumulate inside the epidermis. Due to this accumulation a bulge or outgrowth form termed as blastema.

(3) Redifferentiation and morphogenesis: The blastema cells continue to divide. The specific pattern and axis (dorsal-ventral, anterior-posterior) will form in the growing blastema. Thus the cells of the blastema redifferentiate develop into different structure of the limb. Edge grows out into new digits.

(4) Growth: New blood and nerve supply develops in the growing limb. The regenerated limb increases in size and attain the normal length.
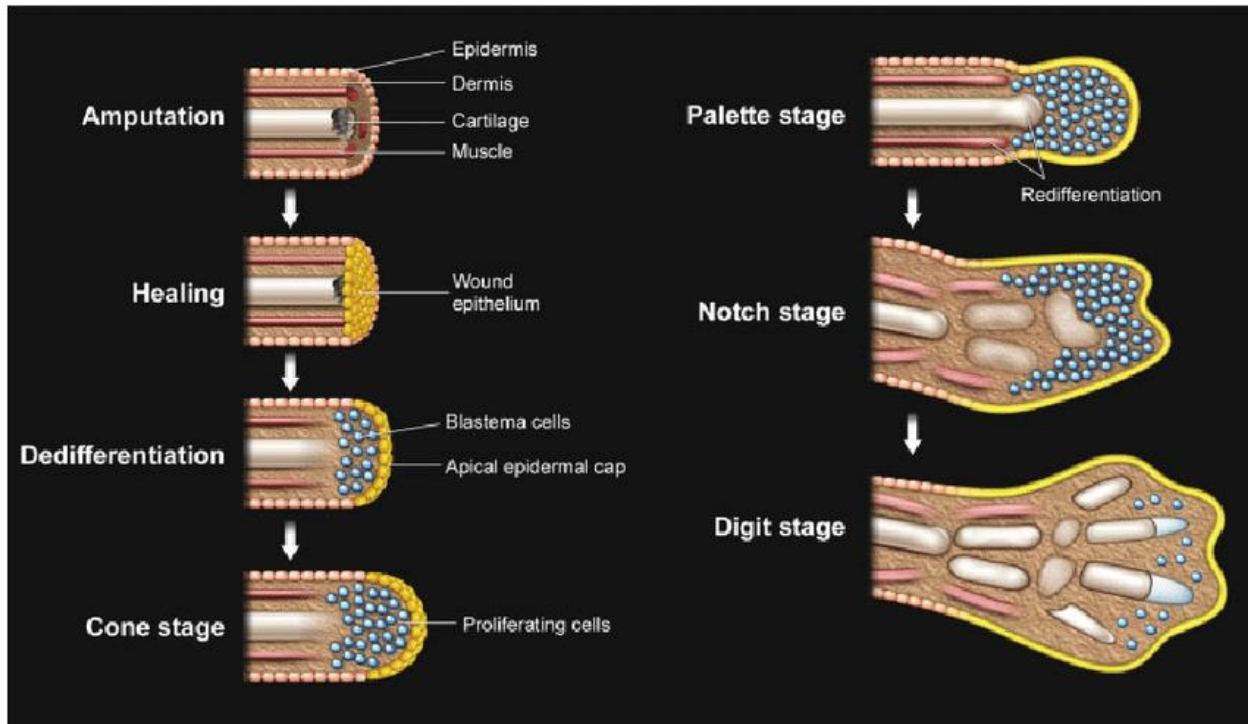
Fig. 6.5: Regeneration of limb of Salamender (Source: Essential Developmental Biology, Slack J. W.)

Mechanism of regeneration of amphibian lens: It is a special kind of regeneration found in urodeles and anurans. In Newt, *Triturus*, if the lens of the eye is removed, a new lens is formed from the uninjured iris. The original lens is developed from epidermal ectoderm but the regenerating lens, formed from the iris is neuroectodermal in origin. Thus regeneration of a part of an organ from tissue other than its original embryonic tissue is called Wolffians regeneration, named after the discoverer Wolf (1935).

It regenerates in the following stages:

a) After the removal of injury of the lens the dorsal region of iris thickens and a cleft arises between inner and outer lamellae of the iris.

b) Amoeboid cells moved from the stroma into the cleft followed by marked increase in the RNA and DNA synthesis as well as of mitotic cell division.

c) The pigmented cells of the dorsal region are engulfed by amoeboid cells.

d) The formed non pigmented cubical cells from hollow epithelial vesicle and extend inner and outer lamellae.

e) The inner wall cells of the vesicle elongated into the lumen and form primary lens fibre.

f) Later on the lens especific crystalline proteins are formed.

g) The primary lens fibres push to the front of vesicle to form the nucleus behind the lens epithelium which forms the secondary lens fibre.
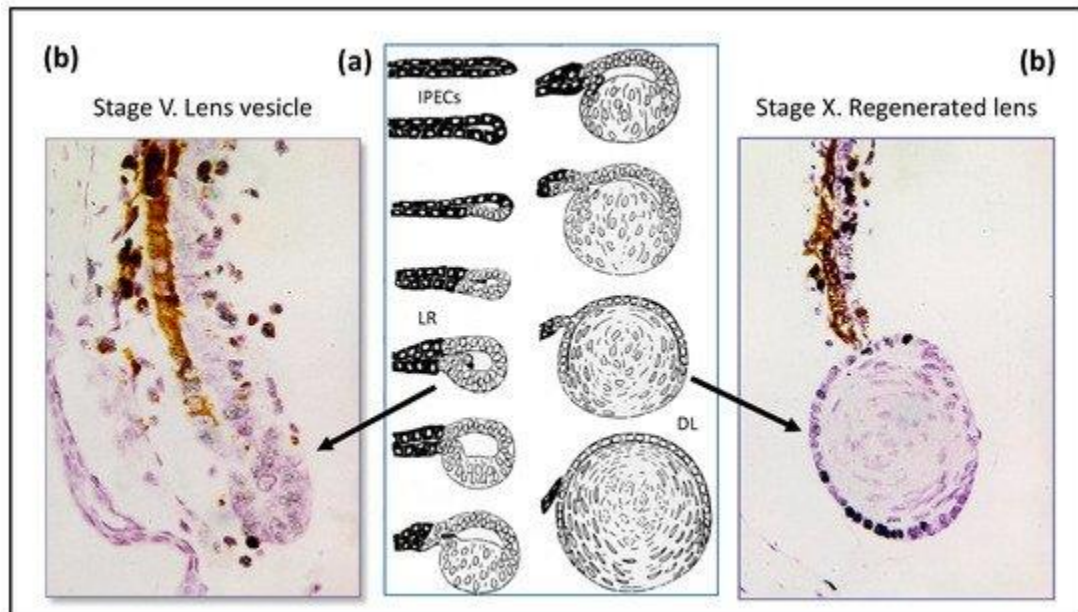
Fig. 6.6: (a) Stages of regeneration of eye lens from the dorsal part of the iris after lens surgical extirpation in the newt. IPECs—iris pigment epithelial cells, LR—lens regenerate, DL—differentiated lens. (b) Histological pictures of newly forming lens (grey and black nuclei—inclusion of 3H- thymidine labelled cells) (Source: JBD)

6.6 METAPLASIA

Metaplasia refers to the replacement of a mature, differentiated cell type by another mature, differentiated cell type that does not typically occur in the tissue in which it is found. Metaplasia typically occurs as a response to chronic irritation of cells, which can be environmental or pathological. Metaplasia itself is a benign, non-cancerous condition; however, if left untreated, the cells undergoing metaplasia can become dysplastic (i.e., atypical in shape and size), which can eventually lead to cancer. Metaplasia can be better understood with the below example;

Intestinal metaplasia: Intestinal metaplasia refers to a transformation in cell type in the upper digestive tract, which includes the stomach and esophagus. The nonkeratinized squamous epithelium that typically covers the esophagus transforms into nonciliated columnar epithelial cells. This condition is also known as Barrett's esophagus and is a consequence of gastroesophageal reflux disease (GERD), which occurs as a result of stomach acid flowing backward into the esophagus. Barrett's esophagus can be reversed by treating the underlying GERD. However, if the condition persists, esophageal cells can become dysplastic. On the other hand, intestinal metaplasia that occurs in the stomach is typically associated with a bacterial infection known as Helicobacter pylori (*H. pylori*). H. pylori can affect the protective mucus lining of the stomach, allowing the acidic contents to irritate the underlying stomach epithelial cells, ultimately leading to metaplasia.

Causes of metaplasia: More often, metaplasia is caused by stressors (i.e. stomach acid) that initiate the transformation into a new type of cell that is better adapted to handle the increased stress. More specifically, intestinal metaplasia can be caused by H. pylori infection, alcohol consumption, and chronic acid reflux. Squamous cell metaplasia of the respiratory tract is typically induced by smoking which turns into the accumulation of toxins. Human papillomavirus is the leading cause of cervical metaplasia and is mainly transmitted through sexual contact.

Metaplasia reversibility: Metaplasia is reversible. Removing the offending stimulus, such as smoking cessation in the case of respiratory squamous metaplasia or administering antibiotics and acid-reducing proton pump inhibitors in the case of intestinal metaplasia. While metaplasia is a risk factor for cancer, it is not cancer. If metaplasia undergoes another stage of transformation, the cells will become dysplastic. Dysplastic cells are considered a precancerous cell type and, if left untreated, will typically become cancerous.

6.7 SUPER-REGENERATION AND HETEROMORPHOSIS

Super regeneration: The development of a superfluous number of organs or parts of the body (such as Heads, tails, and limbs) as a result of regeneration are known as super regeneration. When a deep incision is made on the head end of planaria or earthworm, additional heads will develop. Incisions in the middle part cause the development of both heads and tails.

Heteromorphosis: Heteromorphosis refers to situations where an organ or tissue is different from the expected, either because of (embryonic) development anomalies, or after reparative regeneration following a trauma. The difference includes an abnormal location or an abnormal shape. It should not be confused with homeosis, which means a big change in the tissue structure of an organ. Heteromorphosis is an example of the imperfection of some manifestations of the regenerative capacity.

Many organisms from protozoans to the chordate may have heteromorphosis examples, but it is easier to find in lower forms of animals:

- Earthworm: distortion of polarity: replacement of removed tail with the head end

- Actinia: development of a cut into a second mouth

- Decapods: the replacement of removed eyes with antennae

6.8 SUMMARY

Regeneration can be defined as the natural ability to live organisms to replace worn-out parts, repair or renew damaged or lost parts of the body, or reconstitute the whole body from a small fragment during the post-embryonic life of an organism. Regeneration thus is also a developmental process that involves growth, morphogenesis, and differentiation. Many organisms show the ability or power of regeneration. If the tail of a house lizard is cut, the missing part develops again from the remaining part of the tail. In some cases, regeneration is so advanced that an entire multicellular body is reconstructed from a small fragment of tissue. Our body spontaneously loses cells from the surface of the skin and is replaced by newly formed cells. This is due to regeneration.

6.9 TERMINAL QUESTIONS AND ANSWERS

6.9.1 VERY SHORT QUESTIONS

Q 1. What is regeneration?

Ans. The process of repair and renewal of lost parts of the body is known as regeneration.

Q. 2. What is heteromorphic regeneration?

Ans. In heteromorphic regeneration, the regenerated organ is different from the one that has been renewed.

Q. 3. What is Blastema?

Ans. A few days after the healing of the cut or wound, the epidermis covering the wound bulges out forming a stumpy outgrowth. This is known as blastma or regeneration bud.

Q. 4. Name the factors which affect regeneration.

Ans. Temp., food, oxygen concentration, radiation, electric current, and seawater.

Q. 5. Which factor affects the metabolism of carbohydrates and proteins?

Ans. Injury.

Q. 6. What is responsive tissue?

Ans. This is the tissue that responds to a morphogen of the inductor.

Q. 7. What is embryonic induction?

Ans. Embryonic induction is the process in which one tissue causes the differentiation of another tissue during the development of an animal embryo.

Q. 8. What is necessary for regeneration in oliates?

Ans. Macromolecules

6.9.2 MULTIPLE CHOICES QUESTIONS

1. Regeneration is similar to:

a. autotomy

b. differentiation

c. Cleavage

d. division

2. Morpahallaxis occurs only in:

a. higher animal groups

b. lower animals group

c. both a and b

d. none of these

3. In *Salamander* complete limb is regenerated within:

a. 35 days

b. 45 days

c. 50 days

d. 75 days

4. Entire organs cannot regenerate in:

a. Fishes

b. Mammals

c. Birds

d. Reptiles

5. Autonomy is seen in the tail of:

a. Tadpole

b. Rat

c. Gecko

d. All of these

6. All the physiological changes are under:

a. Neural control

b. Hormonal control

c. Both a and b

d. None of these

7. *Salamander* and *Axolotl larva* regenerate:

a. Limbs, eye structure and intestine

b. Jaws and external gills

c. Both a and b

d. Trunk

8. Regeneration in animals was reported by:

a. T. H. Huxley

b. A. G. Trembley

c. T. H. Morgan

d. T. Muller

9. Tissue regeneration is found in:

a. Mammals b. Reptiles

c. Birds d. Amphibians

10. Regeneration in micro-organisms and protozoans cannot take place without:

a. Micronucleus

b. Nucleolus

c. Flagella

d. Macronucleus

11. Various types of cells aggregate into masses and organize into a whole organism in:

a. Coelentrates

b. Sponges

c. Vertebrates

d. Protozoans

12. Among annelids, the following animals never regenerate:

a. Earthworm

b. Allolobophora

c. Leech

d. Polychaetes

13. Destructive metabolic phase in regeneration is:

a. Catabolic

b. Anabolic

c. Respiratory

d. Excretory

14. Metaplasia is:

a. Non-reversible change

b. Reversible change

c. Both a and b

d. None of these

15. What are the common symptoms of metaplasia:

a. Loss of appetite

b. Weight loss

c. Belching

d. All of the above

16. Which of the following are the types of epithelial metaplasia:

a. Squamous metaplasia

b. Columnar metaplasia

c. Both a and b

d. None of the above

17. What kind of response does metaplasia give:

a. Adaptive response

b. Non-adaptive response

c. Both a and b

d. None of the above

Answers: (1)a (2)b (3)d (4)b (5)c (6)b (7)c (8)b (9)a (10)d (11)b (12)c (13)a (14)b (15)d (16)c (17)a

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UNIT 7: METAMORPHOSIS

CONTENTS

7.1 OBJECTIVES

After studying this module, you shall be able to learn and understand:

- To understand metamorphosis.
- Types of metamorphosis.
- Physiological and biochemical changes during metamorphosis.
- Hormonal control of metamorphosis.
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7.2 INTRODUCTION

The egg of an animal at rest if provided with an enormous amount of food is smaller than the sexually mature adult. The egg, so, does not directly develop into an organism the size of an adult. The environment has adopted many methods to overcome this complication. Some of these methods can be usually classified into the following three categories:

1. Direct development: In lots of animals, the hatched organism resembles an adult, without that it is small in size. It is usually called a juvenile. In such cases, only growth and sexual development occur in the juvenile after its hatching, e.g., certain *Turbellaria, Ascaris, Pheretima,* reptiles, and birds. In the eggs during a large amount of yolk (macrolecithal eggs of reptiles and birds), the growth is direct through no larval stage and no metamorphosis, since the supply of food is sufficient for the whole development.

2. Viviparity: In a few animals, the hatched organism grows and develops inside the body of the mother. It draws its food supply and oxygen from her with the help of a placenta. The remaining growth of a newborn takes place after birth. For example, in eggs with very little yolk (microlecithal eggs of the placental mammals), the development is direct without a larval stage.

3. Indirect development: In many animals, the egg develops into an unusually different organism from the adult. Such an organism leads a self-regulating free life and is known as a larva. Therefore these are with yolk but that is not enough for the complete development, e.g., *Herdmania, Amphioxus*, frog, sea urchin, and insects. The larval stage is free-swimming or free living and feeds excitedly to store food material for additional development. Compared with their adults, the larvae habitually have different habitats and a different way of life. Larvae can be so different from adults that in the past some were inaccurately given a species or a generic

status. For example, the first instar larva of *Meloidae* (entomophagous, oil, or blister beetle) was originally given the generic status of *Triungulinus*. The process of the transformation of larvae into an adult is termed metamorphosis. (Gr., metamorphoun= to transform).

Definition of metamorphosis: Metamorphosis is a post-embryonic extension of the developmental potential and involves dramatic changes in habit, habitat, morphology, physiology, and behavior of the larva so that it is transformed into an adult having an entirely different habitat and structure.

Metamorphosis is a widespread developmental phenomenon that is usually connected with a theatrical change in habitat and succeeding way of life, such as the change from a planktonic to a benthic existence way of the sea-urchin, from an aquatic to a terrestrial survival in frogs and toads, and from non-flying to a flying continued existence in insects. Such changes in atmosphere and behavior demand the equally rapid transformation of the structure and function of the living technology. Metamorphic change during the development cycle is an acceleration or absorption of essentially the same basic process feature of most forms of development. Mainly, it consists of differential destruction of definite tissues, accompanied by an increase in growth and demarcation of other tissues.

Occurrence of metamorphosis: Metamorphosis occurs in the majority of metazoan phyla, starting with Porifera (amphiblastula) and ending with Amphibia (tadpole). Animals with no direct development exhibit a range of larvae that undergo metamorphosis. The procedure of metamorphosis in different animal groups differs equally like alteration and in the mode of causation of the entire sequence, so, it is not possible to explain their metamorphosis in general terms.

7.3 KIND OF METAMORPHOSIS

Among the chordates take place the following two basic types of metamorphosis:

1. Retrogressive metamorphosis: it occurs in ascidians wherever only the larvae (tadpoles) have chordate features, the adults, adapted to a sessile existence, lose all larval locomotory organs and thus give up all traces of chordate relationships.

Metamorphosis is the shape that varies in form during postembryonic development and in several cases, signals a theatrical change in the habitat of the animal such as a change from a

pelagic to benthic survival. Metamorphosis of the ascidian larva is unique and begins about explosively. It involves the transformation of an active, non-feeding, pelagic, lecithotrophic (i.e., that feeds on its food on its yolk reserves) and tailed larva having many advanced features such as axil notochord, dorsal neural tube, and particular sense organs, into an insert, sedentary or sessile, simple (primitive) and planktotrophic filter- feeding adult through only a pharynx with stigmata and endostyle, regularly indicating the chordate features of adult ascidian. This type of metamorphosis which shows degenerative or retrogressive changes starting from larva to adult is called retrogressive metamorphosis. It involves the following two types of change.

1. Retrogressive metamorphic changes: They involve the destruction and disappearance of some of the larval structures such as follows:

i) Long tail of a larva with a caudal fin shortens and finally disappears.

ii) Caudal muscles, nerve cord, and notochord disappear as they break down and are consumed by phagocytes. Thus, with the resorption of the tail go the most obvious traces of tunicate's chordate affinity.

iii) Larval sense organs (the ocellus and the otolith) are lost and the sensory vesicle is transformed into an adult cerebral ganglion.

iv) Adhesive papillae disappear completely.

The biological occurrence in which the structures which are related only to the embryo or larva and have no consequence for the adult animal, are lost at hatching, birth, or metamorphosis, is called caenogenesis (Cohen, 1967). It allows larvae to develop quite different environments from the adults of some species.
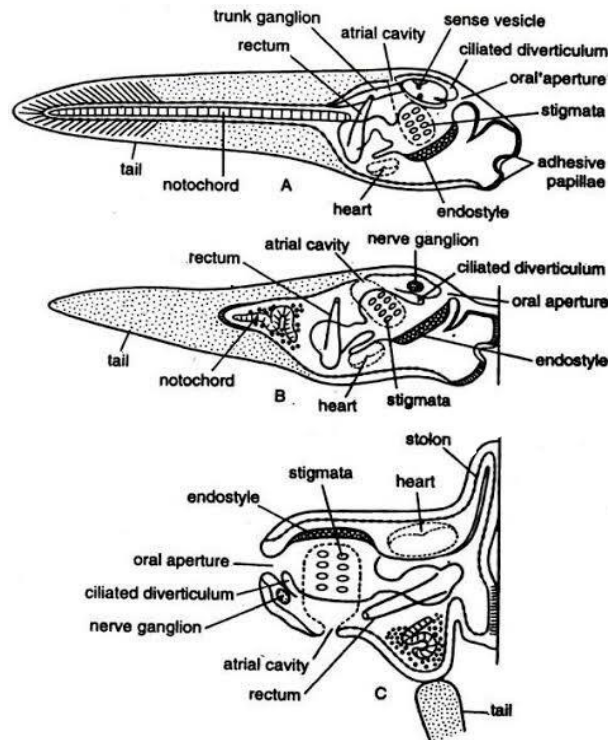
Fig.7.1: Ascidia sp. Metamorphosis- free-tailed larva into a fixed ascidian

2. Progressive growth: Cells in the larval head give rise to adulthood. Some larval features are retained and developed into the particular adult structures:

i) Due to the loss of the tail, the trunk becomes pear-shaped and four larger ectodermal ampullae grow out of its four corners. These ampullae firmly anchor the metamorphosing tadpole to the substratum. Rapidly two smaller ectodermal ampullae appear dorsolaterally.

ii) Anterior region among the point of attachment (adhesive papillae) and mouth exhibits rapid growth, whereas the original dorsal side with atriopore stops growth. This causes shifting of mouth through 90°. The body too rotates so that the general form of the adult sessile organism is unspecified.

iii) Adult neural glands and nerve or cerebral ganglion are produced by the neural tube and trunk ganglioncome to lie mid-dorsally between mouth and atriopore. The trunk ganglion itself persists as a visceral nerve.

iv) With the absorption of its test covering, the mouth becomes functional and filter-mode of feeding by inward ciliary water currents.

v) Pharynx greatly enlarges, develops blood vessels and stigmata multiply rapidly, forming the branchial sac.

vi) Stomach enlarges, intestine elongates and gets curved and liver develops.

vii) Atrial cavity becomes more extensive.

viii) Circulatory system with heart and pericardium develops.

ix) Gonads and gonoducts develop from larval mesodermal cells.

x) Test or tunic spreads to cover the entire animal, becomes thick, tough, and vascular, and attaches the animal by forming a foot if essential.

Therefore, the foregoing metamorphosis changes mark the beginning of a sedentary, actively feeding, sexual adult form.

Conclusion of retrogressive metamorphosis: As an outcome of retrogressive metamorphosis, the free-swimming photo-positive and geo-negative ascidian tadpole larva change into fixed, inactive geo-positive and photo-negative adults. The chordate characteristics of larva similar to the notochord, nerve cord, and sense organs are lost in adults.

2. Progressive metamorphosis: The frog similar to other amphibians and insects has a progressive metamorphosis (in different contrast to the retrogressive metamorphosis of the Ascidians).In the progressive metamorphosis of the frog, the metamorphosis is connected with a transition from an aquatic to a terrestrial mode of life and from an herbivorous to carnivorous manner of feeding. It has a task to change an aquatic, herbivorous, tailed tadpole larva into a terrestrial, carnivorous, and tailless frog. This alteration or metamorphosis involves many structural, biochemical, and physiological changes. These changes comprise the destruction of existing structures, construction of new structures, and modification of larval structures. At the cellular level, metamorphosis is accomplished by cell death, cell proliferation, and cell differentiation. Many genes active in larva become switched off, while many genes immobile in larva start their movement. While metamorphosis requires a harmonious expression of many genes in different tissues, it is controlled by hormones such as TH or thyroid hormones (e.g., thyroxine) In *Rana tigrina* the whole process of metamorphosis is completed within a week (Agarwal and Niazi, 1977). It involves the following two types of changes:

Anatomical Metamorphic changes: Such changes fall into the following three categories:

A. Destructive or regressive changes: The organs or structures that are essential during larval life but no longer desirable in the adults are altered or may disappear completely:

1. The long tail of the tadpole with its dorsal and ventral fin-folds is resorbed and disappears without a trace.

2. The internal gills are resorbed, gill clefts are closed and the peribranchial cavities disappear. The opercular fold too falls off. Some aortic arches are reduced.

3. The horny teeth, labial rings, and horny lining of jaws are shed with the larval skin.

4. Intestine reduced from 9 times body length to 2 times body lengths.

5. Lateral line sensory system disappears and the mauthner cells of the brain also degenerate.

6. Some blood vessels disappear.

7. Resorption of different organs takes place due to autolysis, i.e., production of the lysosomal enzymes and hydrolases (cathepsin, collagenase, nucleases, etc.). Amoebid macrophages of local mesodermal origin engulf (phagocytose) the debris of the decomposed cells.

B. Constructive or progressive changes: These involve the growth and morphogenesis of the following structures:

1. The limbs increase in size and differentiate further.

2. The middle ear develops in connection with the pharyngeal pouch (the pouch situated between the mandibular and the hyoid arch).

3. The tympanic membrane develops, supported by the circular tympanic cartilage.

4. The eyes protrude on the dorsal surface of the head and develop eyelids and nictitating membranes.

5. The tongue develops from the floor of the mouth.

6. The quadrate cartilage is rotated backward so that there is a significantly increased gap enabling the frog to prey upon large insects.

7. With the changes in the organs of respiration, consequent changes take place in the vascular system. The afferent and efferent vessels develop their direct associations and more and more blood passes into the lungs. The heart becomes three-chambered. Aortic arches take on the pattern of the adult frog.

C. Adaptation of larval structures (Adaptive change): Following organs of the frog function both in the larva and the adult, but change their differentiation during metamorphosis:

1. Change in the hardening, pigmentation, and change for dehydration takes place in the skin. The skin of the tadpole is enclosed with a double-layered epidermis. During metamorphosis, the number of cells in the epidermis increases, and the surface layers develop into cornified. Multicellular mucous and serous glands build up as pockets sinking from the surface into the subcutaneous connective tissue layer, the dermis. The pigmentation of the skin to changes and new patterns of colors appear.

2. In a tadpole the intestine is very long, as in mainly herbivorous animals, becomes significantly foreshortened and most of the coils which it forms in the tadpole become straightened out.

3. In the head, widening of mouth, growth of the adult-type jaw, and repositioning of eyes take place. The snout assumes the adult shape.

4. Trunk becomes narrower than the head.

5. The larval pronephros (kidneys) modify into mesonephric kidneys of an adult.

6. Erythropoietic site changes from the kidney to the spleen.

7. The brain becomes more highly differentiated.

8. At the cellular level, cell change is evident in eyelids, limbs, lungs, tongue, eardrum, skin, liver, pancreas, and intestine. Perhaps no cell or tissue or organ remains completely unchanged.

9. There is no change in the lungs throughout metamorphosis.

7.4 METAMORPHOSIS IN AMPHIBIA

Amphibians provide the best example of metamorphosis in vertebrates. In them, metamorphosis incorporates the following ecological, morphological, physiological, and biochemical changes.

Ecological Metamorphosis Changes: In amphibians, ecologically, metamorphosis is associated with a transition from an aquatic to a terrestrial mode of life. Superimposed on this change of environment, a change in feeding habit occurs in the anuran amphibians. For example, the tadpoles of most frogs and toads feed on the vegetable matter- particles of plants, living and decaying- which they scrap off from submerged objects with the aid of the horny teeth surrounding their mouth. Some anurans are detritus feeders and others as the tadpoles of the clawed toad *Xenopus* are plankton feeders. Adult frogs and toads are carnivorous, living on insects, worms, and small vertebrate animals such as small frogs, birds, rodents, etc., the latter is caught overpowered, and swallowed by them. In the case of urodele amphibians (i.e.,

salamanders, newts, etc.), however, there is no substantial change of diet, the larvae being as carnivorous as the adults though naturally, they feed on smaller animals (mainly crustaceans and worms).

Morphological Metamorphic Changes: The changes in the organization or morphology of the animal during metamorphosis are in part progressive and in part regressive and may be grouped into three categories:

1. The organ or structures necessary during larval life but redundant in the adults are reduced and may disappear completely.

2. Some organs develop and become functional only during and after metamorphosis.

3. A third group of structures while present and functional both before and after metamorphosis become changed to meet the requirements of the adult mode of life.

From a morphological point of view, anuran amphibians undergo extensive metamorphic changes because the degree of difference between their larvae and adults remains much more profound. The metamorphosis of anuran amphibians includes the following changes in the organization.

1. **Regressive metamorphic changes of Anura**: Certain adaptive structures formed during embryonic development, namely, the ventral suckers, external gills, and long tail along with fin folds of the tadpole larvae are resorbed during early functional life. Further, gill clefts are closed, peribranchial cavities disappear and the horny teeth of the perioral disc are shed, as well as the horny lining of the jaws. Further, the shape of the mouth changes, the local tube becomes shortened and reduced, and some blood vessels of precociously formed structures, are not a part of the true metamorphic event which occurs much later. They disappear when they have served their purpose.

2. **Progressive metamorphic changes of Anura:** The progressive or constructive metamorphic changes involve the progressive development of the limbs, which increase in size and differentiation. The fore-limbs, which in frogs develop under the cover of the opercular membrane, breakthrough to the exterior. The gill arches become modified into the hyoid apparatus. The middle ear develops in connection with the first pharyngeal pouch. The tympanic membrane develops and is supported by the circular tympanic cartilage. The eyes protrude on the dorsal surface of the head and develop eyelids. The tongue is developed from the floor of the mouth.
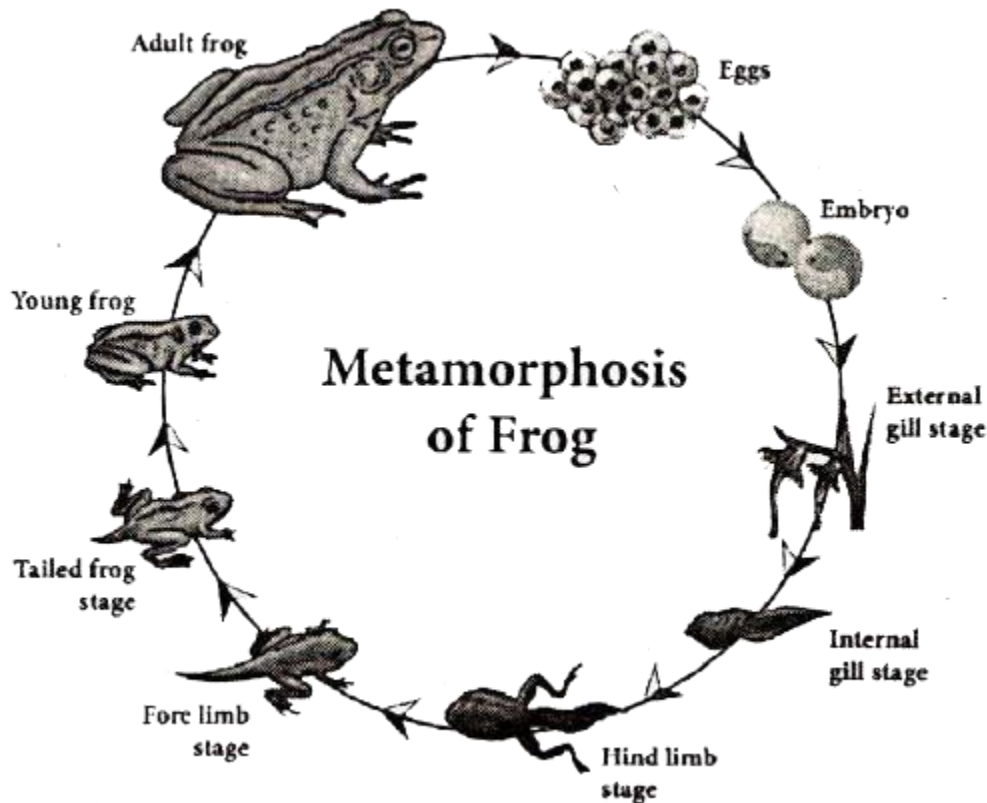
Fig.7.2: Metamorphosis in frog

3. Organs that exist both in larva and adult of Anura: The organs which function both in the larva and the adult, but change their differentiation during metamorphosis are primarily the skin, the intestine, and the brain. The skin thickens and becomes more glandular by possessing multicellular mucous and serous glands, attains an outer keratinized layer, and acquires a characteristic pattern of pigmentation. While the intestine which is very long in tadpole as in most herbivorous animals becomes proportionately shorter and most of the coils that it forms in the tadpole become straightened out. The brain becomes more highly differentiated.

At the cellular level cell modifications are evident in eyelids, limbs, lungs, tongue, eardrum, operculum, skin, liver, pancreas, and intestine. Perhaps no cell or tissue or organ of Anura remains unaffected during metamorphosis remains very rapid and takes only a few days.

Urodele amphibians go through less unusual ecological and morphological metamorphic changes. For instance, in them the tail is retained, only the fin folds disappear. The branchial apparatus is compact the external gills become resorbed and the gill clefts closed. The visceral

skeleton becomes very much reduced. The head changes its shape, becoming more oval. The progressive metamorphic changes occur mostly in the skin and the eyes. The skin becomes cornified and multicellular skin glands become distinguish. The pigmentation of the skin changes. The eyes bulge more on the dorsal surface of the head and develop lids. The legs and intestine suffer no change. In urodeles, therefore the metamorphosis is more gradual and may take up several weeks.

It is worth mentioning that lungs do not undergo drastic changes during metamorphosis in both anurans and urodeles, they develop very gradually and become fully functional in the larval state. Long before metamorphosis, the larvae of frogs and salamanders start coming up to the surface and gulping air into their lungs and thus supplementing their aquatic respiration. This may be of considerable importance when the larvae develop in stagnant and polluted waters, as is often the case.

Time of metamorphosis of Amphibians: Frogs metamorphose after different periods of growth according to the species and it was early known that the achievement of significant species-specific size was much more essential than the duration of growth. Thus in nature, American bullfrog tadpoles metamorphose at the end of the third summer-growth season in the north except at the end of the second summer-growth season in the south, after having attained a certain size, the time required depending on the mean environmental conditions. Further, it has also long been known that the addition of iodine to water or feeding with thyroid gland tissue causes metamorphosis to occur earlier at a smaller size, while the elimination of iodine from the diet postpones it, from the first, therefore the iodine- containing thyroxin of the thyroid gland has been studied intensively about the timing of metamorphosis and the metabolic effect of the hormone.

7.5 PHYSIOLOGICAL AND BIOCHEMICAL CHANGES DURING METAMORPHOSIS

 Morphological changes during metamorphosis go through the following physiological changes:

1. In the frog, the endocrine function of the pancreas starts at metamorphosis and this is associated with the increased role of the liver in the earnings of carbohydrates (glycogen).

2. A thoughtful change takes place in the excretory mechanism. In the tadpole larva, the end creation of nitrogen metabolism is ammonia, which is easily disposed of by diffusion in an aquatic medium except in a terrestrial animal it might accumulate and becomes precarious since of its high toxicity. Metamorphosed frogs, though, excrete most of their nitrogen in the appearance of urea and only a small amount as ammonia. The changeover occurs in the late stages of metamorphosis and is, of course, due to the changed function of the liver which performs the synthesis of urea (Munro, 1939). The biosynthesis of urea from bicarbonate and ammonia involves several enzymes and their activity which is found to be related to the level of thyroxin hormone in the blood rising rapidly during the metamorphic peak.

3. The liver of the froglet starts the secretion of a copper-binding serum protein called ceruloplasmin (Goel, 1984).

4. In the skin begins the synthesis of melanin and serotonin.

5. Peptic activity starts in the stomach of the frog let for the digestion of animal tissue.

6. In the eyes, the modification takes place in the visual pigments- the larval porphyropsin is replaced by adult rhodopsin.

7. In red blood cells, the respiratory pigment hemoglobin also changes. Thus, larval hemoglobin that has a higher affinity for oxygen and sensitivity to acid (i.e., independent from the pH) is replaced by adult hemoglobin which has a lower affinity for oxygen and shows the Bohr effect with pH changes (i.e., highly sensitive to acid).

According to Freiden (1961), the biochemical metamorphic alterations may have direct or indirect adaptive value relating to the transition from freshwater to land. Among the most important adaptive changes are the shift from ammonotelism to ureotelism, the increase in serum albumin and other serum proteins, and the alteration in the properties and biosynthesis of hemoglobins.

The development of certain digestive enzymes and augmentation of respiration also contribute to the achievement of the differentiation process. Throughout metamorphosis, many more important chemical developments may be secondary to primary morphological or cytological alteration which aid in the adjustment to the land. These comprise changes in carbohydrates, lipid, nucleic acid, and nitrogen metabolism. Major change occurs in water balance, visual pigments (vitamin A), pigmentation, and tail metabolism.

7.6 HORMONAL CONTROL OF METAMORPHOSIS

The simultaneous changes in so many parts of the animal's body through metamorphosis propose the survival of some common reason for all the transformations. It has been found that the common cause is a hormone released in large quantities from the thyroid gland of the animal which incoming the stage of metamorphosis. The first identification of this was obtained when Gundernatsch (1912) fed some frog tadpoles on the dried and powdered sheep thyroid gland and observed that they metamorphosis precociously. The confirmation that the thyroid hormone is the source of metamorphosis in normal development was further given by the following two experiments: In an experiment when the rudiment of the thyroid gland was removed in frog embryos in the tail bud stage, then, it was found that the operated tadpoles were possible and showed normal growth but failed to metamorphosis (Allen.1918). The thyroid-less tadpoles continued to grow and attained a much larger size than normal. It was thus proved that metamorphosis cannot set in without a stimulus coming from the thyroid gland. In the final experiment, thyroid-less tadpoles were provided with thyroid hormone either by feeling them on the thyroid gland or by immersing them in water containing soluble extracts from thyroid glands. The tadpoles treated in this way immediately proceeded to metamorphosis, thus showing that their thyroid glands are not essential so long they are supplied with thyroid hormone (Allen, 1938). Related experiments were carried out on urodele amphibians by Marx (1935).

The process of metamorphosis is essentially under hormonal control. Hormones from the hypothalamus (e.g., neurosecretions like TRF or thyrotropin-releasing factor and PIH or prolactin-release-inhibiting factor or hormone), the hypophysis (e.g., TSH or thyroid-stimulating hormone and the PH or prolactin hormone) and the thyroid (e.g., thyroid hormones such as thyroxin) regulate the process of metamorphosis. Thyroxin hormone affects the tissues directly, causing the degeneration and necrosis of some cells and stimulating the growth and differentiation of others. Iodine is not only essential for the working of thyroxin hormone; it has been found that iodine alone can cause a metamorphosis in the frog. If the tadpoles are kept in water containing the elements iodine or if a weak solution of iodine is injected or an iodine crystal is implanted in a tadpole, it undergoes a metamorphosis.

Certain ecological factors also affected the process of metamorphosis in the frog. These factors include starvation, temperature, crowding, illumination, chemicals, and dietary supplements.

Throughout metamorphosis, synchronized changes in all body parts propose the survival of hormones released in large quantities from the thyroid gland of the animal. This indication was predictable by Gundernatsch (1912) whereas who fed some frog tadpoles on the dried and powdered sheep thyroid gland and observed their metamorphosis precociously. Thyroid hormone is the cause of metamorphosis in usual development was further proved experimentally.

The amphibian metamorphosis is below neuroendocrine control, connecting neurosecretory cells in the brain (the hypothalamus) and two endocrine glands, the pituitary (anterior pituitary) and the thyroid. The start of metamorphosis could be an environmental signal touching the larval brain through the nervous system, or there may be an endogenous 'clock' in the hypothalamus. In a way, the hypothalamus integrates the information received from the body through environmental information.

Neurosecretory cells in the hypothalamus are stimulated to create TRF or thyroid-releasing factor which stimulates the anterior pituitary gland to produce a TSH or thyroid-stimulating hormone which causes an orderly increase of thyroid secretion. An increase in thyroid hormone then trips the arranged sequence of tissue changes that transform the tadpole larva into the frog.

Another pituitary hormone, called prolactin is also found to be involved as an inhibitor in the overall control of metamorphosis. Developmental control is affected by a balance between inhibition and disinhibition rather than stimulation at the level of endocrine action. Thyroid hormones are also known to affect the process of protein synthesis at the levels of transcription and translation and to have a role in cytodifferentiation.

7.7 SUMMARY

Metamorphosis is a post-embryonic extension of the developmental potential and involves dramatic changes in habit, habitat, morphology, physiology, and behavior of the larva so that it is transformed into an adult having an entirely different habitat and structure. Metamorphosis is a widespread developmental phenomenon that is usually connected with a theatrical change in habitat and succeeding way of life, such as the change from a planktonic to a benthic existence way of the sea-urchin, from an aquatic to a terrestrial survival in frogs and toads, and from non-flying to a flying continued existence in insects. Such changes in atmosphere and behavior demand an equally rapid transformation of the structure and function of the living technology.

Metamorphic change during the development cycle is an acceleration or absorption of essentially the same basic process feature of most forms of development. Mainly, it consists of differential destruction of definite tissues, accompanied by an increase in growth and demarcation of other tissues.

Metamorphosis occurs in the majority of metazoan phyla, starting with Porifera (amphiblastula) and ending with Amphibia (tadpole). Animals with no direct development exhibit a range of larvae that undergo metamorphosis. The procedure of metamorphosis in different animal groups differs equally like alteration and in the mode of causation of the entire sequence, so, it is not possible to explain their metamorphosis in general terms.

In species with small eggs, a larval stage is interposed as feeding adaptation to support the continued development of adult structures. As soon as adult tissues mature, the larva undergoes dramatic metamorphic changes as larval tissues are shed and transformed into an adult.

Two basic types of metamorphosis:

1. Retrogressive metamorphosis: it occurs in ascidians wherever only the larvae (tadpoles) have chordate features, the adults, adapted to a sessile existence, lose all larval locomotory organs and thus give up all traces of chordate relationships.

2. Progressive metamorphosis: It occurs in amphibians and includes a change of simple larval organization into the more complex organization of the adult.

The process of metamorphosis is essentially under hormonal control. Hormones from the hypothalamus (e.g., neurosecretions like TRF or thyrotropin-releasing factor and PIH or prolactin-release-inhibiting factor or hormone), the hypophysis (e.g., TSH or thyroid-stimulating hormone and the PH or prolactin hormone) and the thyroid (e.g., thyroid hormones such as thyroxine) regulate the process of metamorphosis. Thyroxine hormone affects the tissues directly, causing the degeneration and necrosis of some cells and stimulating the growth and differentiation of others. Iodine is not only essential for the working of thyroxine hormone; it has been found that iodine alone can cause a metamorphosis in frogs. If the tadpoles are kept in water containing the elements iodine or if a weak solution of iodine is injected or an iodine crystal is implanted in a tadpole, it undergoes a metamorphosis.

7.8 TERMINAL QUESTIONS AND ANSWERS

7.8.1 Multiple Choice Questions:

1. In amphibians, metamorphosis is done by:

a) Pituitary gland

b) Thyroid gland

c) Adrenal gland

d) Pineal gland

2. Another word for metamorphosis is:

a) Survival

b) Evolution

c) Modelling

d) Change

3. Retrogressive metamorphosis occurs in:

a) Urochordata

b) Cephalochordates

c) Vertebrates

d) Cyclostomes

4. Retrogressive metamorphosis is found in:

a) *Amphioxus*

b) Ascidia

c) *Rana tigrina*

d) Protochordata

5. The ascidian tadpole larva undergoes:

a) Progressive metamorphosis

b) Retrogressive metamorphosis

c) Partial metamorphosis

d) Complete metamorphosis

6. What is metamorphosis?

a) Process of transforming a larva into an adult

b) Process of transforming a pupa into an adult

c) Process of modification

d) Process of regeneration

7. Another term for a fertilized ovum is:

a) Placenta

b) Sperm

c) Fallopian

d) Zygote

Answers: 1 b, 2 d, 3 a, 4 b, 5 b, 6 a), 7 d)

7.8.2. Short Answer Question:

1. Definition of metamorphosis.

2. What are two basic types of metamorphosis?

3. Explain retrogressive metamorphosis.

4. Write about the ecological metamorphosis changes in amphibians.

7.8.3. Long Answer Question:

1. What is metamorphosis? Describe the hormonal control of metamorphosis in amphibians.

2. Discuss the role of various hormones that control metamorphosis in a frog.

3. Explain the significance of metamorphosis.

4. Discuss the morphological, anatomical, and biochemical changes involved in the metamorphosis of the tadpole of the frog.

5. Discuss the role of various hormones that control metamorphosis in the frog.

7.9 GLOSSARY

Amnion: (Gk. *amnion*=foetal membrane) An extraembryonic inner, fluid-filled sac composed of a thin double membrane that surrounds the embryo in reptiles, birds, and mammals. It provides a kind of private aquarium to the embryo and protects it from mechanical shocks such as pressure, abrasion, irritation, and desiccation.

Amniote egg: an egg that is isolated from the environment by a more or less impervious shell during the period of its development and which is completely self-sufficient, requiring only oxygen from the outside.

Embryo: The early developmental stage of an organism produced from a fertilized egg. In the mammal, the later embryonic stage is called a fetus.

Fetus: An unborn or unhatched vertebrate that has passed through the earliest development stages.

Macrolecithal: referring to a large amount of yolk stored in the egg, e.g., eggs of insects, reptiles, birds, and monotreme mammals.

Mesolecithal refers to a moderate amount of yolk stored in the egg.

Metamorphosis: Abrupt transition from larval to adult; it includes certain morphological, anatomical, physiological, and behavioral, hormonally regulated changes in the larval form to transform it into the adult form.

Microlecithal: referring to a small amount of yolk stored in the egg, e.g., Amphioxus, eutherian mammals.

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UNIT 8: TERATOGENESIS

CONTENTS

8.1 OBJECTIVES

After going through this unit you shall be able to:

- Understand the concept of teratogenesis
- Types of teratogenesis.
- What are phenocopies?
- Developmental mechanisms of teratogenesis.

8.2 INTRODUCTION

Prenatal toxicity characterized by structural or functional defects in the developing embryo or fetus is known as Teratogenesis. It too includes intrauterine growth retardation, death of the embryo or fetus, and transplacental carcinogenesis (in which chemical exposure of the mother initiates cancer development in the embryo or fetus, resulting in cancer in the offspring after birth).

Intrauterine human development has three stages: implantation, post-implantation, and fetal development. The first two stages of implantation and post-implantation are the embryonic stages and last through the first eight weeks after conception. The fetal development stage begins in the ninth week and continues to birth.

The developmental stage depending on chemical contact with the mother can result in different degrees of toxicity in the embryo or fetus. In the preimplantation phase, a toxic chemical can kill some of the cells in the blastocyst, resulting in the death of the embryo. Throughout the post-implantation period, chemical-induced cell death leads to one of two outcomes. If death is controlled by those cells undergoing active cell division at the moment, the analogous organs are affected, resulting in malformation. If the cell death is widespread with no significant replication by the remaining cells to sustain life, the embryo dies. During the third, fetal, period, chemical damage can retard growth or, if severe sufficient, kill the fetus.

A congenital malformation is a gross structural present at birth. Its incidence is about 2.5% in all infants born. But, only half of these deformities are noticeable at the time of delivery, most of the remainder coming to light during the first postnatal year. The term congenital anomaly is

reserved for a minor congenital disorder such as a deformed finger or ear lobe. Anomalies are found in a further 2.5 percent of live-born infants.

Individuals of a species (including humans and other animals) exhibiting minor deviation variation from each other are considered normal. Individuals that show gross deviation from each other are considered normal due to congenital malformations and are known as monsters or terata. The abnormal development or formation of terata is called teratogenesis and the science which is concerned with the investigation of terata and teratogenesis is called teratology.

Teratogenesis is the formation of an abnormal organism. A teratogen is any manager that physically or chemically alters developmental processes and produces inherited deformities. The nature of the teratogen and the developmental stage during which the variation occurs is critical to the type and severity of abnormality it will produce. Biological factors such as the organism's gestation process, developmental pathways, and life-cycle characteristics also control the exposure and effect of a teratogen. Mechanical disruptors, environmental factors, and chemical contaminants are the primary categories of teratogens disturbing wildlife species. The introduction of an embryo, fetus, or larva to a teratogen may result in death, structural malformation, functional disorder, or growth retardation. The most commonly described teratogenic effects taking place in ecosystems are external malformations. Wild organisms have always been subject to teratogenic insult; however, current anthropogenic activities have increased the prevalence of deformities. Herein, the current state of teratogenesis knowledge concerning amphibians, reptiles, birds, fishes, mammals, and invertebrates is discussed with emphasis on structural malformations resulting from exposure to chemical contaminants.

8.3 TYPES OF TERATOGENESIS

Teratogenesis is Genetic teratogenesis and environmental teratogenesis. Genetic teratogenesis has been studied both in human beings and animals.

Genetic teratogenesis in human beings: Abnormal genes may be inherited from one or both parents and they may be dominant or recessive. In the majority of the afflicted individuals, however, a Mendelian pattern of inheritance cannot be observed, the abnormalities merely occur more frequently among relatives than in the general population. In those occurring among relatives, there may be a complex interplay between several genes.

Deformities due to abnormal dominant genes are rare. In most of these, the skeleton is affected and the deformities include achondroplasia (insufficient growth of long bones), and arachnodactyly (abnormally long hand and foot bones).

A teratogen is a substance that may lead to birth defects in an embryo or fetus. During pregnancy, contact with certain chemicals, infections, and drugs may increase the risk that a person will miscarry or that the embryo or fetus could have a developmental abnormality.

Alcohol and smoking are two common teratogens. Contact with either of them can lead to developmental anomalies, miscarriage, stillbirth, preterm labor, and a variety of other pregnancy complications.

The contact of teratogens with pregnancy or a fetus depends on numerous factors. The timing and length of exposure, the stage of pregnancy when the exposure happened, whether a parent's genes make them more at risk, and the type of agent they were exposed to all contribute to the risk. Teratogens commonly fall under the following categories:

1. Drugs
2. Infection
3. Physical Agents
4. Environmental Toxins
5. Maternal Health Conditions

8.3.1 Mechanisms of genetic and environmental teratogenesis

8.3.1.1 Genetic teratogenesis in animals: Genetic teratogenesis can be considered under the following two headings:

1. Gene-phene relationship. Several different genes can cause the same terata, though not necessarily by the same route. For example, there are more than twenty genes that affected eye color in *Drosophila melanogaster*. The mutants causing the same defect may be either recessive or dominant. For example, in fowl, the trait of rumplessness (absence of tail) is controlled by either a recessive or dominant gene. In some cases, the same mutation may behave as recessive or dominant depending on the genetic background. Thus, in mice, the fused gene (for fusion or absence of ribs and or absence of tail) is dominant in *Mus musculus* but recessive in *Mus musculus bactrianus.*

The penetrance (i.e., the proportion of affected individuals in a population) and expressivity (i.e., degree of effect) of mutant genes is dependent on both genetic and environmental factors. For

example, fowl carrying rumples gene can be selectively bred to produce a 'normal' tail phenotype. Similarly, in *Drosophila* carrying the Bar eye gene, the size of the eye and the number of facets in the eye decreases by about 100 facets with an increase in temperature (15° to 31°C) during the development.

2. Autophene, allophone, and pleiotropy. Not all genetic terata are the result of the intrinsic action of genes in the affected tissue. The following two examples will make the point clear:

(1) Creeper mutation (cp/cp) in fowl affects the limbs (called phocomelia or abnormally short limbs) and the eyes (called microphthalmia or small eye), the embryo does not survive till hatching. Transplants of the cp/cp limb rudiments in the normal hosts produce phocomelia limbs. However, transplants of cp/cp eye rudiments in the normal hosts produce normal eyes. Therefore, the cp gene intrinsically affects limb development (it is called autophene) but only indirectly affects eye development (it is called allophone).

(2) The multiple effects of one gene (pleiotropy) are also now better understood. Any given gene mutation essentially affected the production or structure of one transcribed RNA molecule. The translation product of this RNA (i.e., mRNA), the defective protein, may ultimately result in various defects due to the correlation of various biochemical reactions in the body (i.e., by a pedigree of causes), e.g., death in rats due to grey lethal mutant gene and sickle cell anemia in human beings.

8.3.1.2 Environmental teratogenesis: Almost any environmental factor can be teratogenic. The environmental factor may be either biological (e. g., viruses) or non-biological such as physical factors (e.g., temperature, irradiation, mechanical disturbance) and chemical factors (e.g., drugs, environmental chemicals, and dietary imbalances or malnutrition).

1. Teratogenesis due to infection: The most dangerous known teratogenic organism is the virus of German measles (rubella). Contraction of the disease by the mother during the first month of pregnancy appears to carry about a 50 percent risk of producing a congenital abnormality, during the second month the risk is about 25 percent, and during the third month about 7 percent. The virus tends to affect the developing eyes, ears, and palate. The triads of congenital cataracts (blindness), heart disease, and deafness have become known as the rubella syndrome. Rubella also knows to cause terata such as mental deficiency due to a very small brain, cleft palate, and hare-lip.

Cytomegalovirus (CMV) infection is found all over the world. Like the rubella virus, it passes through the placenta to infect the fetus. The CMV is show signs of disease. It may cause deafness, mental retardation, epilepsy, liver disease or cerebral palsy (paralysis due to damage to the brain), or a variable combination of these. Other teratogenic organisms are toxoplasmosis and syphilis. Syphilis is known to cause stillbirth or other congenital abnormalities.

2. Teratogenesis due to Drugs: The potential danger of new drugs to the fetuses was exemplified by two unrelated drugs, thalidomide and meclizine. Thalidomide is a mild sedative tranquilizer that was prescribed for use in pregnancy in many European countries in the late 1950s. The German scientist Lenz (1961) reported a possible connection between this drug and an increased frequency of a human congenital abnormality of the limbs known as Amelia (no limbs) and the closely related **phocomelia** (no development of long bones of limbs and flipper-like hands or feet attached directly to the trunk). A daily intake of thalidomide for one week during early pregnancy was sufficient to induce limb defect. From 1959-1961 thousands of babies in West Germany and hundreds in other countries such as Japan, were born with partial or complete absence of limbs or limbs with defects. This has led to extreme caution in the introduction of new drugs for commercial distribution, for pregnant mothers, since they may cause irreparable harm to human embryos.

 Antimitotic drugs used in cancer therapy would be especially harmful to the rapidly growing embryo. However, only **aminopterin** (a folic acid antagonist) has proved to be teratogenic in man. This chemical has been used to bring about abortion. When it fails to induce abortion, the offspring is likely to show multiple malformations. Teratogenic effects of certain other drugs such as quinine (for malaria), **busulphan** (for leukemia), and **chlorambucil** (for Hodgkin's disease) have also been reported.

3. Teratogenesis due to radiation: It is recognized that the use of roentgen rays or radium in the treatment of pregnant patients having pelvic tumors, is possible to produce skeletal abnormalities in the fetuses. The contact of pregnant women with severe atomic radiation, as in the Hiroshima and Nagasaki explosions, led to a fetal death rate of about 40 percent. The infants who survived tended to show confirmation of brain-cell damage.

4. Teratogenesis due to autoimmunization: Significant interest was aroused by the examination that mothers of infants born without thyroid glands, i.e., thyroid cretins, may have antithyroid antibodies in their blood. This observation suggests that products of embryonic organ

primitive may cross the placenta, inducing s maternal antibody production the antibodies may return to the embryo and interfere with organ differentiation.

5. Teratogenesis due to malnutrition: It is a well-known fact that deficiency of various vitamins causes several diseases in man the deficiency of vitamin A causes night blindness, the deficiency of vitamin D leads to abnormal development of bone and teeth and the deficiency of vitamin K causes abnormalities in the clotting of blood. It has been studied that if rats, rabbits, and other animals are given a diet deficient in vitamin A, B, and D, they may produce various teratogenic effects such as hare- lip, cleft palate, spina bifida, skeletal defects, brain defects, etc. Likely, such vitamin malnutrition in pregnant mothers of the human species may also cause similar congenital malformation.

Sensitive period of the teratogen: The developmental stage at which the embryo is treated by a teratogen has great relevance to teratogenesis. In general, the very early stages of development are not much affected by the teratogen, possibly due to the considerable regulatory capacity of the embryo. Similarly, the later stages of development, when maturation and growth of organs occur, are also comparatively resistant to teratogens. The main teratogenic period starts with the creation of germ layers and continues up to organogenesis. For example, in rats **trypan blue** (20 mg intravenous injection to the mother) and **actinomycin D** (0.3 mg/kg body weight to the mother) are maximally teratogenic on the 8th and 9th day of gestation, respectively. Both chemicals are ineffective after 10th day and only minimally teratogenic before the 6th day of gestation.

Specificity of the teratogen: Almost any teratogen can produce almost any terata if applied at the right time in the experimental animal. However, each teratogen produces its typical syndrome. For example, the micromelia (short limb) in fowl can be caused by a deficient or imbalanced diet, (i, e., riboflavin (or biotin deficiency), insulin, thallium, boric acid, pilocarpine, propanediol-1, 3 sulphanilamides or serine sulfate. A similar situation occurs in the case of tetraptera (four wings) in *Drosophila melanogaster, i.e*., phenocopies of tetraptera can be obtained by treating the early embryo with either ether or 40°C heat shock.

However, the types of micromelia produced in fowl by different teratogens are only superficially similar.

The type of malformation produced by a teratogen is also dependent on the developmental stage of an embryo at the time of treatment. For example, insulin (2 units in yolk sac), causes

rumplessness in the young fowl embryo (24 hours) but micromelia and abnormal beak in the older embryos (70 to 170 hours) of fowl. Thus, each organ system appears to have its sensitive period for a given teratogen.

Teratogenic dose: A teratogen may be quite ineffective at a very low dose, while at its high doses it may be lethal to all embryos. A teratogenic dose range comprises doses that produce at least some malformed and living embryos. It is also necessary to discriminate between the teratogenic and the toxic effects of an agent. By definition, an agent is toxic, if it causes embryonic death by direct action and not through teratogenic interaction. **For example**, trypan blue (50 mg/kg) injected into 8.5-day pregnant rats affects 65 percent of the embryos. But with advancing gestation, out of these 65 percent of the embryos the number of dead one increases at the expense of live and malformed individuals. Moreover, when trypan blue is injected outside the teratogenic period, it does not result in appreciable deaths. Trypan blue therefore is a teratogenic and not toxic chemical.

Interaction of Teratogens with other Environmental Factors: The effect of any teratogen depends on other factors present in the environment of an organism. Many types of communication between environmental factors do occur. **For example**, the teratogenic effects of insulin on a 96 120- hour-old fowl embryo are almost completely by the simultaneous injection of nicotinamide.

The synergistic effects of agents are also well known. The incidence of both the micromelia and abnormal beak in the insulin-injected fowl embryos is significantly enhanced by chlorpromazine, a non- teratogenic but slightly toxic chemical. Likewise in another type of interaction the non-teratogenic dos of two teratogens, sulphanilamide and 6-aminonicotinamide, can cause teratogenesis when injected altogether.

8.3.2 Phenocopies

A **phenocopy** is a variation in phenotype (generally referring to a single trait) that is caused by environmental conditions (often, but not essentially, during the organism's development), such that the organism's phenotype matches a phenotype that is determined by genetic factors. It is not a mutation type, while it is non-hereditary.

The term was coined by Richard Goldschmidt in 1935. He used it to refer to forms, produced by some experimental procedure, whose form duplicates or copies the phenotype of some mutant or combination of mutants.

Examples:The larvae of *Drosophila melanogaster* have been originating to be particularly exposed to environmental factors which generate phenocopies of known mutations; these factors include temperature, shock, radiation, and various chemical compounds. In the fruit fly, *Drosophila melanogaster*, the normal body color is brownish gray with black margins. A hereditary mutant for this was discovered by T.H. Morgan in 1910 where the body color is yellow. This was a genotypic character that was constant in both the flies in all environments. However, in 1939, Rapoport discovered that if larvae of normal flies were fed with silver salts, they develop into yellow-bodied flies irrespective of their genotype. The yellow-bodied flies which are genetically brown are a variant of the original yellow-bodied fly.

An environmental teratogen can cause a normal genotype to produce a phenotype similar to an already known mutant that is a phenocopy of the mutant. Landauer has proposed that a true phenocopy should satisfy two criteria:

 (1)The development stage affected by the mutant gene and the environmental teratogen must be the same that is both should cause terata by altering the same development pathway.

(2) Modifications in the genetic background should have similar effects on the incidence and expressivity of both the mutant gene and the environmental teratogen. On these criteria, the insulin-induced rumplessness appeared to be a true phenocopy of the recessive rumples mutant. In both cases, the abnormal tail structures are formed and later degenerate.

8.3.3 Developmental mechanisms of teratogenesis

Each phase of development is prone to a defect. The chronological nature of development is based on networks of interacting systems, what happens at one stage is essential to all consequent stages. Defects initiated at an early stage may be expressed at later stages because intermediate steps are abnormal. The magnitude of a defect will depend on the stage of development at which it originates and the development process that is a specific target. Lesions arising at early stages usually have more cells are affect (Grant, 1978).

Since in terata any organ and any tissue may be affected so the mode of production of terata is much varied. The development processes that may be affected by teratogenesis include competence, induction (evocation), determination, histogenesis and morphogenesis, cell growth,

cell division, cell death, and cell locomotion. The genetic and molecular studies on development indicate that each teratogen affects the developmental processes by essentially affecting cell metabolism and gene expression. This is brought about by several independent or inter-related mechanisms such as:

1) Production of defective, deficient, or no proteins

2) Production of proteins at an inappropriate developmental juncture

3) Production of defective, deficient, or no rRNA or tRNA of a particular kind or

4) Alteration in membrane permeability.

For example, in the case of sickle cell anemia, the production of a defective protein (β chain of hemoglobin) leads to sickle cell syndrome.

8.4 SUMMARY

A congenital malformation is a structural or anatomical abnormality present at birth. Congenital malformations may be caused by genetic factors or environmental insults or a combination of the two that arise during prenatal development. Most common congenital malformations display multifactorial inheritance with a threshold effect and are determined by an arrangement of genetic and environmental factors. During the two weeks of gestation, teratogenic agents generally kill the embryo rather than cause congenital malformation. Major malformations are more common in early embryos than in newborns; however, the most severely affected embryo is spontaneously aborted through the first six to eight weeks of gestation. During organogenesis between days 15 to 60, teratogenic agents are more probable to cause major congenital malformations.

Teratogenesis is the formation of an abnormal organism. Teratogens are any manager that physically or chemically alters developmental processes and produces inherited deformities. The nature of the teratogen and the developmental stage during which the variation occurs is critical to the type and severity of abnormality it will produce. Biological factors such as the organism's gestation process, developmental pathways, and life-cycle characteristics also control the exposure and effect of a teratogen. The introduction of an embryo, fetus, or larva to a teratogen may result in death, structural malformation, functional disorder, or growth retardation. The most

commonly described teratogenic effects taking place in ecosystems are external malformations. Wild organisms have always been subject to teratogenic insult; however, current anthropogenic activities have increased the prevalence of deformities. Herein, the current state of teratogenesis knowledge concerning amphibians, reptiles, birds, fishes, mammals, and invertebrates is discussed with emphasis on structural malformations resulting from exposure to chemical contaminants.

8.5 TERMINAL QUESTIONS AND ANSWERS

8.5.1. Multiple Choice Questions:

1. What is a teratogen?

a) A noxious substance found in soil that crosses the maternal placental barrier

b) A hormonal trigger that stimulates excessive cell growth in the embryo

c) An allergen that affects both mother and fetus

d) A substance or environmental influence that affects the development of the fetus and results in physical abnormalities

2. A teratogenic action is:

a) Toxic action on the

b) Negative action on the fetus causing fetal malformation

c) Toxic action on the blood system

d) Toxic action on kidneys

3._____are substances that can harm the fetus.

a) Testosterone

b) Telomeres

c) Teratogens

d) Testifiers

4. Which of the following is NOT a teratogen?

a) Testosterone

b) Nicotine

c) Radiation

d) Alcohol

5. Exposure to some maternal hormones before birth may result in the child having:

a) Borderline personality disorders in later life

b) Lower levels of emotional response

c) An insecure attachment

d) None of these

Answers: 1d, 2 b, 3 c, 4 a, 5 a

8.5.2. Short Answer Question:

1. What is environmental teratogenesis?

2. What are Phenocopies?

3. Explain genetic teratogenesis in animals.

8.5.3. Long Answer Question:

1. What is teratogenesis?

2. Describe the genetic bases of teratogenesis.

3. Describe the Developmental mechanisms of teratogenesis.

8.6 REFERENCES

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8.7 GLOSSARY

Malformation: Abnormality occurring during the formation of the structure (during the gestation period), resulting in a complete or partial absence or alteration of normal structure conformation. Results as a morphological defect of an organ, part of an organ, or a larger region, in severe situations with defects in all the body.

Teratogen: A product that could promote a birth defect. It could be biological, environmental, toxic, chemical, or physics.

Teratology: Study of defects that could be congenital malformations or anomalies, present at birth and can be structural, behavioral, functional, or metabolic disorders.

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