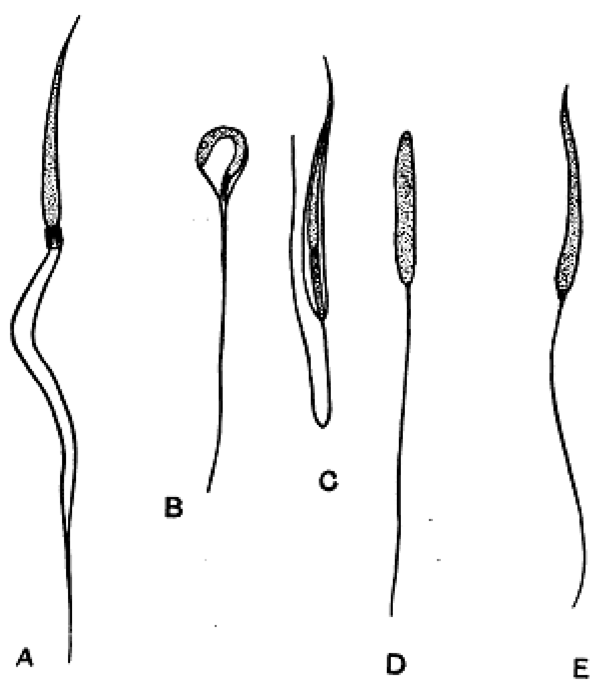
DEVELOPMENTAL BIOLOGY OF FROG

In frog the sexes are separate, female being larger than male. Male has a nuptial pad at the base of the first finger of forelimb and also possesses a pair of vocal sacs. When frogs mate, the male grasps the female's trunk with his forelimbs. The technical name for this special kind of embrace is **amplexus**. Frogs and toads don't have penises. During amplexus the female discharges eggs, usually into water, while the male sheds sperms over the eggs.

Sperm

The mature sperm measures on an average 0.03mm in length. It has an elongated solid head with an anterior bead-like acrosome. The short middle piece is invisible but the tail appears as a gray filamentous extension about four or more times the length of the sperm head.



Amphibian spermatozoa

Egg

The egg for frog is about 2mm in diameter at the time of ovulation. It is surrounded by two accessory egg membranes in addition to the **plasma membrane**. Just outside the plasma membrane is a non living transparent membrane called **vitelline membrane** developed by the ovum itself. Outer to vitelline membrane is the **jelly coat** or **albumen** secreted by the walls of the oviduct. As soon as the egg reaches the water, the jelly coat swells up by the imbibitions of water and it protects the egg from injury and against infection by bacteria and other microorganisms.

Frog's egg exhibits a well developed polarity and radial symmetry. The cytoplasm has two regions, the cortex and endoplasm.

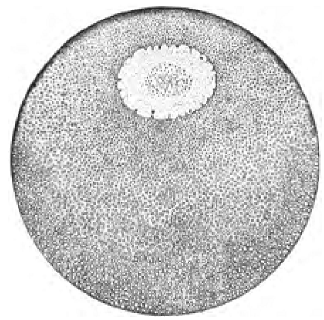
1. Egg cortex

A jelly like viscous layer of cytoplasm adherent to the plasma membrane is called ectoplasm or egg cortex. It possesses some membrane bound spherical bodies called **cortical granules** containing acid mucopolysaccharides. These remain arranged in a layer close to the plasma membrane. Dark-brown **pigment granules** are present in the egg cortex on the animal hemisphere. The presence of these granules imparts a dark brown colour to the entire animal hemisphere. The vegetal pole is whitish with little pigment granules.

The cortical layer of egg is stable and is not shifted by streaming movement of cytoplasm or centrifugation force. It plays an important role in the development of egg. The egg cortex is responsible for establishing polarity, bilateral symmetry and general organization of the developing egg.

2. Endoplasm

The inner ooplasm with its nucleus is called endoplasm which is colloidal in nature. This portion contains cell organellae like mitochondria and ribosomes, and also organic and inorganic substances. Endoplasm contains a cup shaped mass of white yolk platelets called **vitelline cupola**. The **germinal vesicle** or **nucleus** is located near the animal pole. The yolk granules are little and small sized in the animal pole while they are heavily deposited in the vegetal pole. Frog's egg is said to be **mesolecithal** and **moderately telolecithal** since it contains a moderate amount of yolk which is distributed unevenly in the cytoplasm, the vegetal pole having the highest concentration.



Amphibian egg

Fertilization

Fertilization is the fusion of sperm with egg resulting in the formation of zygote. It is characterized by the following events.

1. Fertilization is external.
2. It is monospermy, i.e. only one sperm fuses with the egg.
3. The fertilized egg rotates in such away 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 40^0 from the centre of animal pole.
7. Immediately after the entry of the sperm 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 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. 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**.

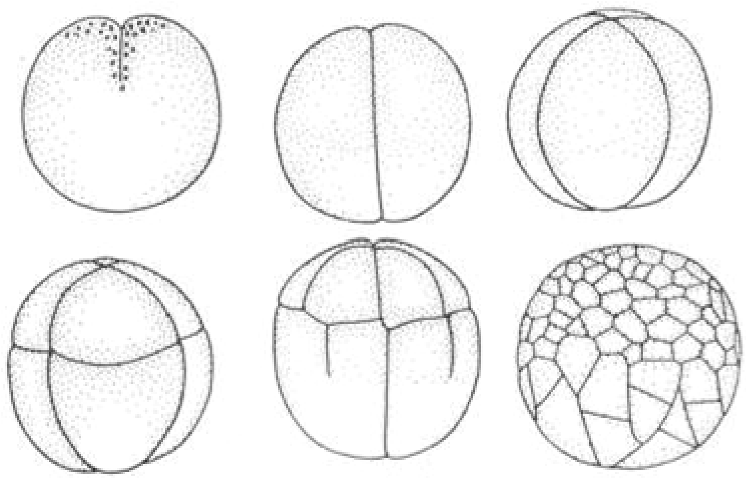
Grey Crescent (Gray Crescent)

1. Grey crescent is a crescent-like and grey colored 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 the 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 in to the chordamesoderm.

Cleavage

The first cleavage of frog's egg was observed by Swammerdam in 1738. The entire process of cleavage in frog's egg was studied by Prevost and Dumas in 1824. In frog's egg the cleavage is **holoblastic** and **unequal**. The cleavage occurs as follows.

1. The first cleavage plane is **meridional**. Initially, a furrow appears at the animal pole. It gradually extends towards the vegetal pole of the egg. It cuts the egg through its median animal-vegetal polar axis and results in two equal sized blastomeres.



Cleavage in frog's egg

2. The second cleavage furrow is again **meridional**. It bisects the first cleavage furrow at right angles. It is a holoblastic cleavage affecting both the blastomeres of the first cleavage. It results in the formation of four blastomeres.

3. In the next stage a **latitudinal/horizontal** furrow is formed above the equator nearer to the animal pole. Such a furrow is due to the influence of yolk concentration in the vegetal pole. The latitudinal furrow uniformly affects all the blastomeres. It results in the formation of eight blastomeres. Four of them remaining in the vegetal pole are large. They are named as **macromeres**. Another four blastomeres remain in the vegetal pole. They are named as **micromeres**. The micromeres are smaller in size than the macromeres.

4. The fourth set of cleavage planes are **meridional** and double in nature. They are unequal. They divide yolkless micromeres more rapidly than yolk-rich macromeres. These cleavages result in the production of 16 blastomeres.

5. The fifth cleavage is latitudinal /horizontal and double, dividing the micromeres as well as macromeres so that four tiers of blastomeres are formed.

6. As a result of further cleavages, a ball of several small blastomeres results. A closer observation reveals that, while the blastomeres above the equator are small and remain as micromeres, the blastomeres of the vegetal pole remain progressively larger. The larger blastomeres are called the macromeres.

Initially the continued division of blastomeres forms a ball like structure which is solid. It is called the **morula** stage, as this has superficial resemblance to a mulberry fruit. Very soon however the morula stage gives rise to a stage called the blastula which is a hollow ball like structure.

Blastulation

At the end of cleavage the solid ball of cells give rise to blastula which consists of a number blastomeres. The characteristic features of the blastula stage are the presence of a well defined cavity called the **blastocoel**. This is the beginning of the primary body cavity. The process of the formation of blastula is called **blastulation**. The blastula of frog is called amphiblastian as the cavity is confined to only the animal pole. The vegetal pole however is composed of a solid mass of non pigmented yolky cells.

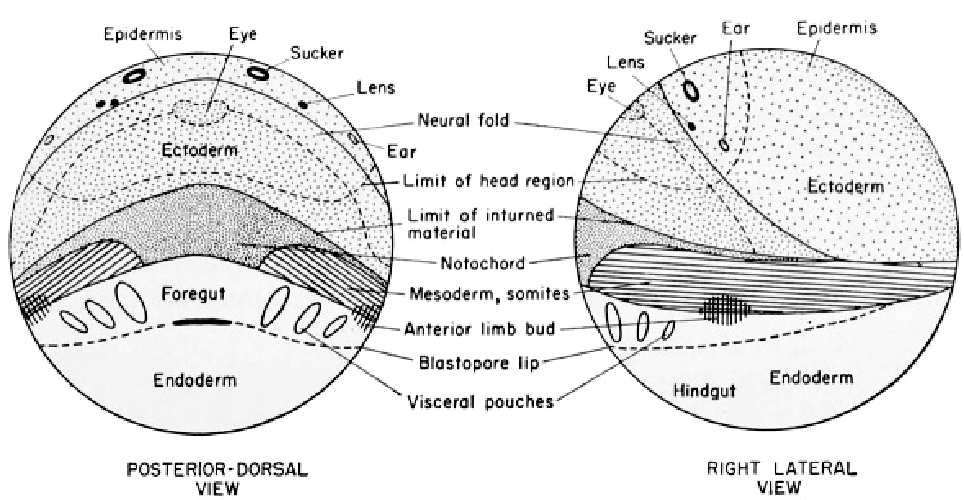
In the thirty two cell stage, the blastula consists of a single layer of cells and is called the early blastula. The pigmented cells (micromeres) are found in the anterior half while the yolky megameres are present in the posterior half. As has been already pointed out, the blastocoel lies entirely in the anterior half. The blastula of frog is hollow and has a very well developed blastocoel. It is said to be a **coeloblastula**.

As segmentation proceeds, the number of cells in the blastula increase; so also the blastocoel. The floor of the blastocoel is flat while its top portion is arched. The roof is made up of three to four layers of pigmented micromeres while the floor is formed by yolky megameres. Between the micromeres and the megameres and along the equator is found a group of cells which are intermediate in size (between megameres and micromeres). These cells constitute the **germ ring**. The germ ring is formed in the region of the grey crescent.

Fate Map

Wather Vogt (1925) used vital staining method for the construction of fate maps of amphibians. Vital stains do not interfere with the normal processes. A piece of agar or cellophane (stain carrier) is used and is pressed against the chosen area of blastula for a short period. Cellophane is better than agar as it can be cut easily into desired size and shape. The stain does not diffuse into the neighboring cells. The blastula of amphibian embryo is round and has three distinct regions:

1. The vegetal region is the pigment free macromere region. It represents **presumptive endoderm** and contains the material for the formation of midgut and hindgut of embryo.
2. Second region is that of animal pole of egg which consists of micromeres. It gives rise to future ectoderm of the animal and forms two main regions:
    a. Region of **prospective ectoderm** which develops into the epidermis of skin.
    b. Region of **prospective central nervous system** which forms brain, spinal cord and sense organs.
3. Third region is the marginal region of gray crescent. It forms the **presumptive mesodermal cells**. It consists of the following subregions:
    a. **Presumptive notochordal** region which is present on the dorsal side and gives rise to notochord.
    b. Below the notochordal area is the portion which forms the part of foregut.
    c. Region of **presumptive somites** which develops on both the sides of notochordal area.
    d. Ventrolateral mesodermal area which lies on lateral and ventral part of marginal zone and forms the **mesodermal lining** of the body cavity, kidney and reproductive organs.



Gastrulation

Gastrulation is the process of formation of hollow gastrula from blastula. It involves dynamic movement and rearrangement of blastomeres. Such movements of blastomeres along specific paths during gastrulation are called as morphogenetic movements. Three types of morphogenetic movements can be found- **invagination, involution** and **epiboly**.

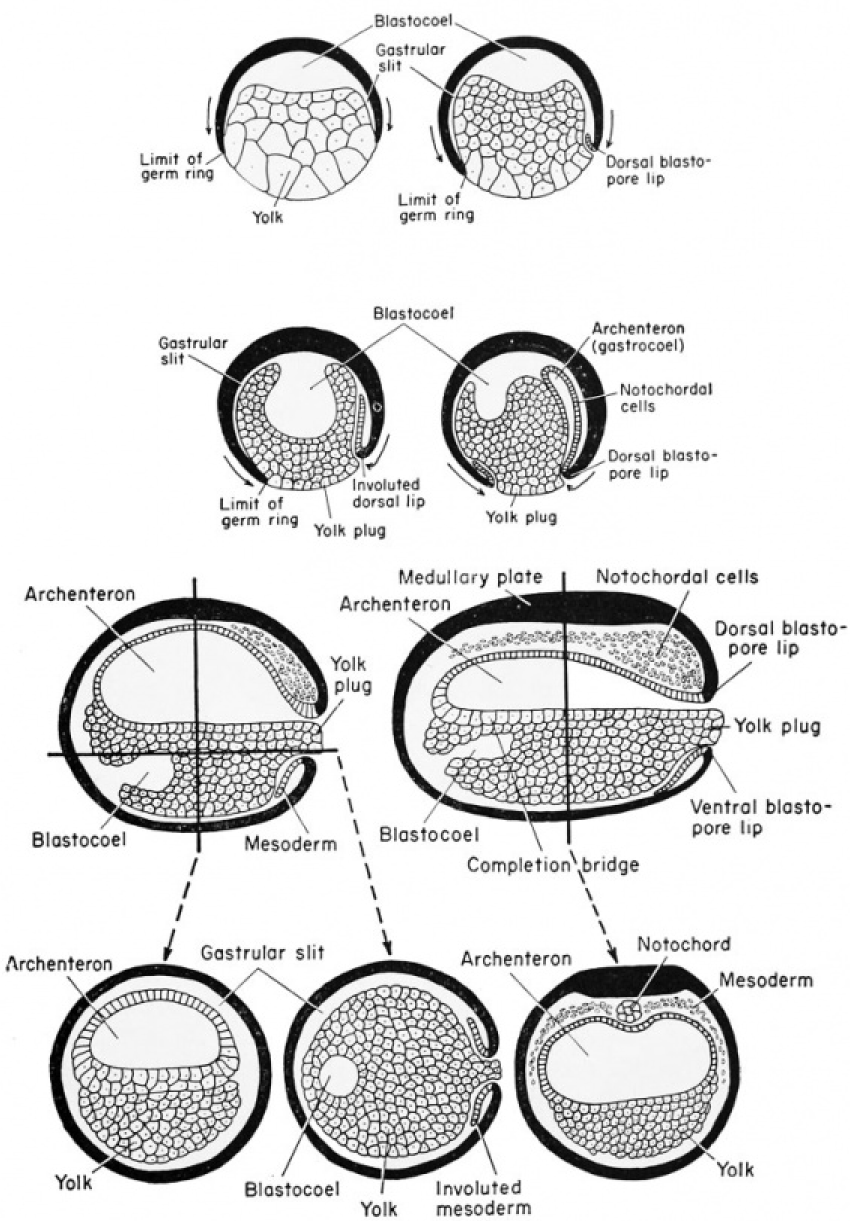
Invagination: Invagination is an active infolding of blastomeres. During invagination, few blastomeres near grey crescent are pushed inward to form a slit or groove. The opening of this groove is called as **blastopore** and the cavity is called as **gastrocoel** or **archenteron**. The blastopore gradually assumes a crescentic shape. Finally it becomes circular. The region dorsal to the blastoporal opening is called the '**dorsal lip**'. The lower edge may be called the '**ventral lip**'. Due to enlargement of archenteron, blastocoel is gradually reduced.

Involution: Involution is the process of rolling in movement of blastomeres. During this process the micromeres multiply and migrate to the dorsal lip of blastopore and roll inside or turn into the archenteron and arrange themselves on the roof of the archenteron. Involution is completed by convergence and divergence. During this, the micromeres multiply rapidly and move towards the blastoporal end, process called convergence. Thus converged cells in the blastopore start to involute slowly and diverge towards the roof of the archenteron. This process is called as divergence. Thus involuted cells develop into chordamesoderm. The archenteron gradually widens which pushes the blastocoel narrow. The crescentic blastopore becomes complete circle.

Epiboly: Epiboly means growth of one layer of cells over another. During epiboly, micromeres of animal pole divide rapidly and move over the macromeres of vegetal pole. This layer forms ectoderm. As a result of these morphogenetic movements, three primary germ layers are formed. The cells which cover the gastrula externally form ectoderm. Those involuted cells into the roof of archenteron give rise to mesoderm and cells of sides and floor of the archenteron will develop into endoderm.

Some other internal changes are also taking place along with those morphogenetic movements. As the archenteron is enlarging, the yolky megameres are pushed out towards the blastopore. This structure is called as **yolk plug**. The process of gastrulation is completed in 36 hours of fertilization.

The process of gastrulation converts the blastula into a spherical, bilaterally symmetrical, triploblastic gastrula. Gradually the gastrula undergoes the process of **tubulation** or **neurulation** to become a **neurula**.



Neurulation

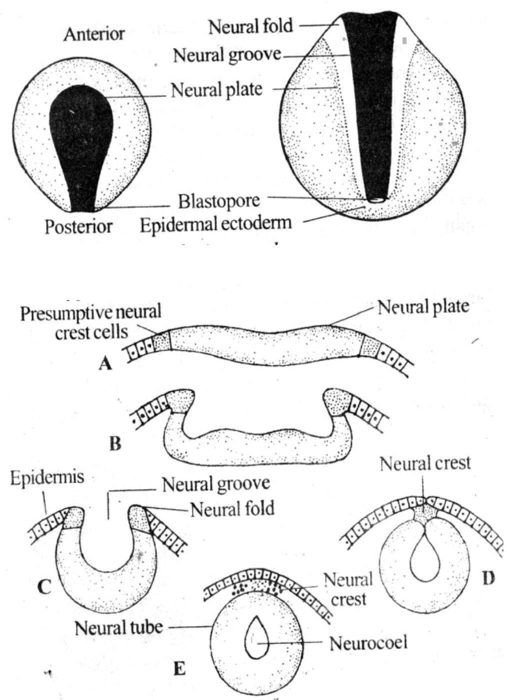
Neurulation accomplishes three major things:

(1) It creates the neural tube, which gives rise the central nervous system.

(2) It creates the neural crest, which migrates away from the dorsal surface of the neural tube, and gives rise to a diverse set of cell types.

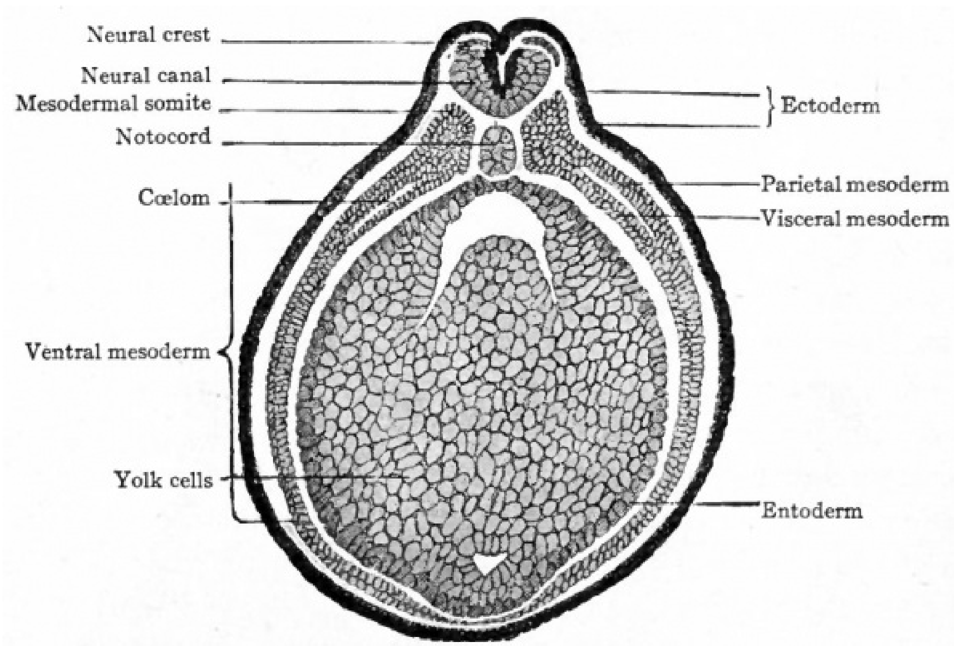
(3) It creates the bona fide epidermis, which covers over the neural tube once it is created.

In the gastrula, the presumptive material for nervous system lies on the mid-dorsal line as a plate called **neural plate or medullary plate**. It extends from the dorsal lip of the blastopore to the anterior end. Soon, the edges of the neural plate become thickened and raised above as **neural folds or medullary folds**. The neural folds of the two sides are continuous anteriorly to form the transverse neural folds. The neural folds enclose a shallow groove called **neural groove**. The neural folds increase their elevation and bend towards one another until their edges meet and fuse. Thus a tube is formed called **neural tube**. It encloses at canal called **neurocoel**. The fusion first starts just behind. Anteriorly, the neural tube opens to the exterior for some time by anterior **neuropore**. It becomes closed soon. Posteriorly the neural folds enclose the blastopore in such a way that the neurocoel communicates with the archenteron through the blastopore. The short narrow canal connecting the archenterons and neurocoel is **neurenteric canal**. Later it also disappears. After, the neural folds have fused in the median line. The neural tube separates itself completely from the overlying epidermis. The free edges of the epidermis fuse together, so that the epidermis becomes continuous over neural tube. As the neural tube is separated from the ectoderm, a certain number of loose cells are liberated from the neural folds in the space between the ectoderm and the neural tube. These cells arrange themselves as two longitudinal bands on the dorso-lateral wall of the neural tube. These cells constitute **neural crest**. Later the neural crest cells differentiate into the ganglia of the cranial and spinal nerves, melanophores (chromatophores), adrenal medulla and visceral skeleton. The anterior portion of the neural tube differentiates into the brain and the posterior part into the spinal cord. The embryonic stage which is having the neural plate or the neural tube is called **neurula**.



Development of Notochord or Notogenesis

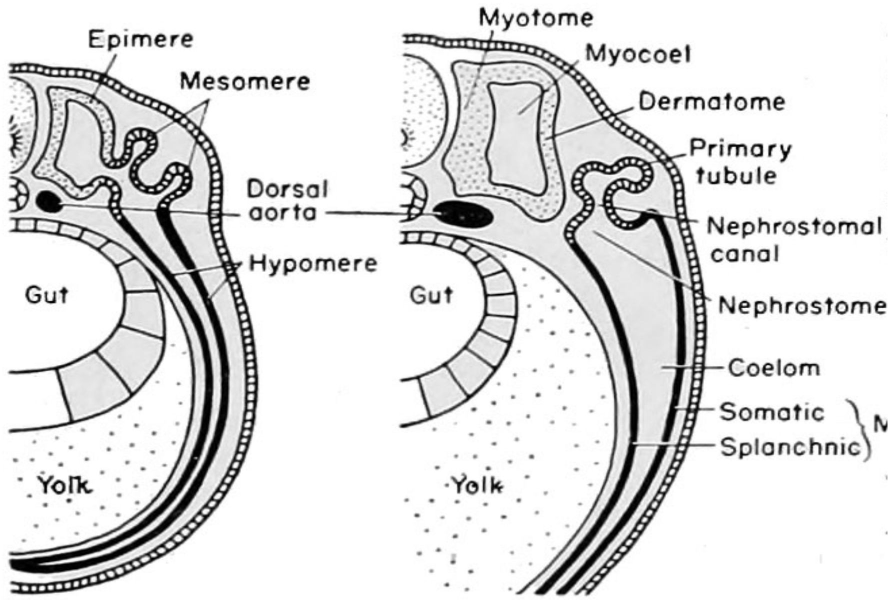
The presumptive notochordal material is present in the roof of archenteron as a longitudinal strip. It becomes separated from the adjacent mesoderm and underlying endoderrn. The cells arrange themselves to form a cylindrical rod called notochord. In this stage, the notochord is attached to the underlying endoderm by a band of cells called hypochordal or sub-notochordal rod. It develops only in amphibian embryo and degenerates immediately. The notochord exceeds from the pituitary body of the brain to the end of the tail. At first, the cells of notochord are closely packed but later they fuse together and become vacuolated except a layer of peripheral cells. Later sheaths are formed around the notochord. There are mainly three sheaths a) Primary sheath is formed by superficial chordal cells, it is an elastic sheath of connective tissue. b) The secondary fibrous sheath is formed by the chordal epithelium and c) The skeletogenous sheath derived from the sclerotome.

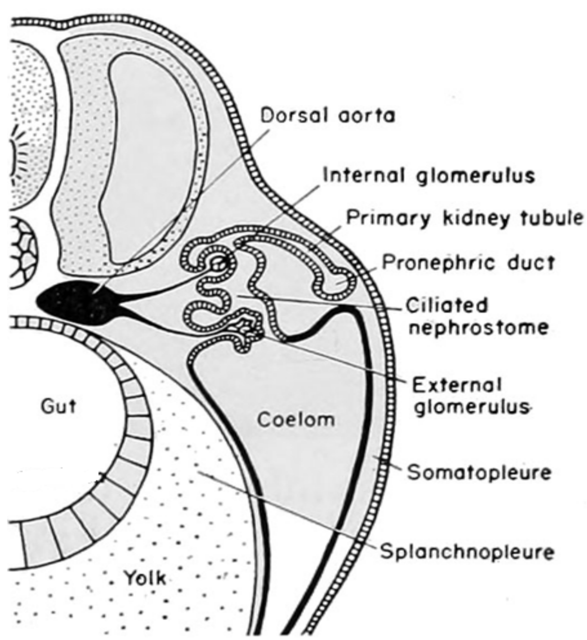


<u>Differentiation of Mesoderm</u>

During notogenesis, the mesoderm separates from the chorda mesoderm mantle and lies on either side of the notochord. Immediately, they subdivide into segments-**somite, nephrotome** and **lateral plate mesoderm.**

Subsequently, the part of each somite next to the notochord separates to form the **sclerotomes** or skeleton forming tissue around the notochord while the major outer portion of somite differentiated into **myotome**, the cells of which develop into striated muscles. The outermost narrow strip of somite beneath the epidermis becomes the **dermatome** differentiating into dermis.





Development of Brain

The anterior part of neural tube is distinguished as encephalon which develops into various parts of the brain through thickening, thinning, evagination and invagination

The primary embryonic brain of the frog has three main subdivisions called **prosencephalon** (forebrain), **mesencephalon** (midbrain) and **rhombencephalon** (hindbrain). The cavities of three primary divisions are known as **prosocoel, mesocoel** and **rhombocoel.**

Prosencephalon

The **prosencephalon** becomes further subdivided into two regions, the **telencephalon** and the **diencephalon**. At the posterior limit of prosencephalon the brain bends ventrally around the anterior end of the notochord to form **cranial flexure** which remains as a prominent feature of brain of vertebrates. The area of cranial flexure thickens to develop the **tuberculum posterius** that marks the posterior limt of forebrain ventrally.

The most anterior division of the forebrain is the telencephalon with its original cavity, the **telocoel**. The anterior limit of telencephalon is the **lamina terminalis** which will separate the future cerebral hemispheres by a longitudinal groove. Actually, the lamina terminalis represents the anterior fused neuroporal area.

The telencephalon is the embryonic cerebrum. Its cavity expands laterally to give rise to the right (first) and left (second) **lateral ventricles** and the surrounding thick-walled cerebral hemispheres, at about the 12 mm. stage. These ventricles are laterally compressed. In the frog the cerebral hemispheres are first differentiated at the 7 mm. stage but never become very large. The two telencephalic vesicles are partially constricted off from each other but remain connected by way of the tubular **foramen of Monro**, which opens into the common (intermediate) **third ventricle**. The third ventricle overlaps and connects the telocoel and the diocoel.

The roof of the cerebral lobes thickens to give rise to **cortex** or **pallium** and the floor and sides of which form the **corpora striata**. The **olfactory lobes** arise as a pair of evaginations from the anteroventral part of telencephalon. Subsequently, they become fused medially. The nerves originating from the olfactory lobes innervate the nasal epithelium or olfactory placode. The ventricle enclosed by the olfactory lobe is called **olfactocoel**.

Diencephalon (Thalamencephalon or Between-brain).

The structural derivatives of this diencephalon include the **posterior commissure**, just anterior to the dorsal limit of the mesencephalon. Anterior to this is the **epiphyseal recess**, and the dorso-medial saccular outgrowth known as the **epiphysis**. This continues to grow forward and becomes separated from the brain as a small knob of cells which remain in the adult as the brow spot. It is presumably homologous to the **pineal gland** of higher vertebrates.

Anterior to the epiphysis, in the roof of the diencephalon and between it and the **anterior choroid plexus**, are the **habenular ganglion** and **commissure**. In front of this there later develops a dorsal outgrowth know as the **paraphysis**. In the floor of diencephalon anterior to tuberculum posterius there develops a vesicular evagination callede **infundibulum**. The cells of the infundibulum will combine with the approximated and pigmented cells of the ingrown **hypophysis** to form the pituitary gland of the adult. The infundibulum cells give rise to the posterior part of the pituitary gland and retain a hollow infundibular stalk connection with the brain. The hypophysis becomes the anterior part of the pituitary gland. During metamorphosis the individual lobes of the pituitary gland differ, both in gross morphology and in finer structure. Between the infundibulum and the tuberculum posterius is a secondary and posteriorly directed pocket known as the **mammillary recess**.

A pronounced thickening appears in front of the infundibulum called **optic chiasma**. A depression is developed anterior to the optic chiasma known as **optic recess**. In front of the optic recess there appears a ventral thickening called **torus transverses**.

The optic vesicles begin to develop very early as ventro-lateral outgrowths of the diocoel. The expansion of the diocoel provides a temporary and slight thinning of the walls of the **optic vesicles**. However, as these vesicles make contact with the lateral head ectoderm, that portion of the vesicle in contact begins to thicken and then invaginate to form a 2-layered **optic cup**. The most lateral and invaginated portion of the cup will become the **retina**, the medial layer will become the **pigmented layer** of the eye, and the connecting and somewhat constricted tube the **optic stalk**. The nervous elements of this optic stalk will join in the **optic chiasma** which contains the optic nerve fiber tracts from the two sides. The stalk will develop around an inverted groove (the **choroid fissure**) which will contain, within the groove, accessory nerves and blood vessels which feed the retina.

Mesencephalon(Midbrain)

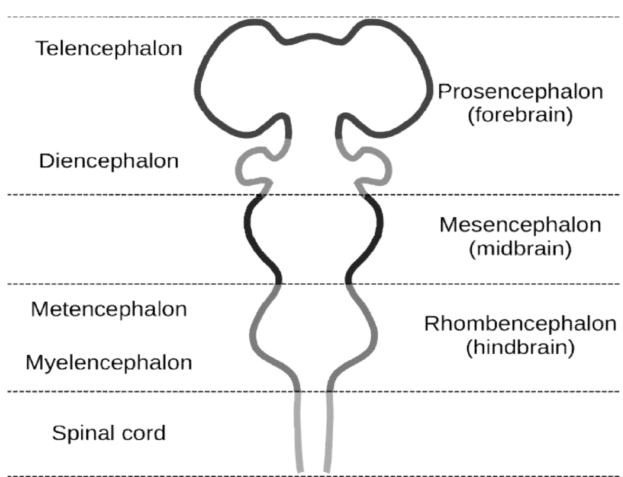
This portion of the brain functions largely as a pathway of nerve tracts between the anterior prosencephalon and the posterior rhombencephalon. These tracts are found principally within the paired ventro-lateral thickenings of the walls and floor on either side of the tuberculum posterius. They are known as the **crura cerebri**.

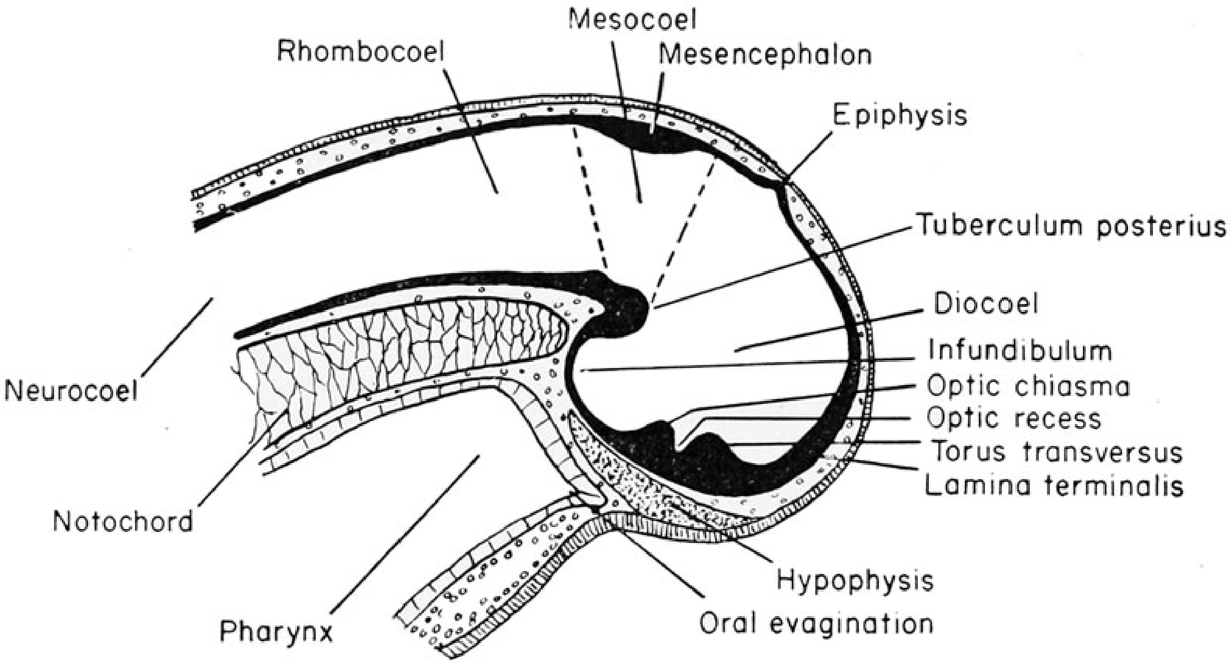
The original dorsal thickening becomes subdivided by a median fissure into paired dorso-lateral thickenings. These are known as the **optic lobes** or **corpora bigeniina**. They do not reach their full development until the time of metamorphosis. Anterior to these lobes is the posterior commissure. From the posterior limits of the mesencephalon and optic lobes may be seen the **valvulae cerebelli** and the fourth pair of cranial nerves (trochlear) which emerge from the dorso-lateral wall. The original cavity of the midbrain (mesocoel) connects the rhombocoel (fourth ventricle) with the third ventricle, which becomes narrow and is known as the **aqueduct of Sylvius**.

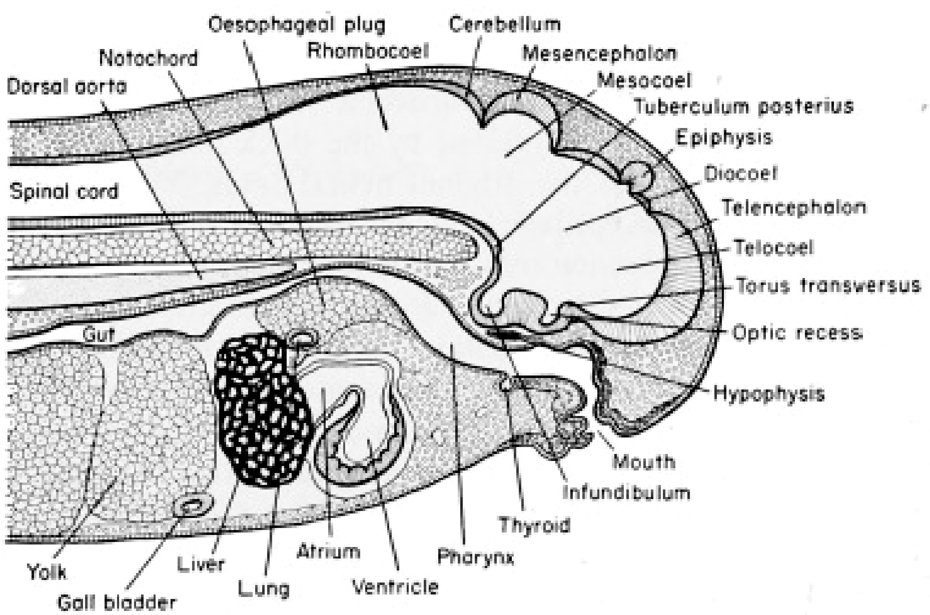
Rhombencephalon (Hindbrain)

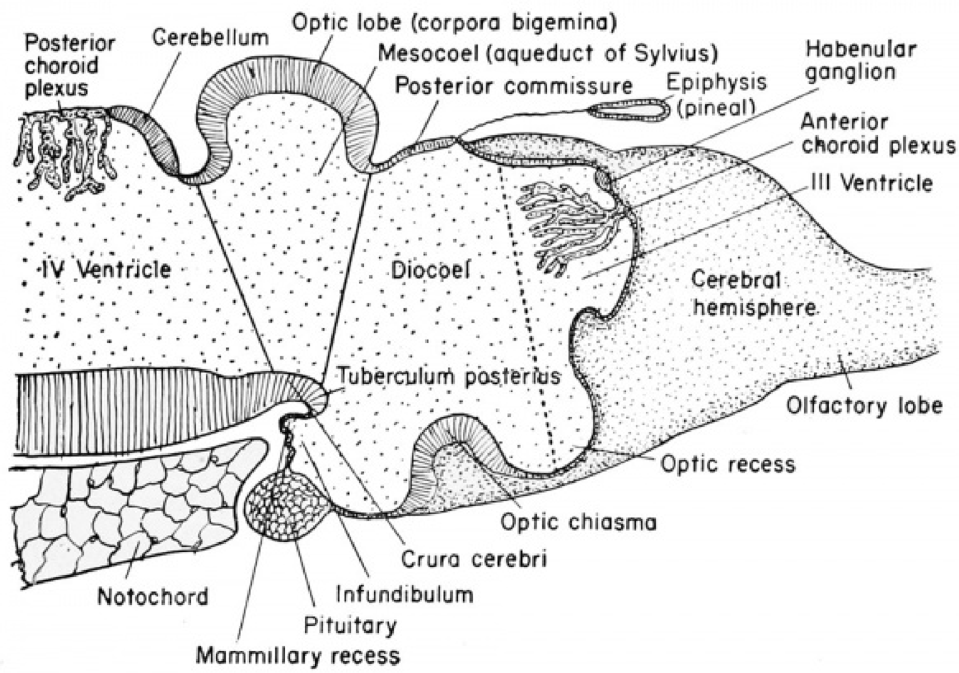
This portion of the brain is clearly marked off from the mesencephalon by a transverse constriction in the roof of the brain, at the posterior limit of the dorsal thickening. It is not clearly divided farther. There appears a slight transverse thickening in the roof of the rhombencephalon which corresponds to the metencephalon of higher forms and develops into the small **cerebellum**. Posterior to this the roof becomes broad, thin, and vascular, and folds into the rhombocoel (fourth ventricle) as the **posterior choroid plexus**. The ventral and ventro-lateral walls of the rhombencephalon are known as the **medulla oblongata** from which arise the cranial nerves V to X inclusive. The walls become thickened by fibers which form numerous pathways from the brain and cord.

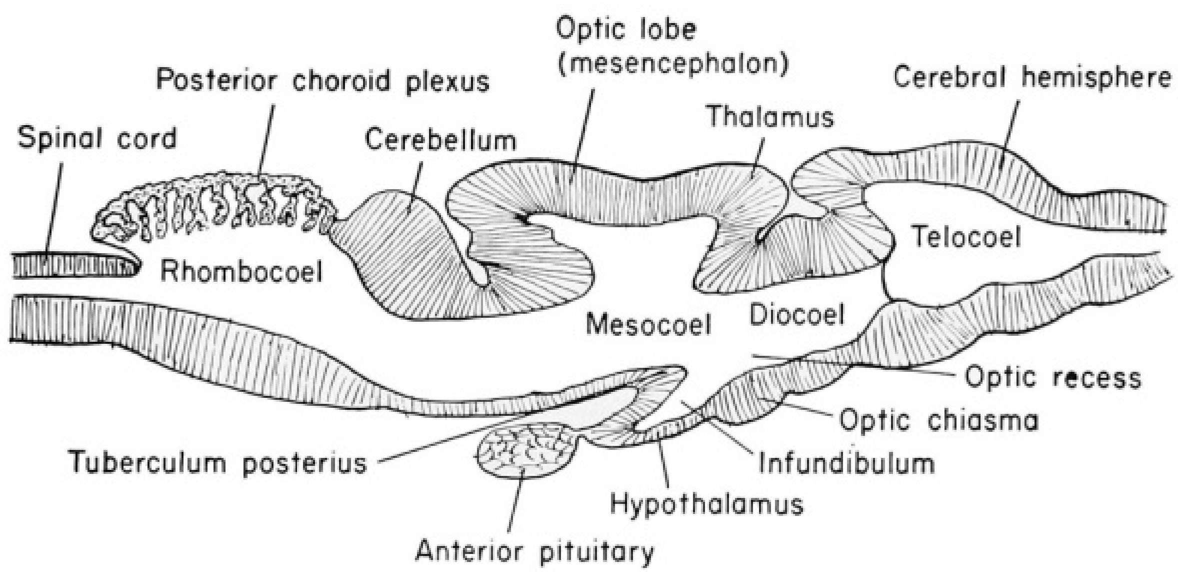
The rhombocoel or cavity of the hindbrain is known as the **fourth ventricle** which communicates posteriorly with the central canal of the spinal cord and anteriorly with the **aqueduct of Sylvius** of the mesencephalon.




Mesocoel
Rhombocoel
Mesencephalon
Epiphysis
Tuberculum posterius
Diocoel
Neurocoel
Infundibulum
Optic chiasma
Optic recess
Torus transversus
Lamina terminalis
Notochord
Hypophysis
Pharynx
Oral evagination


Oesophageal plug
Cerebellum
Notochord
Rhombocoel
Mesencephalon
Dorsal aorta
Mesocoel
Tuberculum posterius
Epiphysis
Diocoel
Spinal cord
Telencephalon
Telocoel
Torus transversus
Gut
Optic recess
Hypophysis
Mouth
Infundibulum
Thyroid
Atrium
Pharynx
Yolk
Liver
Lung
Ventricle
Gall bladder





Development of Eye

The eye is a photoreceptor. It is an ectodermal derivative. Its development begins even at the gastrulation stage. However the first sign of eye formation appears with the development of two optic vesicles from the lateral walls of the embryonic diencephalon.

Formation of optic cup

The eyes develop as two lateral outgrowths of the prosencephalon called **optic vesicles**. The cavity of the optic vesicle is called **optocoel**. The connection of the optic vesicle with the brain becomes a narrow stalk like structure called **optic stalk**. The optic stalk becomes connected with the ventral side of the optic vesicle rather than at its centre. The optic vesicles extend outward and reach the ectoderm. The wall of the optic vesicle next to the ectoderm is gradually flattened and later invaginates to form a double walled cup called **optic cup**. The optic cup consists of two layers. The inner layer (derived by invagination) gives rise to the nervous region, the **retina**.

The outer layer will be a thin, black **pigmented layer** for the absorption of light. Initially the opening of cup is very large. Soon its rim bends inward and converges, so that the opening is reduced. This opening is called the **pupil**. The rim of the optic cup surrounding the pupil becomes the **iris**. Later on, large amount of pigment is deposited in the outer epithelial layer of iris. A groove extends along the ventral side of the optic cup. It is called the **choroid fissure**. It extends to the middle of optic stalk. It serves for the entry of blood vessels and mesenchyme cells into the posterior chamber of eye.

The retina develops a membrane on its inner most surface called the **internal limiting membrane**. The outermost cells of the neurosensory retina differentiate into **rods** and **cones**. The inner cells of the retina differentiate into **neuroblasts** or nerve cells.

Development of lens:

When the lateral surface of the growing optic vesicle comes in contact with the ectoderm it gives off stimulus of some kind, which causes the ectodermal cells to elongate, forming a disc shaped thickening. This is called the **lens placode** or **lens rudiment**.

It curves into a cup and finally separates from the ectoderm. The free edges of the cup fuse to form a globular hollow **lens vesicle**. The lens vesicle comes to lie in the cavity of the optic cup.

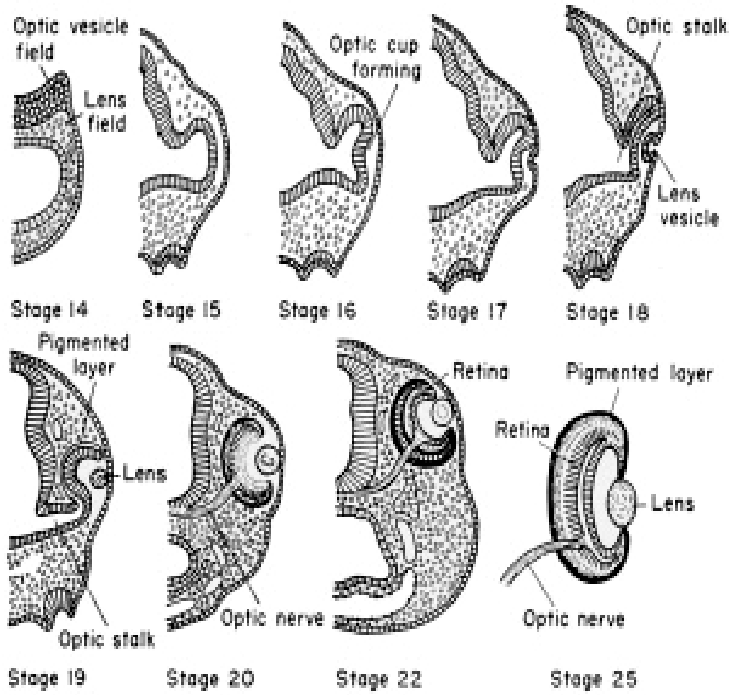
The cells of the inner side of the lens vesicle elongate, become columnar and are finally transformed into long fibres. Their nuclei degenerate and cytoplasm becomes hard and transparent making it refractile. These cells are called **lens fibres**.

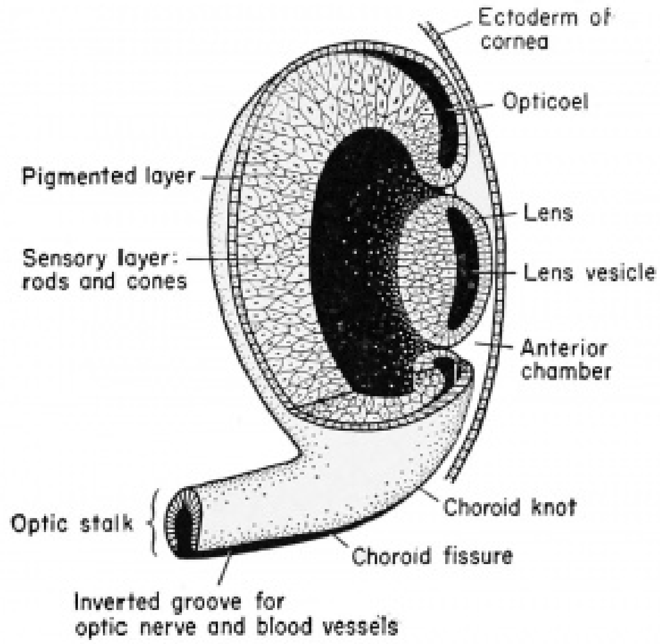
The outer layer of the lens remains unchanged and becomes the **lens epithelium**. The junction between the lens fibres and the lens epithelium represents the growing point of lens. Here the epithelial cells are continuously transformed into lens fibres.

When the lens is formed, the free margin of optic cup touches the edges of the lens and grows in front forming iris. Thus lens hangs in the opening of optic cup. Soon after the development of lens the overlying ectoderm closes over and differentiates to become the **cornea**. It is continuous with the skin. The transformation of the skin into cornea is caused by an induction arising from the optic cup and lens. The ectodermal cells covering cornea form an extremely thin, transparent membrane. This is known as **conjunctiva** of the eye ball. In adult this becomes continuous with the inner lining of upper and lower eyelids. The space between lens vesicle and the overlying presumptive anterior epithelium of cornea represents the anterior chamber. It contains cellular material called anterior **vitreous body**.

The choroid and sclerotic coat of eye develop from the mesenchyme cells accumulating around the eye ball. The interior layer of mesenschyme cells give rise to a net work of blood vessels surrounding the pigmented retinal layer and are called **choroid coat**. The outer layer of mesenchyme form fibrous capsule, the **sclerotic coat** or **sclera** around the eye. The sclera provides protection to eye and the eye muscles.

The ectoderm from above and below the original lens placode region grows out as two folds. These folds grow over conjunctiva and come to touch each other forming a complete layer of ectoderm. At a later stage these folds separate along the line of fusion to form the regular upper and lower eye lids.


Optic vesicle field
Lens field
Optic cup forming
Optic stalk
Lens vesicle
Stage 14
Stage 15
Stage 16
Stage 17
Stage 18
Pigmented layer
Lens
Optic stalk
Optic nerve
Retina
Pigmented layer
Retina
Lens
Optic nerve
Stage 19
Stage 20
Stage 22
Stage 25


Ectoderm of cornea
Opticoel
Pigmented layer
Lens
Sensory layer: rods and cones
Lens vesicle
Anterior chamber
Choroid knot
Optic stalk
Choroid fissure
Inverted groove for optic nerve and blood vessels

EMBRYOLOGY OF CHICK

The fully formed and freshly laid hen's egg is large. It is 3cm. in diameter and 5cm. in length. It contains enarmous amount of yolk. Such egg is called macrolecithal egg. The egg is oval in shape. The ovum contains a nucleus. It is covered by yolk free cytoplasm. It is 3mm. in diameter. It is seen on the animal pole. The entire egg is filled with yolk. This yolk has alternative layers of yellow and white layers. They are arranged concentrically around a flask shaped structure called latebra. Below the blastodisc the neck of latebra expands. This is called nucleus of pander. Yellow yolk got its colour because of carotenoids White yolk layers are thin and yellow yolk layers are thick. Yolk is a liquid. It contains 49% water and 33% phospholipids 18% proteins, vitamins, carbohydrates are present.

The entire ovum is covered by plasma membrane. It is called plasmalemma. It is lipoprotein layer. This is ovum is covered by egg membranes.

Primary membranes: These membranes develop between oocyte and follicle. The primary membranes are secreted by follicle cells. It is called vitelline, membrane is come from two origins. Inner part is produced by ovary. Outer part is from the falopian tube.

Secondary membranes: Oviduct secretes secondary membranes. Above vitelline membrane albumen is present. It is white in colour and it contains water and proteins. The outer layer of albumen is thin. It is called thin albumen. The middle layer of albumen is thick. It is called thick albumen, or dense albumen. The inner most albumen is very thick. It develops into chalazae. The chalazae are called balancers. They keep the ovum in the centre.

Shell membranes: Above the albumen two shell membranes are present. Towards the broad end of egg, in between the shell membranes an air space is present. This air space is formed when egg is laid cooled from 60°C to lesser temperature.

Shell: Above the shell membranes a shell is present, it is porous in nature. It is calcareous. This porous shell is useful for exchange of gases. In a freshly laid hen's egg shell is soft. Very soon it becomes hard.

Laying of the egg: Between 9 A.M. and 3 P.M., the egg is expelled from the cloaca of hen. At the time of laying formation of endoderm is completed. For further development it is to be incubated.

Incubation: When the egg is laid, the development is stopped. For further development it is to be kept at 38°C. This is done by hen by sitting over the egg. This is called incubation. Artificially eggs are incubated in incubators. For the hatching of egg 21 days are required.

In the upper region of oviduct fertilization will takes place. One sperm will penetrate into hens egg and fertilizes with the egg. The fertilized egg will travel through oviduct. It takes nearly 22 hours. Hence the early development of egg will take place in oviduct.

CLEAVAGE

Cleavage is restricted to **blastodisc** and the yolk remains uncleaved. Such cleavage is called **meroblastic or discoidal** cleavage. The central part of blastodisc is whitish and circular. It is surrounded by a darker marginal zone known as the **periblast,** which merges with the underlying white yolk.

I.Cleavage: After five hours of fertilization the first cleavage will appear. It is confined to the centre of blastodisc. It is meridional. lt cannot completely divide the blastodisc. Blastomeres are not formed.

II.Cleavage: It takes place at right angles to first cleavage. Even because of second cleavage clear blastomeres are not formed.

III.Cleavage: It is vertical and parallel to the first division. It is in the two sides of first division. As a result of this division eight blastomeres are formed. But they do not show boundaries.

IV.Cleavage: It takes place in such a way that eight central blastomeres and eight peripheral blastomeres (marginal blastomeres) will form. Only at this stage of division definite cells are formed. The central eight cells are completely separated from yolk. After fourth cleavage the cleavages are irregular and a blastoderm is formed.

In all these cleavages, the furrows do not extend right upto the edge of the disc so that the blastomeres in the central area have distinct boundaries and those of the outer area have no outer boundaries but they merge with the unsegmented syncytial periblast.

The marginal syncytial periblast brings the yolk and the growing mass of cells in the blastoderm into nutritive contact. The central cellular area expands by the addition of cells from the periphery. Later the horizontal divisions take place and the central area becomes two or three cell thick. It gets separated from the underlying yolk by a space, the **subgerminal cavity** (**blastocoel)** which develops either by splitting or separation of the upper layer from the lower layer that retains connection with the yolk mass.

BLASTULA

These cells will undergo further division quickly. Hence above the segmentation cavity mass of cells will be present, in several layers. These cells have complete boundaries. The cells present towards the periphery are not separated from yolk. They are called marginal cells. This region is called **zone of junction**.

Area Pellucida & Area opaca:

The central cell mass of the blastoderm will be in four to five layers, they are lifted from the yolk. Hence the central part of the blastoderm is free from yolk. This region is transparent.

It is called **area pellicida**, which is destined to become the embryo proper. At the zone of junction the cells are in contact with that region is opaca. That region is called **area opaca** which gives rise to extra-embryonic structure.

The area opaca later becomes differentiated into three more or less distinct zones. The blastomeres produced from the original periblast constitute a **germ wall**. Due to the addition of more and more blastomeres on the periphery, an outer ring called **margin of overgrowth** is formed. The blastomeres of this zone have no well defined boundaries. Inner to the germ wall area and lying in close contact with the yolk are a group of cells without complete cell boundaries. This region is known as **zone junction**.

The blastoderm at this stage contains two types of cells-relatively large yolk-laden blastomeres and small yolk-free blastmeres. Now follows a segregation wherein the yolk rich blastomeres gradually accumulate at the under surface of the blastoderm, leaving the smaller yolk poor blstomeres at the surface. The upper layer is called **epiblast** and the lower layer, the **hypoblast**. A narrow cleft called blastocoels appears between th epiblast and hypoblast. The separation of epiblast from the hypoblast is called **delamination**.

Cleavage results in the conversion of the blstodisc into a disc shaped blastula called **discoblastula** which floats a top the yolk mass.

GASTRULATION IN CHICK

Gastrulation begins within four or five hours after the onset of incubation and it is completed by about 22 hours. Gastrulation in chick embryo can be divided into two phases- 1. Endoderm formation and 2. Primitive streak formation and movement of chordamesodermal elements.

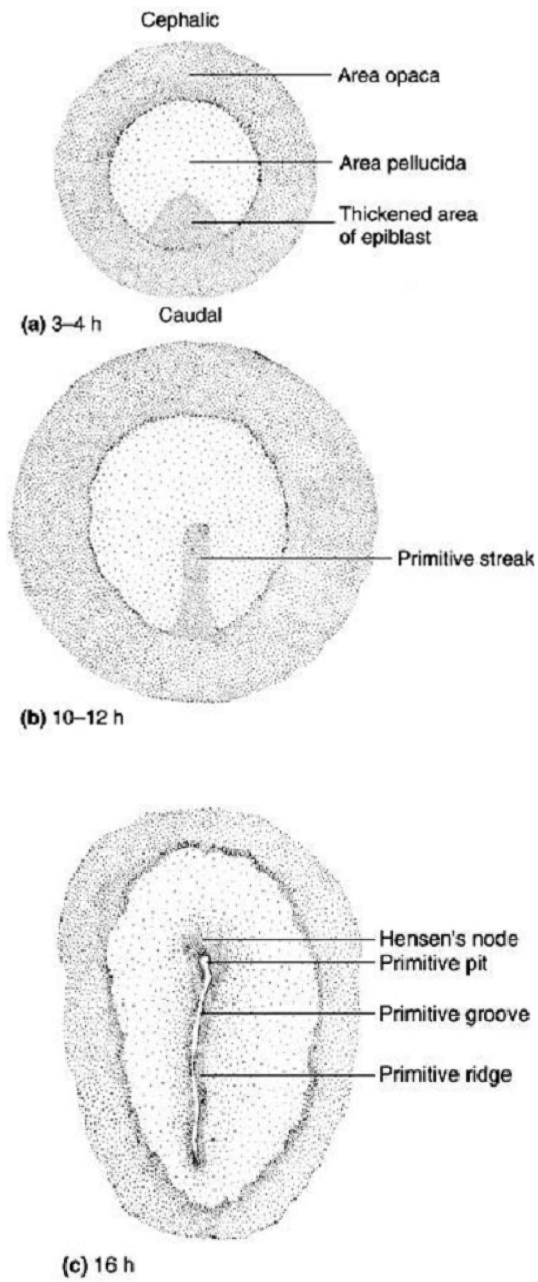
Formation of endoderm: Endoderm of hypoblast develops as a single layer of cells in side of blastocoel. After the formation of endoderm, upper layer is called epiblast. There are different theories to explain the formation of endoderm.

Infiltration theory: This was proposed by Peter in 1923. According to this theory some cells in blastoderm which are loaded with yolk will fall into blastocoel. It starts from posterior end of blastoderm. From there the cells migrate forward one behind another and endoderm is formed.

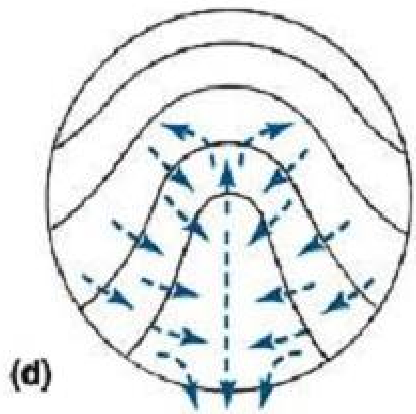
Delamination theory: It was proposed by Spratt in 1946. Blastoderm is two or three layered thick. The lower layer will separate from the upper layer by splitting and the lower layer is called endoderm, upper layers are called ectoderm. In between ectoderm and endoderm blastocoel is present.

Theory of of involution: In 1909 Peterson Proposed this theory. According to this theory a slit like opening at the posterior side of blastoderm forms. Through this opening the blastoderm cells will role into the primary blastocoel. It forms an endoderm.

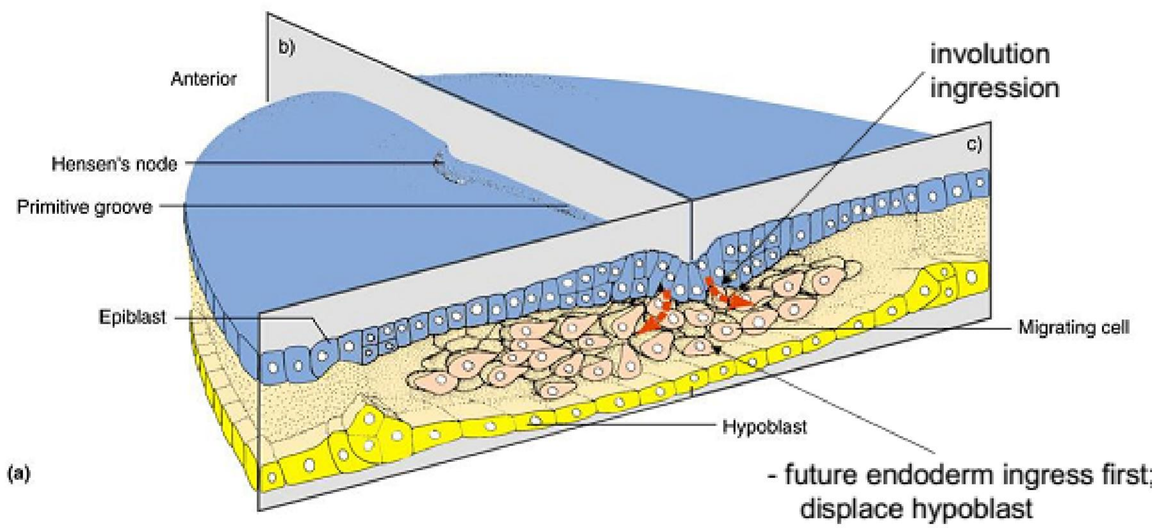
Theory of invaginaton: This was proposed by Jockobson in 1938. According to this theory the posterior end of blastoderm will invaginate in blastocoel as a small pocket. This becomes endoderm. In this way endoderm is formed.

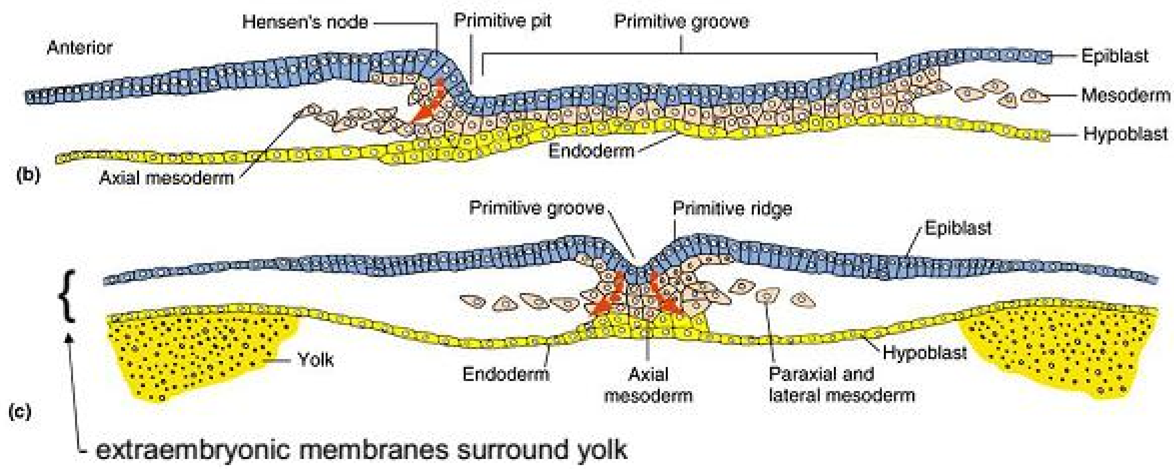


Elongation of primitive streak and formation of primitive groove & primitive pit anterior end of primitive ridges are thickened = Hensen's node



Major gastrulation events occur at the primitive pit & primitive groove functional equivalent of amphibian blastopore





The second step in gastrulation is the **formation of primitive streak**. At the posterior region of area pellucida in the mid dorsal line primitive streak will appear as a thickened area. It starts eight hours after incubation. The thickening is because convergence of cells of blastoderm towards the centre. Usually in the early stages the primitive streak is short and broad. It gradually extends to the middle of blastoderm. At eighteen to nineteen hours of incubation, primitive streak is well developed. It is called definite primitive streak. Along the middle line of primitive streak a narrow furrow is developed called **Primitive groove.** The edges of groove are thick. They are called **primitive folds**. At the anterior end of groove a mass of closely packed cells will be present. It is called "**hensen's node**" or **primitive knob**. In the centre of this node a pit is present. It is called **primitive pit**. It represents the vestige of neurenteric canal. The primitive streak elongates along with this, area pellucida will also elongates. As the primitive streaks growing the cells from this region will involute into space between epiblast and hypoblast This process is called immigration. The immigrated cells will become prechordal plate, notochord, and mesoderm.

The mesoderm is formed as two layers. In front of the primitive streak an area without mesoderm is present. It is called **proamnion.** At this place head will develop. After 48 hours of incubation the proamnion is also occupied by mesoderm. The mesoderm is divided into dorsal and intermediate and lateral mesoderms.

The notochordal cells arrange themselves to form a cylindrical, rod called notochordal process. It will begin at hensen's node and it slowly grows. Because of its growth the primitive streak is slowly reduced. By the end of gastrulation the primitive streak is reduced and incorporate into tail bud.

The dorsal mesoderm is located on either side of notochord. It is divided into segments. They are called **somites**. The first pair of somites will form after 21 hours of incubation.

Afterwards, for every one hour one pair of somite will add. The 24 hours old embryo contains four pairs of somites.

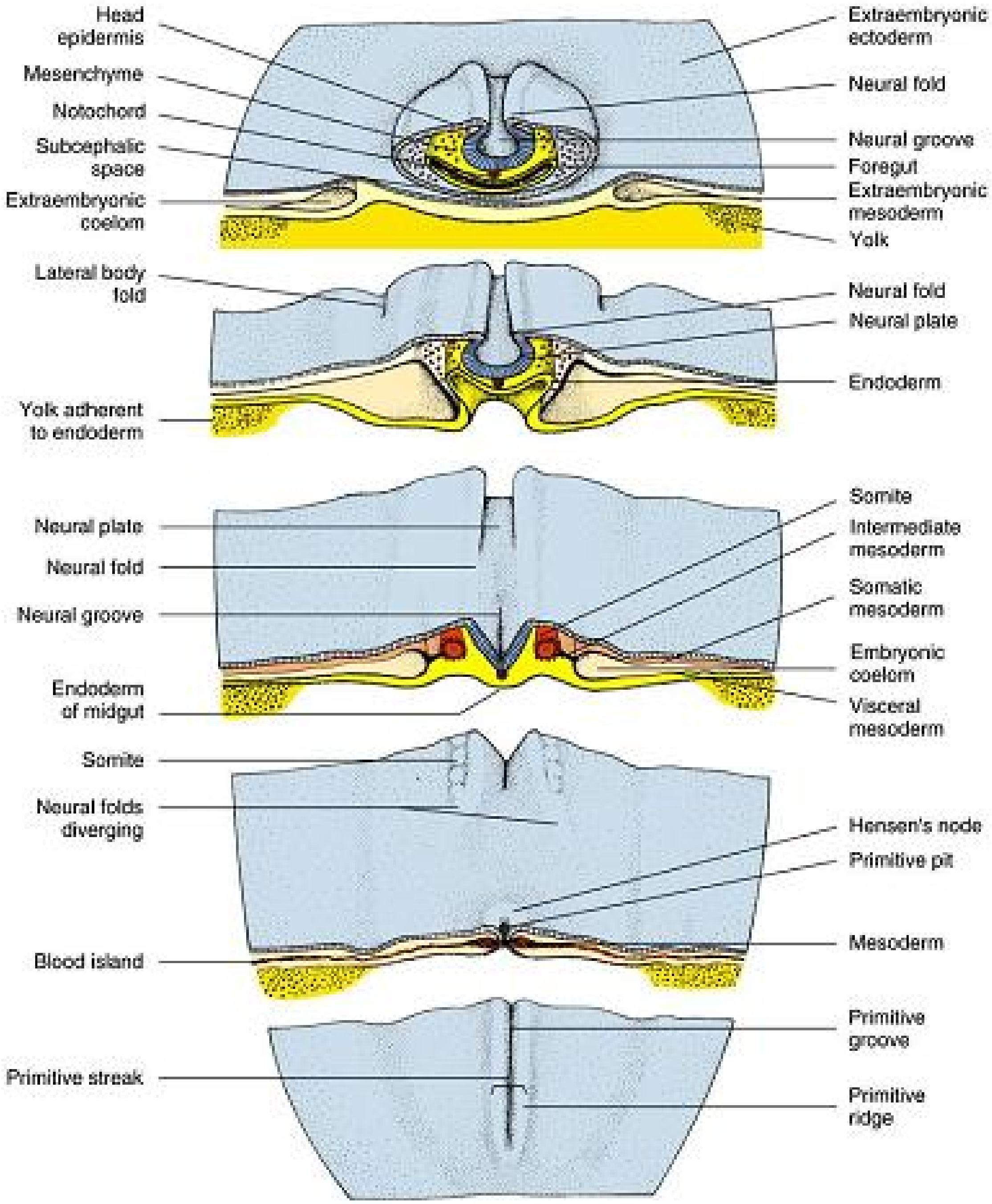
The intermediate mesoderm connects the dorsal mesoderm with lateral mesoderm as a stalk. Afterwards it undergoes segmentation and give kidneys.

The lateral mesoderm extends on periphery of embryo, it is divisible into extra embryonic and embryonic mesoderms. This lateral mesoderm will split into two layers. The upper layer is called **somatic mesoderm** and inner layer is called **splanchnic mesoderm**. Ectoderm and somatic mesoderm will be called **somatopleure**. The splanchnic layer and endoderm will be called **splanchnopleure**. In between the two layers of mesoderm the space is called **coelome**.

Thus at the end of gastrulation specific organ forming areas started to develop.

24 HRS. CHICK EMBRYO

1. At 24 hrs. incubation period the chick embryo is oval in shape.
2. The primitive streak is fully formed and the process of gastrulation is completed
3. The Notochord extends from the from the hensen's node as head process into the mesoderm-free area anteriorly.
4. The head fold and fore-gut develop in the embryo.
5. The mesoderm differentiates into somites, intermediate and lateral plate mesoderm.
6. In the 24 hrs chick embryo four pairs of somites are differentiated from the mesoderm.
7. The coelom begins to develop in the lateral plate mesoderm.
8. The blood islands appear in the area opaca. Pericardial region and primordial of heart are established.
9. The area opaca further modifies into the area vasculosa and area vitellina.
10. The neurectoderm gives rise to the neural folds and neural groove. The fusion of neural folds begins from the mid-region.



Head epidermis
Mesenchyme
Notochord
Subcephalic space
Extraembryonic coelom
Extraembryonic ectoderm
Neural fold
Neural groove
Foregut
Extraembryonic mesoderm
Yolk
Lateral body fold
Yolk adherent to endoderm
Neural fold
Neural plate
Endoderm
Neural plate
Neural fold
Neural groove
Endoderm of midgut
Somite
Intermediate mesoderm
Somatic mesoderm
Embryonic coelom
Visceral mesoderm
Somite
Neural folds diverging
Blood island
Hensen's node
Primitive pit
Mesoderm
Primitive streak
Primitive groove
Primitive ridge

33 HRS CHICK EMBRYO

1. Lengthening of foregut and subcephalic pocket.
2. Formation of neural tube and sinus rhomboidalis.
3. The primary division of encephalon into prosencephalon, mesencephalon and rhombencephalon.
4. Formation of neural crest cells on either side of the neural tube.
5. Development of the infundibulum as a median ventral outgrowth from the floor of prosencephalon.
6. Formation of 13 pairs of somites.
7. Development of heart as tubular structure lying in the midventral region to the foregut.
8. Formation of extraembryonic blood vessels in the area vasculosa.
9. Formation of intraembryonic blood vessels.
10. Disappearance of primitive streak.

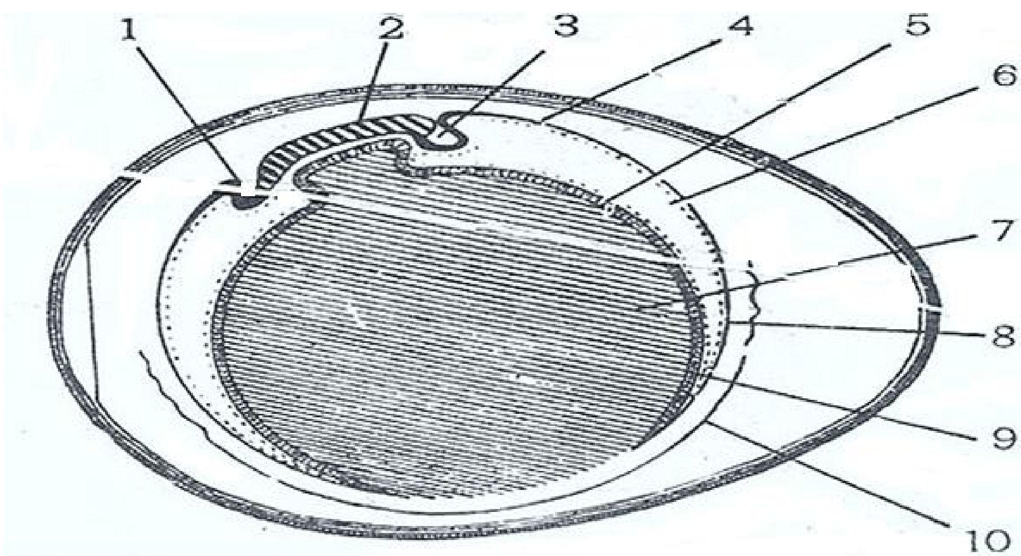
48 HRS. CHICK EMBRYO

1. Appearance of cranial flexure and torsion.
2. Formation of eleven neuromeres, 3 neuromeres in prosencephalon, 2 in mesencephalon and 6 in rhobencephalon
3. Formation of secondary constrictions in the brain.
4. Completion of vitelline (extraembryonic) circulatory system.
5. Development of intra embryonic blood vessels.
6. Formation of two pairs of aortic arches.
7. Twisting of the heart and formationof chambers in it.
8. Commencement of blood circulation.
9. Formation of optic cup and lens vesicle.
10. Formation of visceral cleft.
11. Development of auditory vesicle.
12. Differentiation of 28 somites
13. Development of extra embryonic membranes
14. Formation of pronephros.

FOETAL MEMBRANES OF CHICK

In the development of every vertebrate certain tissues do not directly enter into the formation of the embryo proper. These tisues rather help in the care and maintenance of the embryo. Collectively these parts are refered to as extra-embryonic membranes. In chick a well developed system of extra-embryonic membranes are formed.

In the development of chick these membranes will develop from orginal blastoderm, the central part of blastoderm will give embryo proper, the marginal blastoderm will give extra embryonic membranes. Amnion and chorion will develop from somatopleure, yolk sac and allontois, will develop from splanchnopleure.



1. Tail fold 2. Embryo 3. Head fold 4. Extra embryonic somatopleure 5. Extra embryonic splanchnopleure 6. Extra embryonic coelom 7. Yolk 8. Ectoderm 9. Endoderm 10. Vitelline membrane

Amnion & Chorin:

In the development of embryo amnion and chorion are closely associated, Amnion is bag like covering over the embryo, it separates the embryo from internal environment, Amnion is developed from somatopleuric amniotic folds. These folds are head fold, lateral folds and tail folds.

a) At about 30 hours of incubation, in front of the head of embryo a head fold is developed, it is called amniotic head fold.

b) At about third day of incubation amniotic tail fold is developed. It grows opposite to head fold.
c) Meanwhile lateral folds will develop, they grow dorso-medially.
d) After some time head fold, lateral folds, and tail fold will fuse near posterior end of the embryo.
e) At 72 of incubation they are still not fused. They show an opening called amniotic umblicus, afterwards they unite.
f) The amniotic folds consist of two limbs, an outer and an inner, both consisting of ectoderm and a thin layer of somatic mesoderm. In the outer limb, the ectoderm is external and mesoderm is internal, while the inner limb contains an inner ectoderm and an outer mesoderm. The outer limb constitutes the chorion and the inner limb becomes the amnion.

g) As the amniotic folds grow centripetally, they fuse to form continuous membranes which constitute two sacs around the embryo. At the end of 4th day, the embryo is completely enclosed in a cavity called amniotic cavity bounded by amnion. The space between the amnion and chorion is called the sero-amniotic cavity or exocoel.

Functions of chorion:-

The extra embryonic coelome is filled with a fluid. It gives protection to the developing embryo.
This coelome gives space, for developing allantois.
Chorion combines with allantois and acts as a respiratory organ.

Functions of Amnion:-

Amnion is a sac like structure around embryo. It contains amniotic fluid. It will protect embryo from mechanical shocks and desiccations.
It protects the embryo when the egg is laid. It gives artificial aquatic environment for growth of embryo.

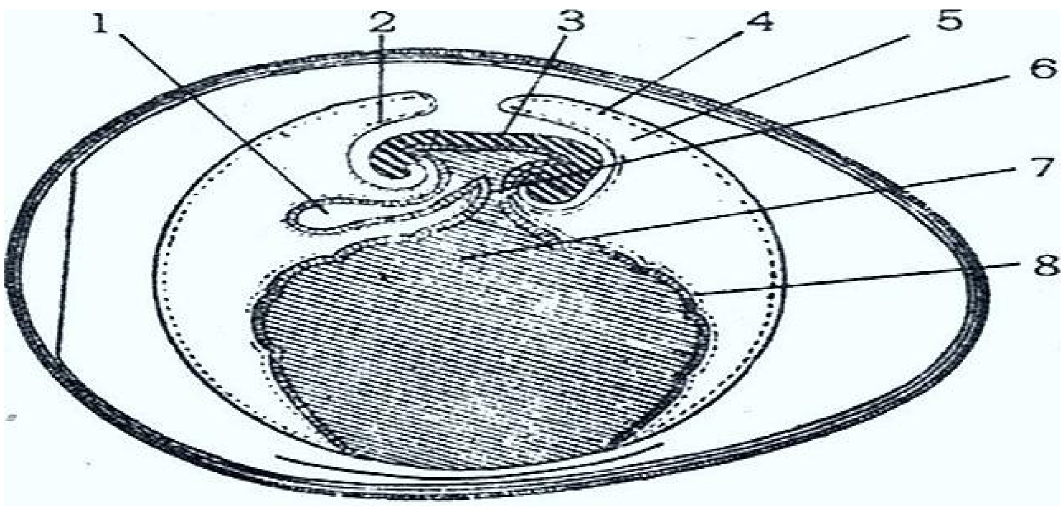
Yolk sac:
At 16 hours of incubation, yolk sac makes its appearance. It develops from splanchanopleure. Splanchanopleure contains endoderm and mesoderm layers. The Splanchanopleure instead of forming a close gut, it will grow over yolk, and becomes yolk sac. The primitive gut is present above the yolk. This yolk region is in contact with midgut. Finally the yolk sac is communicated with midgut through an opening. The yolk sac is connected to the mid-gut by a narrow stalk called yolk stalk or umbilical stalk. The narrow canal within the yolk stalk is termed as the yolk duct.

Functions of Yolk sac:

It digests the yolk, and the digested food will be circulated through blood to the developing embryo. Hence yolk sac is considered as a nutritive organ of the embryo. It also performs respiration during the early stage of development.

Allantois:

In chick it develops from the ventral part of caudal end of the hindgut. It develops at third day of incubation. About the 3^{rd} day of incubation, a region of the floor of the hind gut begins to buldge out as a diverticulum which gives rise to allantois. It consists of an inner endoderm and an outer splanchnic layer of mesoderm. Allantois remains posterior to yolk sac and expands very rapidly penetrating into the extraembryonic coelom. The distal end of allantois enlarges into an allantoic vesicle and the proximal part becomes the allantoic stalk. Unlike amnion and chorion, the allantois arises within the body of the embryo. The mesoderm of the chorion and mesoderm of allantois will unite. It forms chrio-allantoic membrane. As the embryo is growing the allantoic and yolk stalk are brought together. Their mesodermal layers will unite. It is called umblical stalk. It is covered by somatic umblicus.



Early stage in the development of extra-embryonic membrane in chick

1.Allantois 2. Prospective amnion 3. Embryo 4.Amniotic folds 5. Prospective chorion 6. Yolk stalk 7. Yolk 8. Yolk sac

Functions of Allantois :

Allantois is richly vascularised. Hence it works as respiratory organ.
It stores nitrogenous waste material of the embryo.
In later development the allantoic circulation will absorb calcium from the shell. This calcium is used in construction of bones in young ones.
Allantois absorbs calcium from shell. Hence the shell becomes thin. It helps in rupturing the shell during hatching.