

## UNIT – II 11

1. **Gametes of Frog:** a) Structure of sperm; b) Structure of ovum;
2. **Frog Embryology:** a) Fertilization; b) Cleavage; c) Blastulation; d) Gastrulation; e) Formation of three germinal layers;
3. **Regeneration in chordates.**

## Development of Frog

Frog is an anuran amphibian. In frog sexes are separate. There is well developed sexual dimorphism. The males are small containing vocal sacs and nuptial pads. The male has a pair of testes and the female has a pair of ovaries. During breeding season the male copulates with the female and the copulation is called amplexus. The frogs are oviparous. The female lays the eggs in water. Fertilization is external. Development is indirect as there is a larva called tadpole.

### a) Structure of sperm

1. The sperm is the male gamete.
2. It is produced by the testis.
3. It is haploid and flagellate type.
4. The sperm of frog is microscopic and is about 0.03 mm long.
5. Head is divided into three regions, such as head, a middle piece and a tail.
6. The head is cylindrical and long. It is formed of acrosome and nucleus.
7. The middle piece is short. It contains two centrioles and the mitochondria.
8. The tail is long. It contains an axial filament. The tail consists of two regions, namely main piece and end piece.

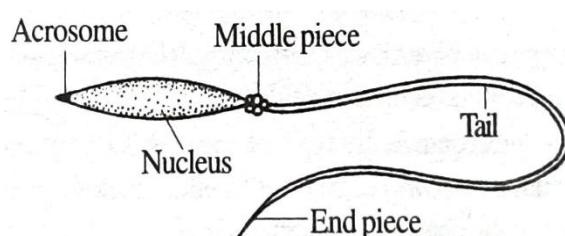


Fig. Sperm of frog.

## b) Structure of ovum (Egg)

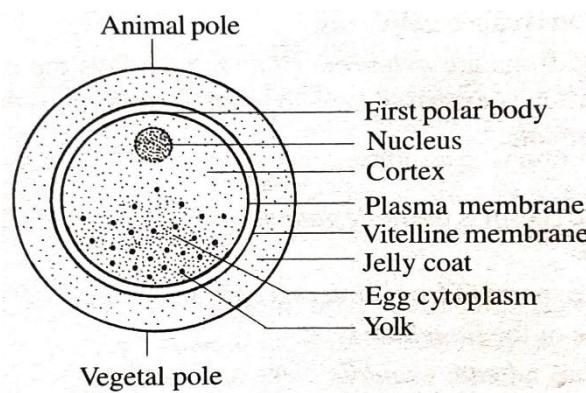


Fig. Egg of frog.

1. The egg is the female gamete. It is also called ovum. It is haploid.
2. It is produced by the ovary.
3. It is a non-cleidoic egg as the shell is absent.
4. It is a megalecithal egg as it contains large amount of yolk.
5. It is a telolecithal as the yolk is present on one side.
6. The egg of frog is spherical in shape. It is about 2 mm in diameter.
7. It is surrounded by three egg membranes, namely an inner plasma membrane, a middle vitelline membrane and an outer jellycoat.
8. The egg cytoplasm is called ooplasm. It has two regions, namely peripheral cortex and central endoplasm.
9. The cortex contains two types of granules. They are dark brown pigment granules and cortical granules. Cortical granules are arranged in layer close to the plasma membrane.
10. The endoplasm contains nucleus on one side and numerous white plate-like yolk platelets on the other side.
11. The egg has two distinct colours. One side is black in colour. This side is called animal pole. The nucleus is situated at this side.
12. The opposite side is yellow in colour. This side is called vegetal pole. It contains yolk.

## Fertilization

Male gamete unite with female gamete to form a diploid zygote is called as fertilization that means, Fertilization is the fusion of sperm with egg resulting in the formation of zygote.

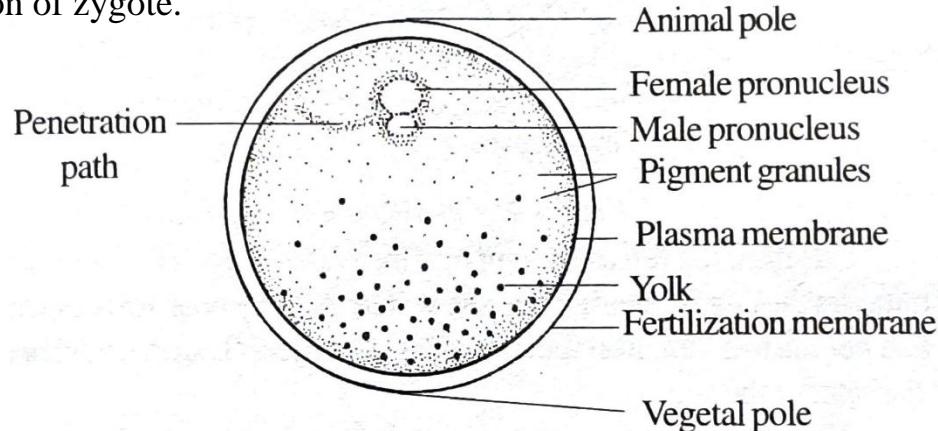
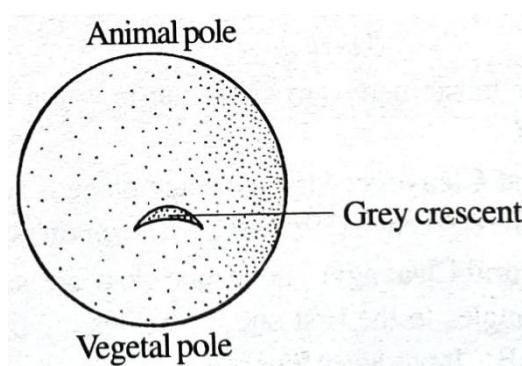


Fig. Fertilized egg of frog (Jelly coat removed).

1. Fertilization is external.
2. It is monospermy, i.e., only one sperm fuses with the egg.
3. The fertilized egg rotates in such a way that the animal hemisphere goes above.
4. The jelly coat swells and increases in thickness.
5. The second meiotic division is completed resulting in the release of the second polar body.
6. The sperm enters the egg in the animal hemisphere at an angle of  $40^\circ$  from the centre of animal pole.
7. Immediately after the entry of the spermatozoon into the egg, the vitelline membrane becomes elevated. This membrane is now called fertilization membrane. The space between this membrane and the surface of the egg is called perivitelline space filled with a fluid called perivitelline fluid. In this fluid, the fertilized egg can rotate freely. The rotation of the egg is inevitable for the normal process of development. Immediately after fertilization, the black pigmented animal pole gets placed above and the yolk-laden vegetal pole below.

8. Before the release of egg into the water, the jelly coat remains thin. As the egg is released into the water, the jelly coat absorbs water and begins to swell until the thickness of the jelly becomes twice the diameter of the egg.
9. The second maturation division is completed immediately after fertilization. As a result, the fertilized egg releases the second polar body.
10. The egg pronucleus and sperm pronucleus fuse together to form the zygotic nucleus. This process is called amphimixis.
11. The entry of sperm causes the movement of black pigments. On one side, just below the equator, a crescent like area appears; it will be grey in colour. This area is called grey crescent. It appears opposite to the point of sperm entry. The region of the grey crescent will become the posterior side and the opposite region will become the anterior side of the future embryo. This leads to the formation of a definite bilateral symmetry in the fertilized egg. The unfertilized egg is radially symmetrical.
12. The sperm penetrates the egg perpendicular to the cortex. After penetration, the sperm moves in the cortex perpendicularly, along the radius of the egg. This path of the sperm is marked by pigment granules. This path of the sperm in the egg cortex is called penetration path. After crossing the cortex, the sperm changes its direction and moves towards the egg-nucleus. This changed path is also marked by pigment granules and is called copulation path, because it leads to the copulation (fusion) of the sperm and the egg-nucleus.

### **Grey Crescent (Gray Crescent)**



*Fig. Fertilized egg showing grey crescent.*

1. Grey crescent is a crescent-like and grey coloured area developing on the surface of amphibian egg opposite to the point of sperm entry
2. It is a surface feature developing as a result of cytoplasmic movements stimulated by sperm entry in the egg.
3. It appears just above the margin where the yellow-white vegetal pole material merges with the darkly pigmented animal pole material.
4. It appears on the surface of the egg opposite to the point of sperm entry.
5. Grey crescent marks the future dorsal side of the embryo.
6. The first cleavage bisects the grey crescent into two equal halves and this plane represents the future median plane of the embryo.
7. The formation of grey crescent, thus fixes up the final symmetry of the egg and the future embryo.
8. In the gastrula, the grey crescent materials are located on the dorsal lip of the blastopore.
9. The grey crescent materials function as the organizer because, when it is removed from the embryo, the embryo fails to develop further. At the same time when a normal embryo is grafted with another grey crescent, two embryos develop.
10. In the late gastrula, grey crescent materials are incorporated into the chordomesoderm.

## Cleavage

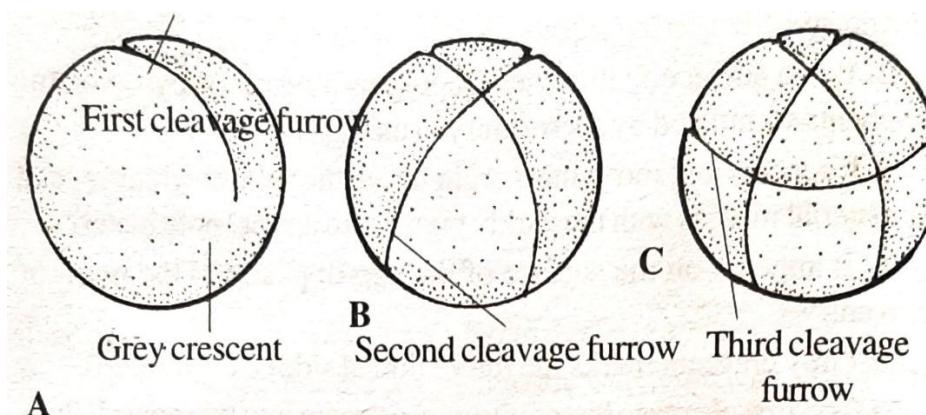


Fig. 13.6: Cleavage in frog. A. 2 cell stage, B. 4 cell stage, C. 8 cell stage.

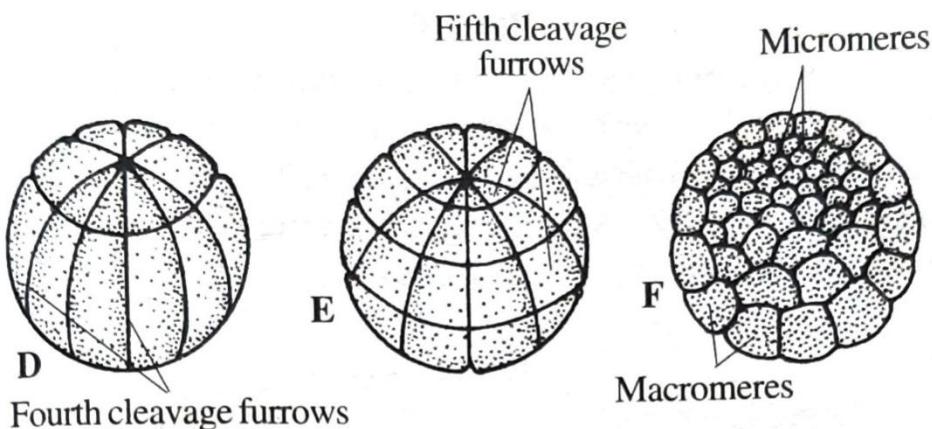


Fig. Cleavage in frog. D. 16 cell stage, E. 32 cell stage, F. Blastula.

Cleavage is the division of zygote into many cellular units called blastomeres. In frog, the entire egg divides and hence the cleavage is called total or holoblastic cleavage. The blastomeres are dissimilar in size and so the cleavage is unequal.

**First Cleavage:** First cleavage plane is meridional. It passes through the egg centre. It results in two equal blastomeres.

**Second Cleavage:** The second cleavage is also meridional, but at right angles to the first one. It divides the first two blastomeres. It produces four cells.

**Third Cleavage:** The third cleavage is latitudinal occurring slightly above the equator i.e., towards the animal hemisphere. This results in the production of eight blastomeres, of which four are smaller and the other four are larger. The smaller blastomeres are situated at the animal hemisphere and are named as micromeres. The larger blastomeres are called macromeres and are located at the vegetal hemisphere.

**Fourth Cleavage:** The fourth cleavage is double. The furrows are meridional. This produces sixteen blastomeres, eight animal pole micromeres and eight vegetal pole macromeres.

**Fifth Cleavage:** The fifth cleavage is also double. The furrows are latitudinal. One bisects the micromeres in the animal hemisphere and the other cuts the macromeres in the vegetal hemisphere.

This results in thirty-two blastomeres being arranged in four tiers. Of these, two tiers are micromeres and the other two tiers are macromeres. From this stage the regularity of the cleavage is lost and the blastomeres divide at differential speeds; the micromeres divide faster than the macromeres.

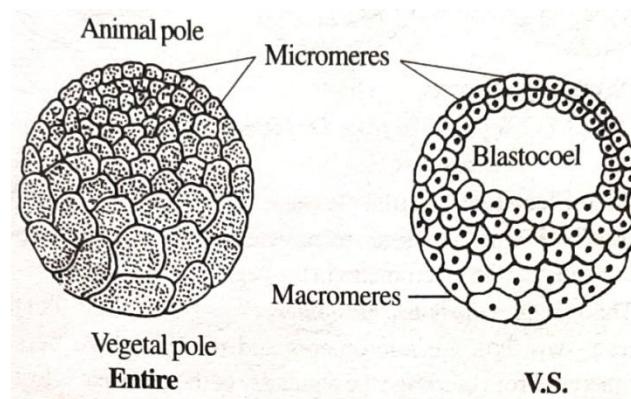
### **Blastulation**

Blastulation is the development of blastula. As cleavage proceeds a small cavity develops among the blastomeres. This cavity begins to develop in the eight cell stage itself. It is called the blastocoel or segmentation cavity. The surrounding blastomeres secrete an albuminous fluid into it. This fluid is named as blastocoel jelly. The blastocoel gradually increases in its volume as more and more blastocoel jelly is poured into it. This stage of the embryo is called blastula.

As the blastocoel increases in volume, the blastomeres arrange themselves in layers around the blastocoel. These layers are called blastoderm. The blastoderm remains two cell thick at the animal pole. The sides and floor of the blastocoel are formed of many layers. The blastula of frog is called coeloblastula, because it is a hollow sphere.

### **Blastula**

Cleavage produces an embryonic stage called blastula. It develops from the zygote. It is spherical in shape with a cavity inside. The cavity is called blastocoel. It is filled with an albuminous fluid called blastocoel jelly. The blastocoel is surrounded by blastoderm formed of blastomeres.



V.S. Fig. Blastula of frog.

The blastomeres are of two types, namely micromeres and macromeres. The blastoderm is two-cell thick in the animal pole and many cell thick in the vegetal pole. Hence the blastocoel is eccentric and not central. As the blastula is a hollow sphere, it is called coeloblastula.

## Fate Map

Fate map is a chart showing the end result of the different areas of an embryo. Towards the end of cleavage the organ forming areas are well marked.

1. The macromeres of the vegetal pole develop into the endoderm.
2. The notochordal area lies in front of the macromeres.
3. In between the endoderm and the notochordal area, lies the prechordal area.
4. On either side of the prechordal materials lies the mesoderm.
5. The micromeres of the animal pole develop into ectoderm.
6. The micromeres present adjacent to the notochord and mesoderm develop into the neurectoderm.
7. The micromeres present away from it develop into the epidermal ectoderm.

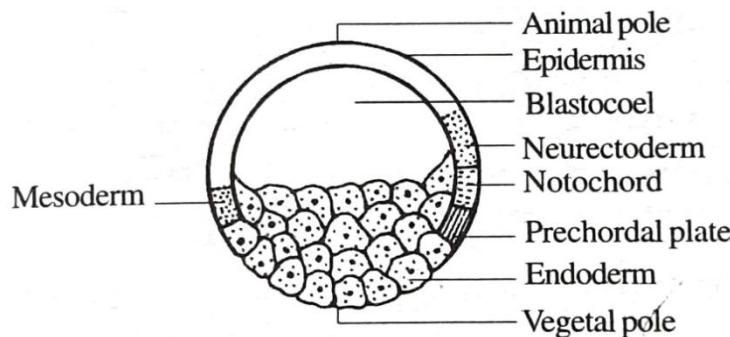


Fig. Section of blastula showing fate map.

## Gastrulation in Frog

Gastrulation is the development of gastrula from blastula. The blastula has a single layer of cells, the blastoderm. But the gastrula has three layers of cells. During gastrulation, the blastomeres (cells) are arranged into three layers, namely ectoderm, endoderm and mesoderm. These layers are called germ layers. During gastrulation, the blastomeres move from one place to other. The movement of cells during gastrulation is called morphogenetic movements. The morphogenetic movements bring out a definite shape to the embryo and hence the name.

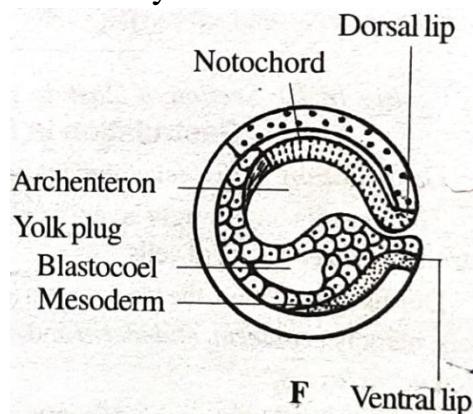


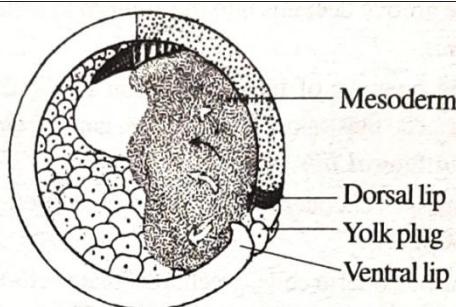
Fig. Mature gastrula

The morphogenetic movements are of two types, namely epiboly and emboly. Epiboly is the movement of cells on the surface of the embryo. Convergence, extension, etc. are epibolic movements. Emboly is the movement of cells into the embryo. Invagination, involution, divergence, etc. are embolic movements.

During gastrulation, the following events occur:

1. A groove appears below the grey crescent. The macromeres move into the embryo through this groove and form endoderm. The movement of endoderm into the embryo is called invagination.
2. The groove deepens into the embryo to form a cavity called archenteron.
3. The opening of the archenteron to the outside is called blastopore. The blastopore has four lips, namely dorsal lip, ventral lip and two lateral lips.
4. A mass of endoderm cells protrude out through the blastopore called yolk plug.
5. The mesoderm cells, prechordal plate cells and notochordal cells migrate towards the blastoporal lips. This movement is called convergence.

6. After reaching the blastoporal lips, these cells roll over the blastoporal lips and move into the embryo. This rolling movement is called involution.
7. The involuted cells move away from the blastopore into the embryo. This movement is called divergence.
8. The prechordal cells and notochordal cells are arranged as a plate in the mid-dorsal roof of archenteron.
9. The mesoderm is arranged as two sheets on either side of notochord.
10. In the early stage, the notochord and mesoderm remain as one continuous sheet called chordomesoderm.
11. Above the notochord the neurectoderm cells are arranged as a plate called neural plate. It is formed by extension.



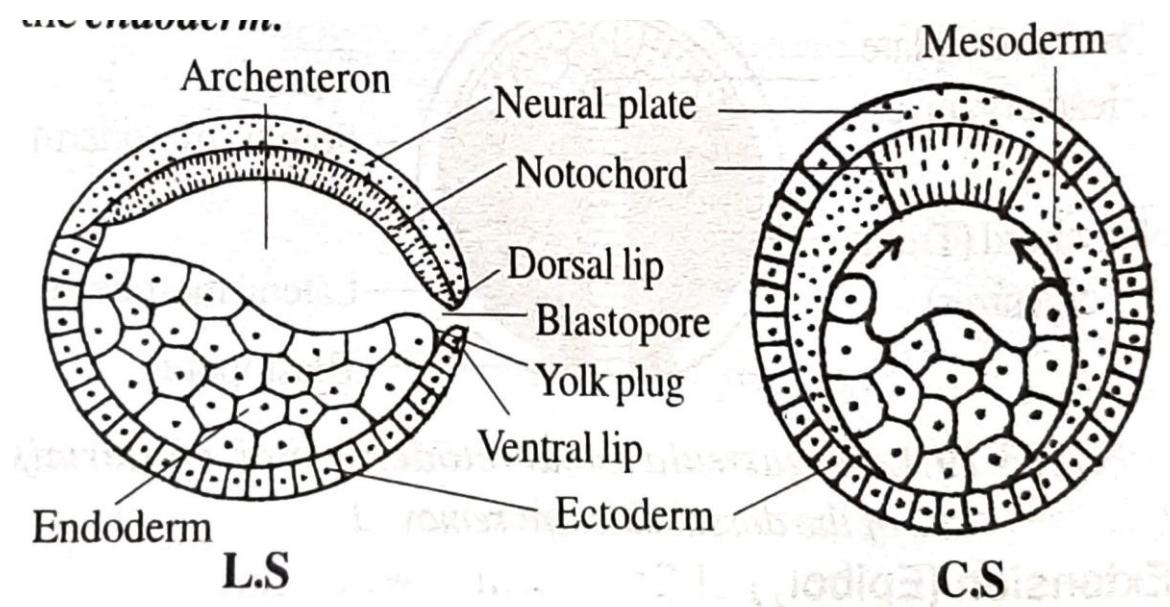
*Fig. Gastrulation in frog*

12. The epidermal ectoderm extends all over the embryo except the area of neural plate and blastopore.
13. The diameter of blastopore is decreasing.
14. The gastrula rotates within the fertilization membrane.
15. The mature gastrula is oval in shape. It has a cavity called archenteron. The archenteron opens to the exterior by a blastopore. A mass of endoderm cells projects through the blastopore. It is called yolk plug. It consists of three layers of cells, namely an outer ectoderm inner endoderm and middle mesoderm. The roof of the archenteron is formed of notochord. The ectoderm lying above the notochord is called neurectoderm.

## Gastrula

Gastrula is an embryo developed from the blastula.

1. The gastrula is oval in shape and it contains three germ-layers, namely ectoderm, endoderm and mesoderm.
2. Internally the gastrula has a cavity called the archenteron.
3. It opens to the exterior by the blastopore.
4. A mass of endoderm cells protrudes through the blastopore. These cells constitute the yolk plug.
5. The floor and the lateral sides of the archenteron are formed of the endoderm.
6. The roof of the archenteron is formed of the chordomesoderm.
7. Externally the gastrula is covered by ectoderm. infra
8. The ectoderm that lies on the mid-dorsal line develops into the nervous system and hence it is called neurectoderm.
9. The remainder of the ectoderm is the epidermal ectoderm.

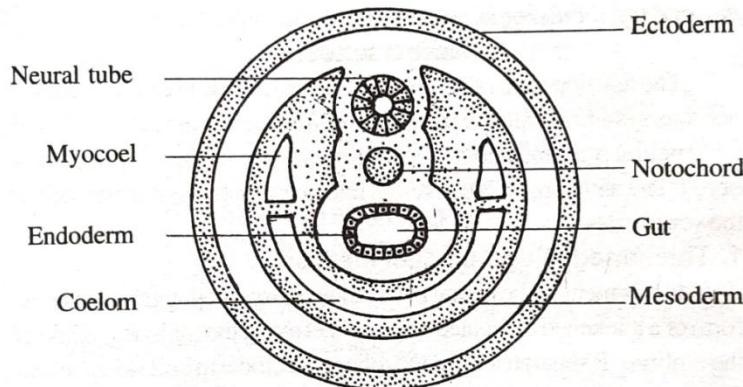


*Fig. Gastrula*

## **Formation of three germinal layers:**

Germ layer is a layer of cells in the embryo. Germ layer refers to a layer of distinctive cells in the embryo, developing into a definite set of structures or organs, when the embryo develops into the adult.

A typical vertebrate embryo has three germ layers, namely ectoderm, endoderm and mesoderm. The animals having three germ layers are called triploblastic animals. Eg. Platyhelminthes to mammals.



*Fig. A typical embryo showing the three germ layers.*

The cnidarians have only two germ layers namely ectoderm and endoderm, the mesoderm being absent. These animals are called diploblastic. The germ layers begin to develop in the gastrula stage. The three layers develop from the blastula by morphogenetic movements.

**Von Baer** proposed a law called germ layer theory. This theory says that each germ layer develops into the same set of organs in different species of animals. Each germ layer is destined to develop into a set of organs.

The **ectoderm** develops into the skin epidermis, nervous system, sense organs, stomodaeum and proctodaeum.

The **endoderm** develops into the epithelial lining of alimentary canal and associated structures like thyroid, lungs, liver, pancreas etc.

The **mesoderm** is destined to develop into the skeleton, connective tissue, muscles, vascular system, coelomic epithelium and its derivatives and urinogenital system.

‘ The three germinal layers arrange themselves in definite areas in the embryos. During organogenesis, they split up into small cellular masses, each of which is destined to develop into a particular organ. These groups of cells are named as organ rudiments.

The process of formation of primary organ rudiments is called tubulation. The tubulation includes neurogenesis, notogenesis, mesogenesis, enterogenesis and so on.

### **Notogenesis**

The development of notochord is called notogenesis. The notochord develops from chordomesoderm.

### **Mesogenesis**

The development of mesoderm is called mesogenesis. It develops on either side of notochord between ectoderm and endoderm.

### **Enterogenesis**

The development of gut is called enterogenesis. It is formed from endoderm.

### **Growth**

Growth is the increase in size of an organism or an organ. They increase in size by the synthesis of protoplasm. The primary organ rudiments grow to attain a maximum size characteristic of adult.

### **Differentiation**

Differentiation is the specialization of a cell or tissue or organ to do a particular function.

## Organogenesis

|                 |                             |  |
|-----------------|-----------------------------|--|
| <b>Ectoderm</b> | Epidermal ectoderm          | Skin epidermis, skin derivatives like sweat glands, sebaceous glands, mammary glands, nail, hair, feathers, claws, inner ear, lens, anterior pituitary, lining of stomodaeum and proctodaeum, etc. |
|                 | Neural tube                 | Brain, spinal cord, cranial and spinal motor nerves, retina and optic nerve of eye, posterior pituitary.   |
|                 | Neural crest                | Ganglia, sensory nerves, adrenal medulla, pigment cells (chromatophores) visceral skeleton.  |
| <b>Endoderm</b> | Archenteron                 | Digestive tube, liver, pancreas, allantois, urinary bladder, yolk sac, thyroid, , thymus, tympanic cavity(middle ear) respiratory system.  |
|                 | Notochord                   | Well developed in Amphioxus; in other chordates it is reduced.   |
| <b>Mesoderm</b> | Dorsal mesoderm<br>(somite) | <b>Myotome</b> -Skeletal muscles and limb-skeleton.  |
|                 |                             | <b>Scleratome</b> - Vertebral column.  |
|                 |                             | <b>Dermatome</b> - Dermis.   |
|                 | Intermediate mesoderm       | Excretory system and reproductive tracts.  |
|                 | Lateral mesoderm            | <b>Somatic mesoderm</b> - Parietal peritoneum.   |
|                 |                             | <b>Splanchnic mesoderm</b> - Visceral peritoneum, circulatory system, muscles of gonads viscera, mesenteries.  |
|                 |                             | <b>Coelom</b> - Body cavities.   |

*Fig. Chart showing the derivatives of germinal layers in vertebrates.*

## Regeneration in chordates.

The replacement of the lost parts of the organism is called regeneration. Regeneration is a complex morphogenetic process. It is a fundamental phenomenon of life. Regeneration is indispensable 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'

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 replaced by newly formed cells. This is due to regeneration.

Regeneration can be defined as the natural ability of living organisms to replace worn out parts, repair or renew damaged or lost parts of the body, or to 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.

### **Regeneration in chordates:**

**Fishes:** In fishes, the ability for regeneration is restricted only to the replacement of fins. Lamprey can regenerate its lost tail.

**Amphibians:** The regeneration power is well marked in urodeles amphibians like salamanders, newts and their axolotl larvae. They can regenerate limbs, tail, external gills, jaws, parts of eye like 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 and feathers.

**Mammals:** In mammals, regeneration is very poor. It includes the replacement of nails, antler, skin, erythrocytes, the internal lining of gut and uterus and so on. 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 of regeneration. If one kidney is damaged or removed, the other enlarges to compensate the lost kidney. This is called as compensatory hypertrophy.