# B.Sc. ZOOLOGY LAB MANUAL

6th Semester


Prepared By
**Biological Science Dept.**
Zoology

## MIDNAPORE CITY COLLEGE

## PREFACE TO THE FIRST EDITION

This is the first edition of Lab Manual for UG Zoology 6$^{th}$ Semester. Hope this edition will help you during practical. This edition mainly tried to cover the whole syllabus. Some hard core instrument based topic are not present here that will be guided by responsive teachers at the time of practical.

**ACKNOWLEDGEMENT**

We are really thankful to our students, teachers and non teaching staffs to make this effort little bit complete.

Mainly thanks to Director and Principal Sir to motivate for making this lab manual.

**MJ-13P: Developmental Biology Lab**

**List of Practical**

MJ-13P: Developmental Biology (Practical) Credits 01

Course Outline:
1. Study of whole mounts and sections of developmental stages of the frog through permanent slides: Cleavage stages, blastula, gastrula, tail-bud stage, tadpole (external and internal gill stages).
2. Study of whole mounts of developmental stages of chick through permanent slides: Primitive streak (13 and 18 hours), 21, 24, 28, 33, 36, 48, 72, and 96 hours of incubation (Hamilton and Hamburger stages).
3. Study of the developmental stages and life cycle of Drosophila from stock culture.
4. Study of different types of placenta (photomicrograph/ slides).

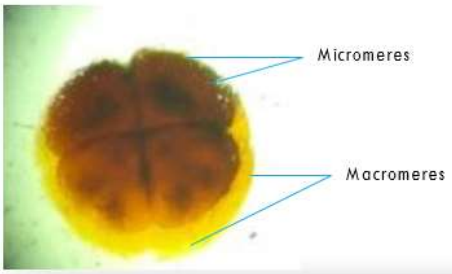
## FROG EMBRYO-THIRD CLEAVAGE (WHOLE MOUNT) 8-CELLED STAGE (ANIMAL POLAR VIEW) POLAR VIEW

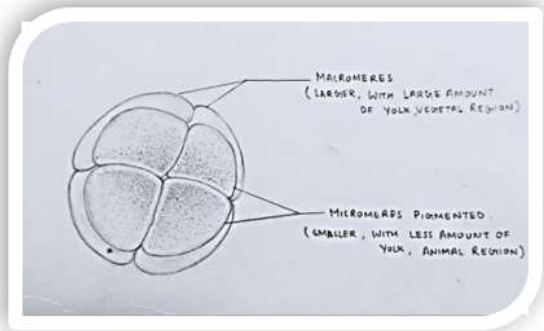
**The polar view shows eight cells formed in two tiers.**

**Upper four cells of animal pole are small called micromeres and appear pigmented.**

**The lower four cells in the vegetal pole are larger with large amount of yolk called as megamers or macromeres.**

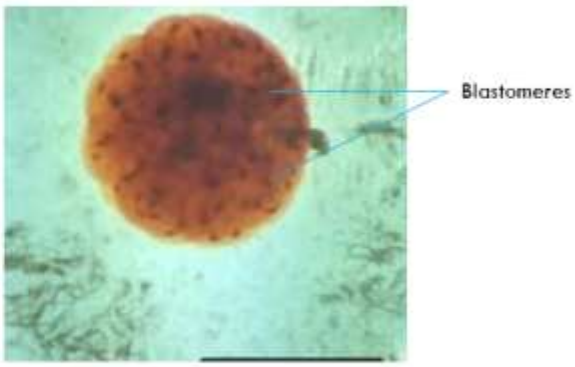
**Third cleavage: This is a latitudinal division from just above the equatorial plane perpendicular to the first two divisions. As a result, eight cells are formed in two tiers.**



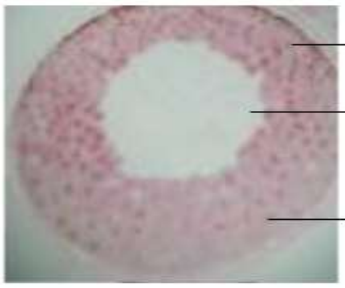

# FROG EMBRYO : MORULA(W.M)

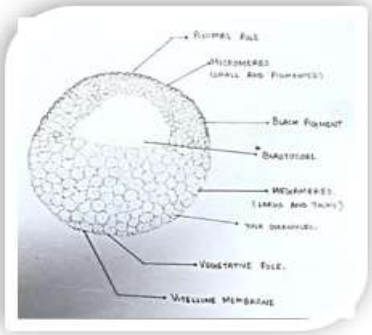
1. The cells formed by cleavage are blastomeres, the upper black blastomeres are called micromeres, and lower white ones are macromeres.
2. Further cleavages divide the micromeres more rapidly than the lower macromeres whose division is hindered by yolk. The blastomeres' mutual pressure flattens their surfaces in contact with each other but free surfaces of each blastomere remain spherical.
3. At this stage the whole embryo acquires a characteristic appearance reminiscent of a mulbery and so it is called morula.



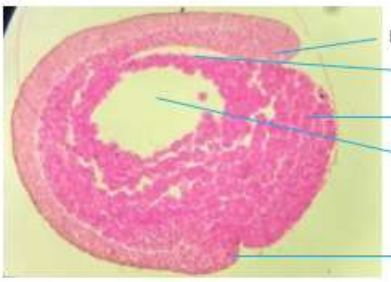
# FROG EMBRYO
# BLASTULA (L.S)

1. **The section of blastula shows a cavity known as blastocoel**
2. **The Blastocoel in blastula is located above the equator, therefore it is called as eccentric in position.**
3. **Blastocoel is surrounded by two types of blastomeres- micromeres in the animal pole and macromeres in the vegetal pole.**

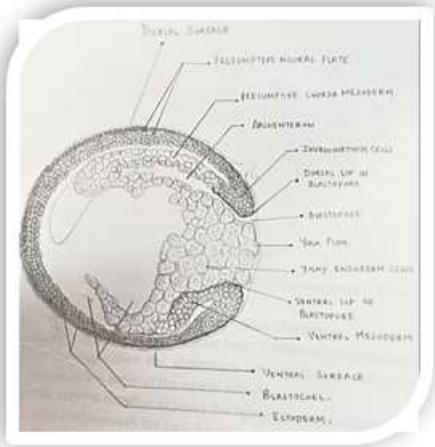


# Frog Embryo -Advanced gastrula/yolk plug stage (L.S)

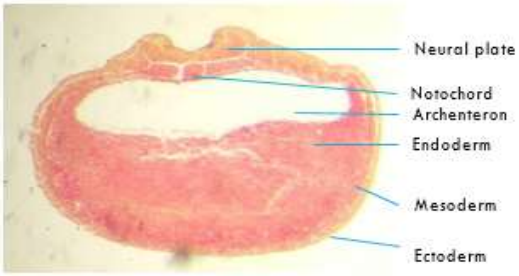


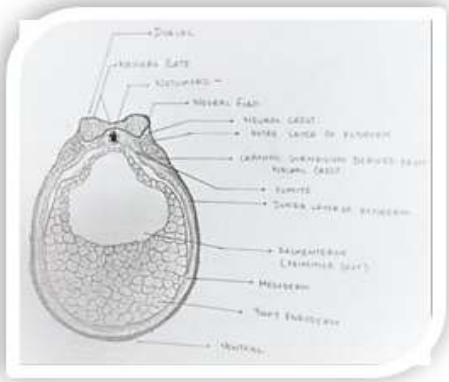

1. Three primary germ layers can be found.
2. Ectoderm is made up of micromeres and surrounds the embryo
3. Endoderm is well developed and internalized
4. Mesoderm is formed from the roof of the archenteron and endoderm, from the
5. floor of the archenteron.
6. Due to enlargement of archenteron, blastocoel is gradually reduced.
7. The yolk laden macromeres are pushed towards to blastopore which forms yolk
8. This stage of the gastrula is called as yolk plug stage of the gastrula.



# FROG EMBRYO-EARLY NEURAL NEURAL PLATE STAGE (T.S)

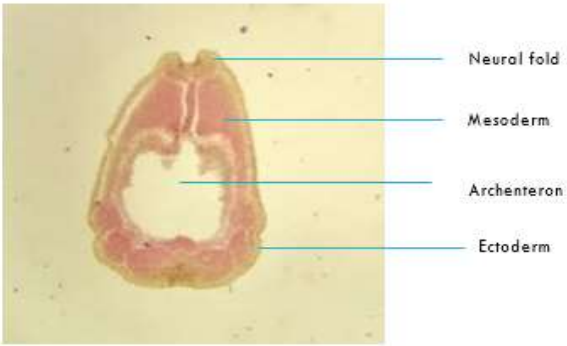
1. During late gastrulation, external changes along the upper surface of the embryo begin to form the neural tube—a process called neurulation.
2. An oval-shaped area on the dorsal side of the embryo marks the presumptive neural tube. This region is the neural plate.
3. During this process the ectoderm along the mid-dorsal line becomes thick and flattens forming plate-like structure known as neural plate (medullar plate).
4. Microfilaments in neural plate cells flatten and thicken the neural plate.

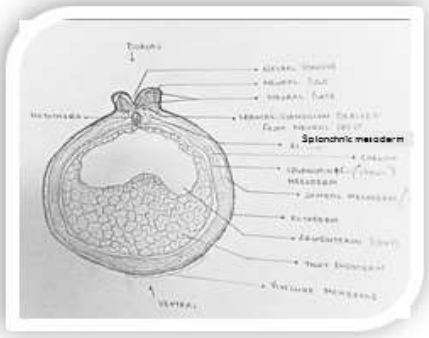


# FROG EMBRYO-MID NEURULA NEURAL FOLD STAGE (T.S)

1. **The edges of the neural plate roll up and over the midline of the neural plate.**
2. **U-shaped neural groove appears in cross section in the centre of the plate, dividing future right and left sides of the embryo**



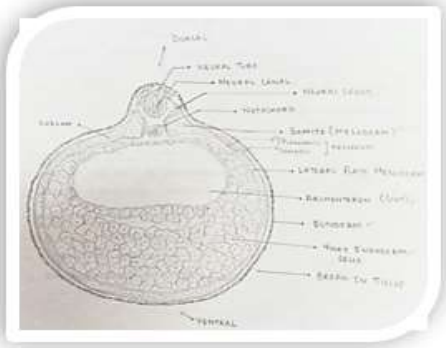
# FROG EMBRYO-ADVANCED NEURULA NEURAL TUBE STAGE(T.S)

The neural folds approach each other towards the midline of the embryo, eventually fusing to form the neural tube beneath the overlying epithelium

A distinct notochord is found in the mid line (axis) immediately below the neural tube

Paraxial mesoderm on both sides of notochord organises into blocks called somites

Downward growth of mesoderm continues

# EXTERNAL GILL-STAGE OF TADPOLE (W.M)

**Tadpole at this stage is about 5.5 mm long and the following structures are visible in it:**

1. Five pairs of branchial, pharyngeal or visceral pouches are formed in the gill-plate area due to out pushing of the endodermal lining of pharynx.

2. Soon from the sides of head in the pharyngeal region three pairs of external gills are projected out, which are feathery extensions of the integument above the gill-slits. There are bathed by the surrounding water.

3. The mouth is surrounded by fringed lips and also acquires a pair of horny jaws. The fringed lips has two rows of tiny, needle-like horny teeth. A 10.5 mm long tadpole contains 3 rows of fringed teeth.

4. Adhesive suckers disappear.

5. The larval gut is differentiated into pharynx, oesophagus, stomach and intestine. Liver and gall bladder also develop. The intestine is very long and coiled like a watch spring due to herbivorous mode of feeding.

6. Myotomes extend up to the tip of tail.

7. Melanophores appear in the skin of dorso-lateral surface of head, trunk and tail.

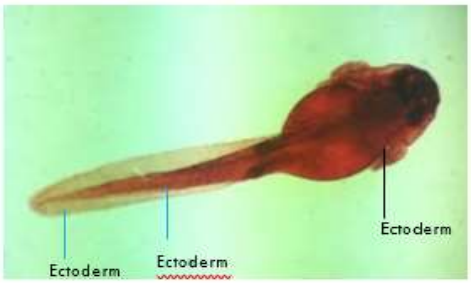
8. The cornea becomes transparent and eye lens is visible.

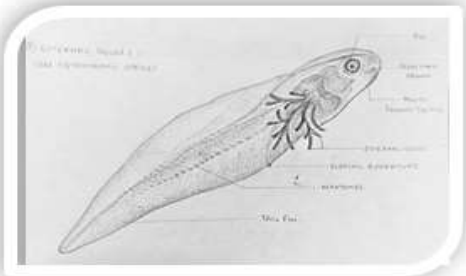
9. Lateral line sensory system is visible on either lateral side of the tail.

11. A pair of pronephric kidneys becomes functional and excretes ammonia.

12. On either side of cloacal aperture, at the junction of trunk and tail, a pair of hindlimb buds appears.

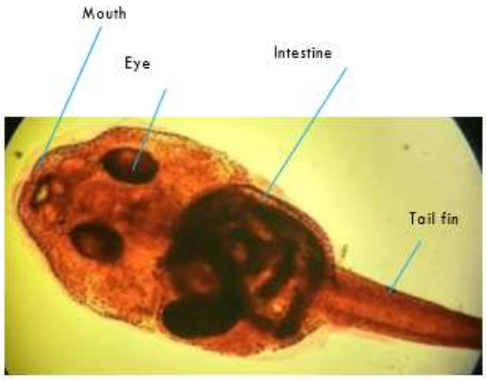
The tadpole swims actively with the help of tail and feeds on algal and other aquatic vegetation.

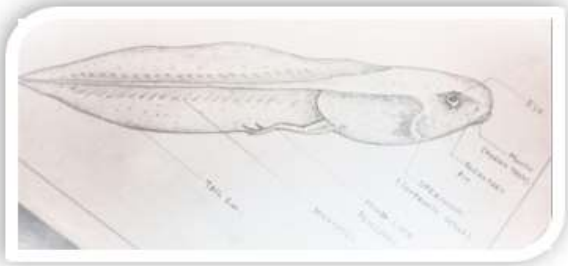


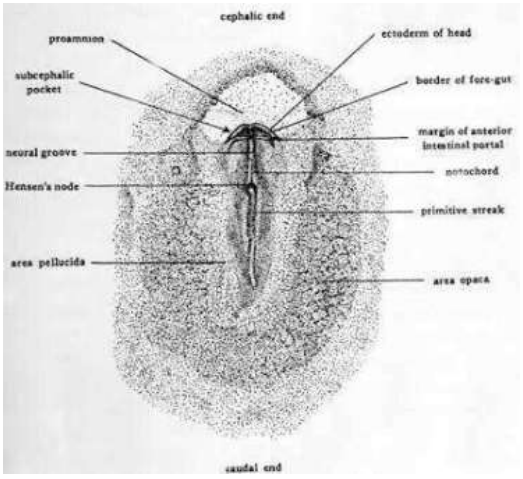
# INTERNAL GILL-STAGE OF TADPOLE:

1. The opercular folds grow backward from the hyoid arch of each side covering the external gills and gill-slits and finally fuse with each other ventrally and with the belly wall. Thus, an operculum or gill-cover is formed enclosing the external gills
2. External gills later fall off and four pairs of filamentous internal gills develop on the walls of gill-slits.
3. Intestine is still coiled and long.
4. Different parts of hindlimbs such as thigh, shank, ankle, foot and five toes become well formed in the tadpole of 40 mm long.
5. A pair of forelimb buds appear behind the head but remain hidden within operculum. As development proceeds, the left forelimb emerges through the spiracle. The right forelimb appears later.
6. In a mature tadpole, a pair of lungs develop from the pharynx. Now the larva breathes by both, the internal gills and lungs.



**Study of whole mounts of developmental stages of chick through permanent slides: Primitive streak (13 and 18 hours), 21, 24, 28, 33, 36, 48, 72, and 96 hours of incubation (Hamilton and Hamburger stages).**




i. 13 hours after incubation the primitive streak becomes so distinct that embryos are characterized as being in primitive streak stage

ii. In fixed and stained slide, w.m. is composed of central furrow, called as primitive groove lined by thickened primitive ridges.

iii. At the cephalic end of the primitive streak, closely-packed cells form thickened area, called as Hensen's node. Part of area pellucida adjacent to the primitive streak shows increased thickness and forms embryonic elliptical shape.

iv. Area pellucida assumes elliptical shape.

v. Elongated primitive streak represents long axis of future embryonic body.

**Whole Mount of 18 Hours Chick Embryo:**

1. It is a W.M. of 18 hours stage of chick embryo.
2. At this stage the dark peripheral area opaca and central translucent area pellucida are distinctly visible.
3. In the anterior part is present the pro-amnion, which is a small and comparatively more translucent region of area pellucida and is characterised by the absence of mesoderm.
4. In the middle of area pellucida, in the posterior half, runs a primitive streak having a primitive groove through its centre. The primitive groove is being bound by primitive folds.
5. In the anterior half of area pellucida, in the middle, runs a neural groove bound by neural folds.
6. The primitive streak and neural groove is separated by a thickening-the Hensen's node having a small depression in the centre-the Hensen's pit.
7. The primitive streak gives rise to an out-growth, the notochord immediately below the primitive groove.

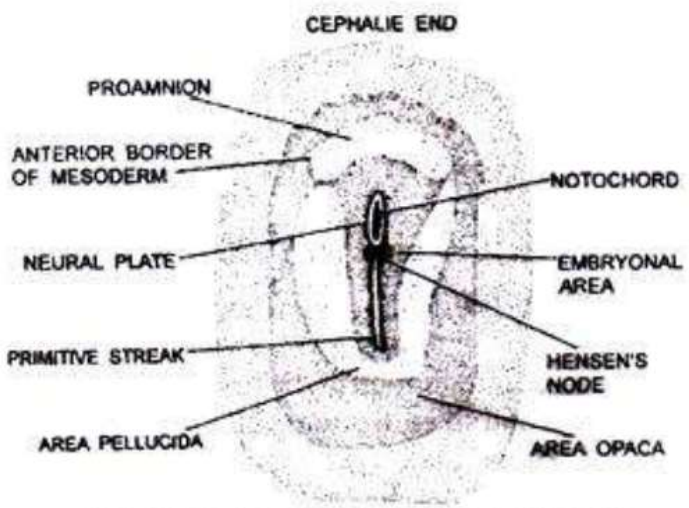



**Fig. 17.3. W.M. of 18 Hr embryo of chick**

**Whole Mount of 21 Hours Chick Embryo:**

1. It is a W.M. of 21 hours chick embryo.

2. At this stage the dark peripheral area opaca and central translucent and colourless area pellucida are distinctly visible.

3. In the anterior part are present the pro-anmnion, which is a small and comparatively more translucent region of area pellucida and is characterised by the absence of mesoderm.

4. In the middle of area pellucida, in the posterior half, runs a primitive streak having a primitive groove through its centre. The primitive groove is being bound by primitive folds.

5. In the anterior half of area pellucida, in the middle, runs a neural groove bound by neural folds.

6. The primitive streak and neural groove are separated by a thickening, the Hensen's nod having a small depression in the centre of the Hensen's pit.

7. The primitive streak gives rise to a small outgrowth, the notochord immediately below the primitive groove and to mesoderm on either side.

8. At this stage embryonic and extra ambryonic regions have also become distinguished in the area pellucida.

9. In the anterior most part the ectoderm has given rise to head fold, which is a pocket-like extension of neural folds.

10. With the ectoderm the underlying endoderm is also transformed into a pocket-like structure the -foregut.

11. The proambion is comparatively reduced in size.

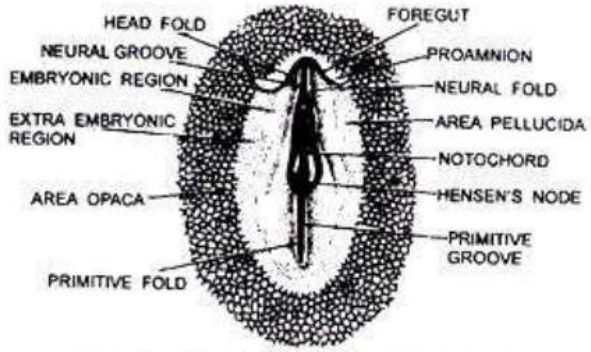



**Fig. 17.4. W.M. of 21 hour chick embryo.**

**Whole Mount of 24 Hours or 4 Pairs of Somites Stage of Chick Embryo:**

1. It is a W.M. of 24 hours 4 pairs of somites stage of chick embryo.

2. At this stage the dark peripheral area opaca and central translucent and colourless area pellucida are distinctly visible.

3. In the anterior part is present the proamnion, which is a small and comparatively more translucent region of area pellucida and is characterised by the absence of mesoderm.

4. In the middle of area pellucida, in its posterior half runs a primitive streak with a primitive groove in its centre. The primitive groove is bound by primitive folds.

5. In the anterior half of area pellucida, in the middle, runs the neural groove bound by neural folds.

6. The primitive streak and neural groove are separated by Hensen's node having a small depression in the centre-the Hensen's pit.

7. Immediately below the primitive groove the primitive streak gives rise to a small out-growth, the notochord and on either side to mesoderm.

8. In the area pellucida embryonic and extra embryonic regions also become distinguished.

9. In the anterior- most part the ectoderm has given rise to head fold, which is a pocket-like extension of neural folds. The underlying endoderm is also transformed into a pocket-like foregut. The proamnion is greatly reduced.

10. In front of Hensen's node the mesoderm of embryonic area differentiated into 3-4 pairs of mesodermal somites.

11. The neural canal, in the region of head fold, gives rise to forebrain.

12. The foregut extends on either side into an amino-cardiac vesicle.

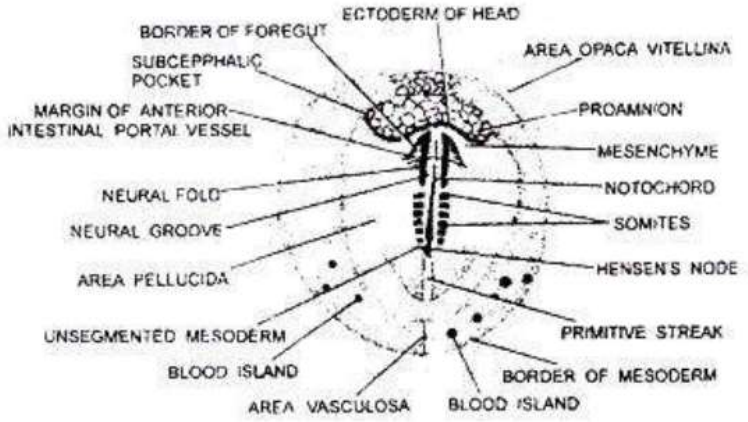



**Fig. 17.5. W.M. 24 hours chick embryo.**

**Whole Mount of 30 Hours of 8-10 Pairs of Somites Chick Embryo:**

1. It is W.M. of 30 hours of chick embryo or 8-10 pairs of somite stage of chick embryo.

2. At this stage the dark peripheral area opaca and central translucent and clourless area pellucida are distinctly visible.

3. In the anterior part is present the proamnion, which is a small and comparatively more translucent region of area pellucida and is characterised by the absence of mesoderm.

4. In the middle of area pellucida, in the posterior half, runs a primitive streak with a primitive groove running through its centre. The primitive groove is bound by primitive folds.

5. In the anterior half of area pellucida, in the middle, runs the neural groove bound by neural folds.

6. The primitive streak and neural groove are separated by Hensen's node having a small Hensen's pit in the centre.

7. Immediately below primitive groove the primitive streak gives rise to the notochord and on either side to mesoderm.

8. At this stage embryonic and extra embryonic regions have also become distinguished in the area pellucida.

9. In the anterior-most part, the ectoderm has given rise to head fold which is a pocket like extention of neural folds. The underlying endoderm has transformed into pocket like foregut. The proamnion is reduced.

10. The mesoderm, in front of Hensen's node, has given rise to 8-10 pairs of somites.

11. In the region of head fold the anterior part of neural canal has given rise to a distinct fore brain.

12. The foregut and cardiac vesicles are sufficiently developed.

13. The extra embryonic area has grown in size.

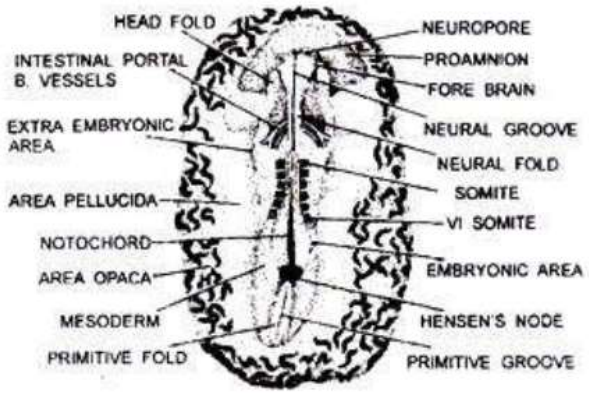



**Fig. 17.6. W.M. of 30 hr chick embryo (8-10 somies)**

**Whole Mount of 33 Hour Chick Embryo of 11-12 Pairs Somites:**

1. It is whole mount of 33 hours chick embryo.
2. At this stage the dark peripheral area opaca and central translucent area pellucida are not distinctly visible.
3. The primitive streak has been comparatively reduced because of great lengthening of neural canal and neural folds.
4. The extra embryonic area has grown in size.
5. The mesoderm, in front of Hensen's node, has given rise to 11-12 pairs of somites.
6. The foregut and cardiac vesicles are sufficiently developed.
7. The brain is differentiated into fore brain, mid- brain and hind brain.
8. The area opaca has changed into area vasculosa.
9. Proamnion has disappeared.
10. Anterior omphalomesenteric vein has developed.

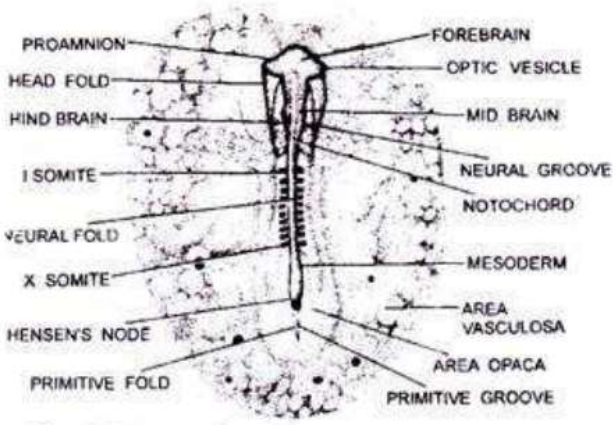



**Fig. 17.7. WM. of 33 hr (11-somies) chick embryo.**

**Whole Mount of Chick Embryo of 13-14 Pairs Somites or 36 Hours:**

1. It is Whole Mount of 36 hours chick embryo.
2. At this stage the dark peripheral area opaca and central translucent and colourless area pellucida are not visible.
3. The extra embryonic area has grown in size.
4. The primitive streak is comparatively reduced because of great lengthening of neural canal and neural folds. The notochord has extended from behind the brain up to the end of body.
5. The mesoderm, in front of Hensen's node, has given rise to 13-14 pairs of somites.
6. The brain is differentiated into fore brain, mid brain and hind brain.
7. In the fore brain region optic vesicles and in the hind brain region optic vesicles have developed.
8. The area opaca has changed into area vasculosa.
9. Proamnion has disappeared.
10. Anterior omphalomesentric vein and vitelline artery have developed.
11. The cardiac vesicle has given rise to heart.

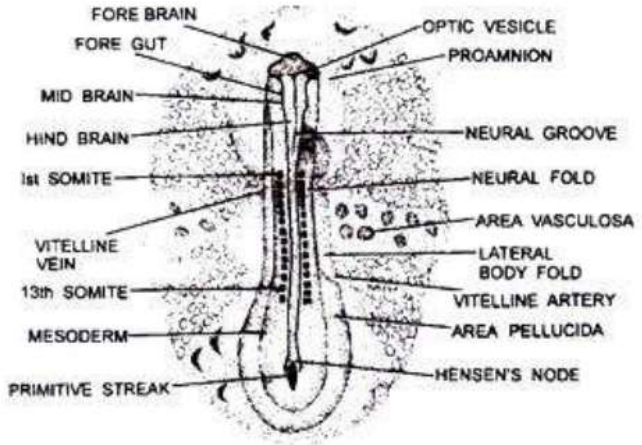



**Fig. 17.8. W.M. of 36 hr (14 somits) chick embryo**

**Whole Mount of of 48 Hours Chick Embryo of 26-28 Pairs of Somites:**

1. It is W.M. of 48 hours chick embryo.
2. At this stage the area opaca and area pellucida are not visible.
3. The extra embryonic area has grown in size.
4. Primitive streak has disappeared.
5. The mesoderm, in front of Hensen's node, has given rise to 26-28 pairs of somites.
6. The brain has differentiated into telencephalon, prosencephalon, mesencephalon, metancephalon and mylencephalon.
7. The heart has been differentiated into ventricle and atrium. Sinus venosus and truncusarteriosus have also started developing.
8. The eye has been differentiated into optic cup and lens and optic vesicle has also developed sufficiently.
9. The head region has curved on right side due to cranial flexion.

10. Three pharyngeal gill-slits have also been differentiated.

11. Behind Hensen' node a tail bud has also developed.

12. Lateral amniotic folds, anterior omphalomesentric vein and vitelline artery have appeared.

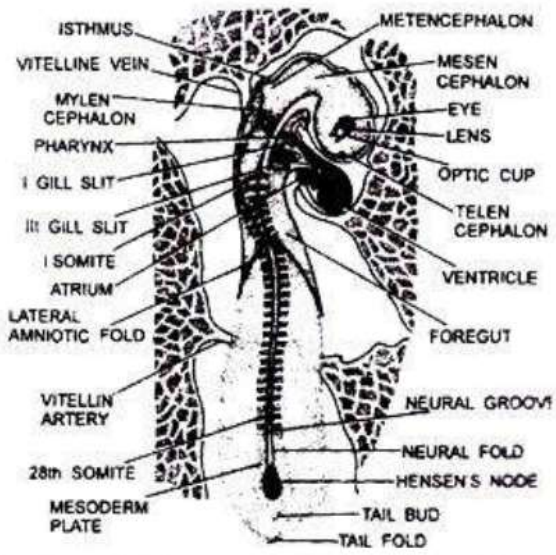



**Fig. 17.9. W.M. of 48 hr (27 pairs somites) chick embryo.**

**Whole Mount of 72 Hours or 36 Pairs of Somites Stage of Chick Embryo:**

1. It is W.M. of 72 hours chick embryo.

2. At this stage area opaca and area pellucida are not visible.

3. The extra embryonic area has grown in size.

4. Primitive streak has disappeared.

5. The mesoderm, in front of Hensen's node, has given rise to 36 pairs of somites.

6. The brain has differentiated into telencephalon, mesencephalon, metancephalon and mylencephalon.

7. The heart has been differentiated into ventricle and atrium.

8. The eye has differentiated into optic cup and lens and optic vesicle has also developed sufficiently.

9. The head region has bent on right side due to cranial flexion.

10. Four pairs of gill-slits have been differentiated.

11. Tail bud is greatly developed and has given rise to allentoic stalk and tail.

12. Lateral amniotic folds, vitelline artery and anterior omphalomesentric vein have developed.

13. In the middle region a pair of fore limb buds and in front of tail a pair of hind limb buds have developed, which will give rise to fore and hind limbs.

14. Olfactory pit, visceral arches, amnion, allantois and amniotic cavity have also developed.

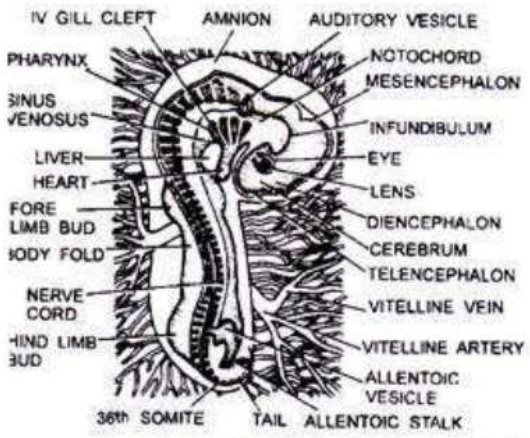



**Fig. 17.10. W.M. of 72 hr (36 pairs somites) chick embryo**

**Whole Mount of 96 Hours Chick Embryo:**

1. In the chick embryo of 96-hours of incubation, the entire body has been turned through 90 degree and the embryo lies with its left side on the yolk.
2. At the end of 96 hours the body folds have undercut the embryo so that it remains attached to the yolk only by a slender stalk.
3. The yolk salk soon become enclogated, allowing the embryo to become first straight in the mid-dorsal region and then convex dorsally.
4. The progressive increase in the cranial, cervical, dorsal and caudal flexures results in the bending of the embryo on itself so that its originally straight long axis becomes C-shaped and its head and tail lie close together.
5. Optic cup shows the more developed lens.
6. Endo-lymphatic duct arises from the auditory vesicle.
7. Visceral arches have become very much thickened.
8. Appendage buds increase rapidly in size and become elongated.
9. The number of somites increases to 41 pairs.
10. Allantois has also appeared.
11. Omphalomesenteric artery and omphalomesenteric vein are also developed.

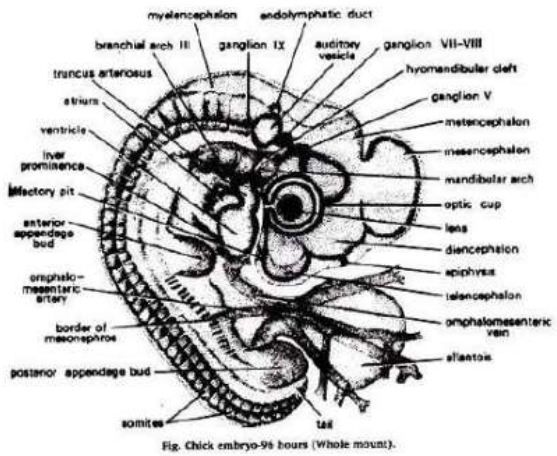
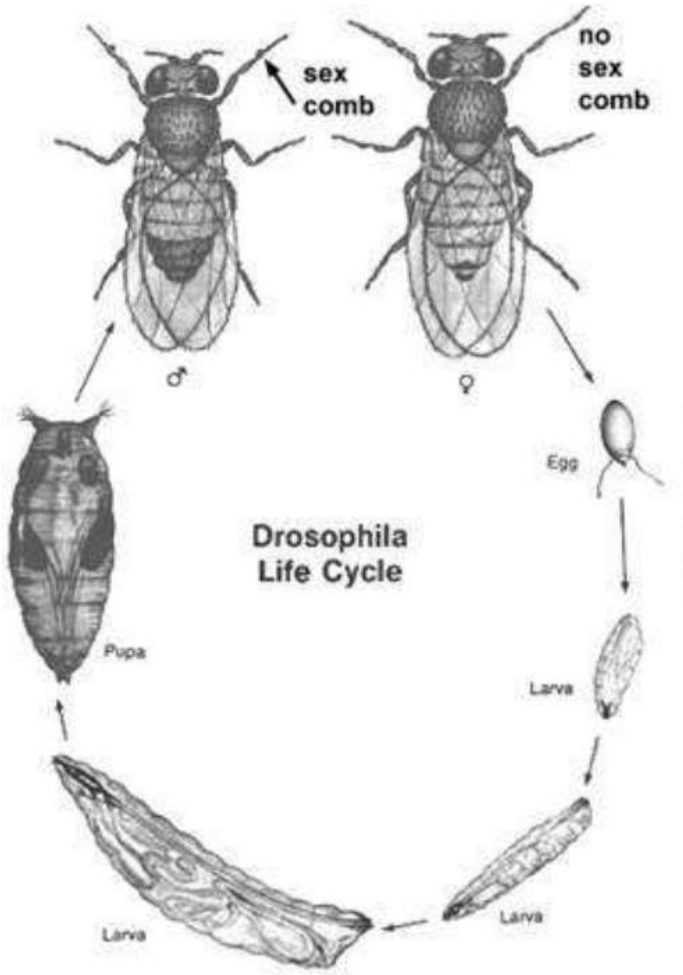



Fig. Chick embryo-96 hours (Whole mount).

**Study of the developmental stages and life cycle of Drosophila from stock culture**

## Life cycle of Drosophila




Fruit flies are holometabolous insects; that is, they undergo complete metamorphosis during their life cycle. The life cycle consists of four distinct stages: egg, larva, pupa, and adult. The rate of development is dependent on temperature, being more rapid at higher temperatures. For instance, at 20$^{o}$C, the life cycle is completed in 14 or 15 days, but at 25$^{o}$C, the cycle lasts about 10 days.

**Mating and Eggs:** Mated females store sperm to fertilize eggs that are subsequently laid. Therefore, to ensure that the desired cross is achieved, it is necessary to place females that are virgins with their intended male mates. Female flies are unable to mate for several hours

after they have eclosed as adults from their pupal cases. Therefore, virgin females can be obtained by clearing all of the flies from a vial and collecting all newly-eclosed females several hours later. These virgin females can be kept separated from males for several days until needed for crosses.

**Oviposition** by the female starts as early as the second day after its emergence from its pupal case. It increases for about a week until a female adult may be laying 50-75 eggs per day for a total of approximately 400-500 eggs in 10 days. The egg is ovoid, covered outside with a thin but strong envelope (chorion) from which project anteriorly two thin stalks whose terminal portions are each flattened into a spoon-like float. The latter serve as "water-wings" to prevent the egg from sinking and drowning in a semiliquid medium. At the anterior end of the egg is a minute pore (micropile) through which the spermatozoa enter the egg as it passes down the oviduct into the uterus. Although many sperm may enter the egg as it passes down the oviduct, only one fertilizes the female pronucleus and the others are soon absorbed in the developing embryonic tissue.

**Larva:** The larva is a white, segmented, worm-shaped burrower with black mouth parts (jaw hooks) in the narrower head region. For tracheal breathing it has a pair of spiracles (air intakes) at both the anterior and posterior ends. Since insect skin will not stretch, the young small larvae must periodically shed their skins (cuticle) in order to reach adult size. There are two such molts in *Drosophila* larval development that are accompanied by shedding of the mouth parts as well as the skins. During each period between molts, the larva is called an instar, i.e. the first instar is between hatching and the first molt. Both the size of the larva and the number of teeth on the dark colored jaw hooks are an indication of which instar the larva has reached. After the second molt, the larva (now third instar) feed until ready to pupate. At this stage, the larva crawls out of the food medium onto a relatively dry place, ceases moving, and everts its anterior breathing spiracles.

**Pupa:** Soon after everting its anterior spiracles, the larval body shortens and the cuticle becomes hardened and pigmented. A headless and wingless prepupa forms. This stage is followed by the formation of the pupa with everted head, wing pads, and legs. The puparium (outer case of the pupa) thus utilizes the cuticle of the third larval instar. The adult structures that seem to appear first during the pupal period have actually been present as small areas of dormant tissues as far back as the embryonic stage. These localized preadult tissues are called **anlagen** (or **imaginal discs**) and because of the ease in which they can be isolated

have often been used in studies of developmental genetics. The main function of the pupa is to permit development of the anlagen to adult proportions. The breakdown of larval tissues to furnish material and energy for this development is therefore a prime feature of pupal metabolism.

**Adult:** Adults exhibit a typical insect anatomy, including compound eyes, three-part bodies (head, thorax, and abdomen), wings, and six jointed legs. The various types of bristles and hairs found on the body are characters that we will use to identify different phenotypes of flies.

## Study of different sections of placenta (photomicropgraph/ slides)

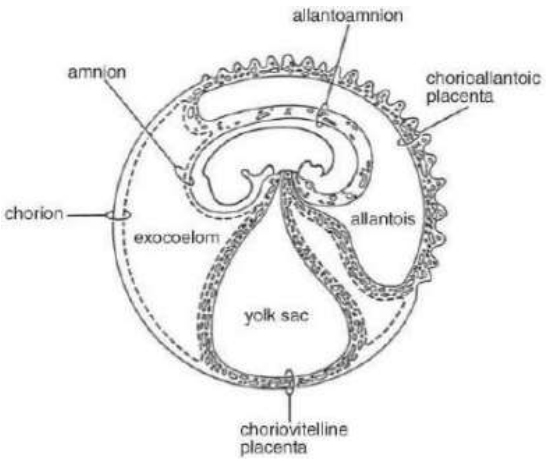
**Depending on different criteria placenta may be divided in following types –**

1. **Depending on the involvement of embryonic tissue:**

   a. **Chorio-vitelline placenta** –( yolksac placenta)

   In this placenta chorion of the embryo, vitelline circulation of yolk sac and epitheliumof the uterus together form an association known as Chorio-vitelline placenta.

   Ex – *Didelphys* sp. *Macropus* sp.



**b. Chorio-allantoic placenta** –

The placenta in which chorion and allantois of the embryo and endometrium of the uterus together form a complex organ and through allantoic circulation nutrition and other essential elements from the maternal blood pass on to embryonic circulation is known as chorio-allantoic placenta.

Ex – All the utherine mammals.

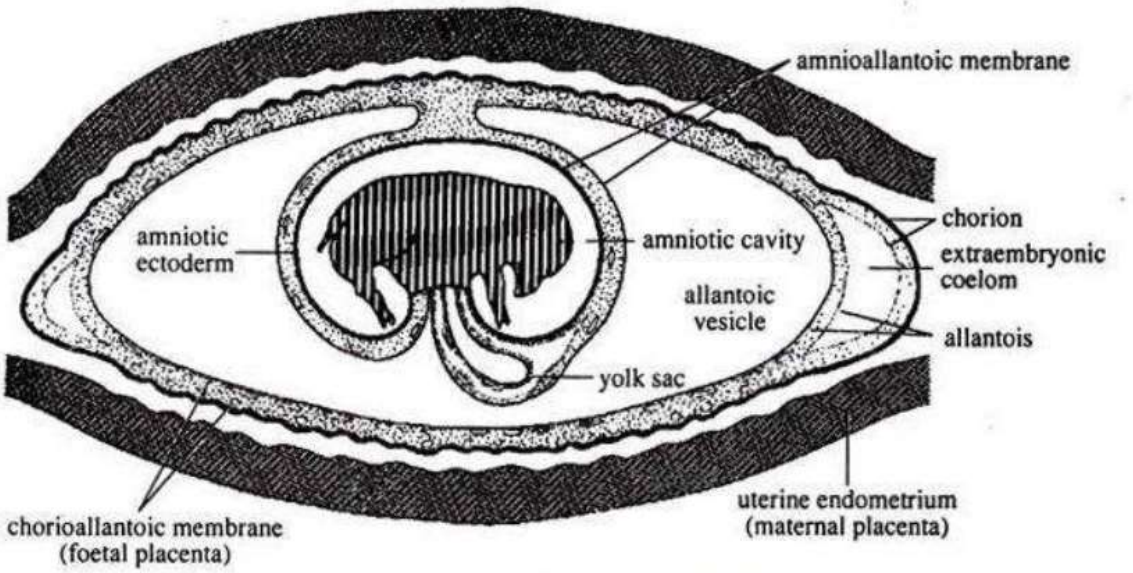



Fig. 5.51 : The mature extraembryonic membranes of the foetal pig, showing composition of foetal placenta (chorioallantoic membrane) and relation to endometrium of uterus.

## 2. Depending on the distribution of villi on chorion :

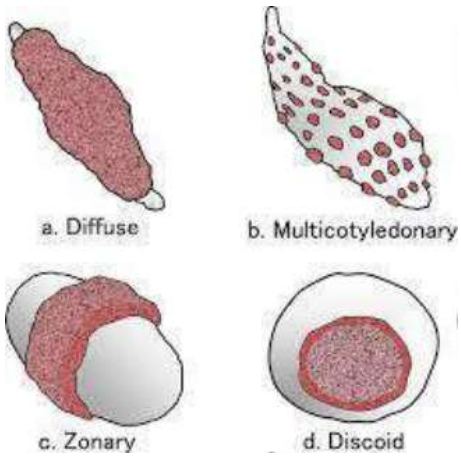
**a. Diffuse placenta:** The villi are numerous and distributed uniformly over the whole surface of the chorion with a diffused appearance. Such placenta are calleddiffuse placenta. Ex – Ungulates ( Pig, Horse etc)

**b. Cotyledonary placenta**: In this placenta chorionic villi are found in groups or patches, while the rest of the surface of chorion remains smooth. The patches of villi appear as cotyledon. So the name cotyledonary placenta has been given.Ex –Ruminant ( Cow, Camel)

**c. Zonary placenta** : In this placenta chorionic villi are found in a particular zone like a band in a semicircular fashion. As the villi are found in definite zone, sothe name has been given. Ex – Carnivores ( Cats, Dogs)

**d. Discoidal placenta**: In this placenta the villi are restricted to a circular disc or plate on the dorsal surface of blastocyst. Ex – Insectivores, bat & rodents.

**e. Metadiscoidal placenta**: Primates have a special type of discoidal placenta in which villi are at first scattered but later on become restricted to one or two discs.




## 2. Based on the degree of involvement of foetal and maternal tissues:

**Epithelio-chorial placenta**: This is a loose association of chorionic villi of the foetus with the epithelium of the uterus. The epithelium of the uterus folds to form pockets and within these pocket chorionic villi rests. So there are six barriers in between maternal and foetal blood. Ex – Pig, Horse, Marsupials etc.

**Syndesmochorial placenta** : The uterine epithelium disappears & the chorion of the foetus comes in direct contact with the connective tissue of the uterine wall of the mother. So there are five tissue barriers in between maternal and foetal blood. Ex. Sheeps, Giraffe, Deers etc.

**Endothelio-chorial placenta**: In this type of placenta the chorion of the foetus comes in direct contact with the endothelium of the uterine capillaries. Because both the epithelium and the connective tissue of uterine disappear, there are four tissue layers in between the maternal & foetal blood. Ex – Dog, Cat, Fox etc.

**Haemo-chorial placenta** : In this type of placenta endothelium of uterine blood vessels is lost so the chorionic epithelium is bathed directly in maternal blood. There are only three barriers of tissue layers. Actually the chorionic villi are surrounded by spaces (sinuses) devoid of endothelial lining, into which maternal blood enters through the arteries of the uterus and from which blood flows into the uterine vein. Ex –Primates including Man, Insectivores (moles, shrews) and Chiropterans (bats).

**Haemo-endothelial placenta** –In this type of placenta chorionic epithelium and connective tissue of foetus are lost as a result the endothelium of the foetal blood vessel come direct in contact with maternal blood and be the only barrier between foetal and maternal blood. Ex – Rodents (mouse, rat, guinea pig, rabbit).