Observations:
Part (A).
Gosner series of developmental stages of the toad,
*Bufo melanostictus* (Schn.),
External features of the embryos and larvae of the toad, 

*Bufo melanostictus* (Schn.) in Gosner stages of its development

Normal room temperature, in which development of the toad proceeded, was 24.5°C. Age of earlier stages is given in minutes and hours, and that of later stages is given in days. Normal size of the embryos or larvae of a stage is given in mm. Size of the embryos those are in cleavage and gastrula stages is expressed by their diameter. Size of the embryos of later stages and of larvae is expressed by their antero-posterior length. (TL means total length). Numbers given to the stages in text as well as in figures, directly refer to the serial number of Gosner developmental stages.

**Stage (1+2);** Uncleaved egg: 00 hrs; Diameter 1.3 mm. About 20 mins. later, after the eggs are laid, the polar pit indicating the expulsion of the second polar body, was observed.

**Stage (3);** Two cells: 2 hrs; Diameter 1.3 mm. The first cleavage furrow, which is vertical, appears in the animal pole, 1.20 hrs after the formation of polar pit. In about 10 mins it completely covers the animal hemisphere. In next 10 mins it completely divides the egg into two cells.

**Stage (4);** Four cells: 2.25 hrs; Diameter 1.3 mm. The second cleavage furrow is also vertical and is at right angles to the first. In 10 mins
after the completion of the previous stage it covers about one third of the egg diameter, and in next 15 mins four cells are formed.

**Stage (5): Eight cells; 2.35 hrs; Diameter 1.3 mm.** The third cleavage furrow is horizontal and originates above the equatorial plane of the egg, dividing the egg into four smaller dark brown micromeres in the animal hemisphere, and four bigger brownish white macromeres in the vegetal hemisphere.

**Stage (6): Sixteen cells; 3.35 hrs; Diameter 1.3 mm.** About 25 mins later, after the previous stage, the four micromeres become divided into eight cells by two synchronous vertical cleavage furrows at right angles to each other. The furrows extended into the macromeres in the next 35 mins dividing the macromeres into eight cells.

**Stage (7): Thirty two cells; 3.55 hrs; Diameter 1.3 mm.** Within 10 mins after the previous stage, eight cells of the upper tier divide into sixteen cells by horizontal cleavage furrow. In next 10 mins of this, horizontal cleavage furrow divides the lower tier cells into sixteen cells. Simultaneously the sixteen cells in the upper tier start to divide irregularly and more speedily than the cells of the lower tier.

**Stage (8); Morula; 4.35 hrs; Diameter 1.3 mm.** The difference between size of the micromeres and macromeres is not conspicuous.
Stage (8): Early blastula; 5.35 hrs; Diameter 1.3 mm. Micromeres are small enough to be easily seen, and macromeres are large.

Stage (9): Mid blastula; 8.35 hrs; Diameter 1.4 mm. Micromeres are very small, but can be easily recognized as dark brown spots surrounded by pale brown boundaries. The whole surface of the animal pole appears smooth (Fig. 9. as viewed from the vegetal pole).

Stage (9⁺): Late blastula just before appearance of dorsal blastoporic lip; 10.35 hrs; Diameter 1.4 mm. Macromeres are also greatly reduced in their size, especially towards marginal zone. Still micromeres can be recognized.

Stage (10⁻): Gastrula with appearance of dorsal blastoporic lip; 11.35 hrs; Diameter 1.4 mm. The area (arrow mark in figure) where the dorsal blastoporic slit forms, is clearly visible by concentration of dark pigment and that pigmented area is slightly concave.

Stage (10): Gastrula with crescent-shaped blastoporic lip; 13.35 hrs; Diameter 1.4 mm. The dark brown pigment of the animal hemisphere covers more than half of the egg’s surface. The dorsal blastoporic lip is clearly seen and is crescent-shaped.
Stage (10\textsuperscript{+}): Gastrula with horse-shoe shaped blastoporic lip; \textbf{15.35 hrs}: Diameter \textbf{1.4 mm}. The dorsal blastoporic lip extends on the lateral sides, and the lip looks like a horse-shoe. The surface of the egg (except yolk plug area) is getting more smoothened. Individual cells are no longer conspicuous. At this time the area where the ventral blastoporic lip is to be formed is clearly marked by dense pigmentation.

Stage (11): Gastrula with large yolk plug; \textbf{16.35 hrs}: Diameter \textbf{1.4 mm}. A circular ring of blastoporic lip is formed by starting of the involution of the cells of the ventral blastoporic lip area.

Stage (12\textsuperscript{-}): Gastrula with middle yolk plug; \textbf{17.35 hrs}: Diameter \textbf{1.4 mm}. The diameter of the yolk plug is reduced by its inward movement.

Stage (12): Gastrula with small yolk plug; \textbf{19.35 hrs}: Diameter \textbf{1.4 mm}. The diameter of the yolk plug is still reduced and the egg surface looks perfectly smooth.

Stage (13): Neural plate; \textbf{24.35 hrs}: TL \textbf{1.4 mm}. About 3.30 hrs. later, after the previous stage the formation of neural plate is started on dorsal side, as a slightly flattened area adjacent to the small yolk plug, which is still visible from outside. After 1 hr. of this, the whole neural plate area was clearly marked by dark pigmentation (arrow mark in figure) all along the inner and outer borders of the neural ridge, which
is slightly elevated from the egg’s surface. Now the gastrula appears to
be very slightly elongated in the antero-posterior direction. Still, the
whole neural plate area can be recognized by its pale colouration
although it is not tabular. Now, the yolk plug has disappeared leaving
a vertical blastoporic slit. Within next 30 mins the neural plate
becomes more conspicuous with more flattening of the area and
appearance of a median line of dark pigmentation (arrow mark in the
figure) where the neural groove is to be formed. From now onwards the
blastopore marks the posterior end of the embryo.

**Stage (14): Neural fold formation; 26.35 hrs; TL 1.5 mm.** The neural
folds become more and more elevated with more elongation of the
embryo. Now the neural plate is key-hole shaped, being slightly
depressed towards the neural groove. The anlage of oral sucker
(adhesive organ)(arrow mark in the figure) has appeared as darkly
pigmented curved band at the antero-ventral side of the embryo.
Blastopore is still visible as a small pore.

**Stage (15): Approach of neural folds; 28.35 hrs; TL 1.5 mm.** By
further deepening of the neural groove and elevation of neural folds,
the neural folds of both sides approach each other. They are closer in
trunk region than in head region.

**Stage (16): Formation of neural tube; 30.35 hrs; TL 1.8 mm.** 1 hr. after
the previous stage, both neural folds touch each other at the same time,
leaving a little gap at the anterior and posterior ends. In next 1 hr. those gaps also disappear and complete neural tube is formed. At this time the jelly coverings are almost dissolved and the embryo covered only by chorion becomes free. Gill plate is faintly seen.

**Stage (16):** Advanced neural tube; **32.35 hrs; TL 2 mm.** Both neural folds are completely fused, but both can be demarcated by a shallow groove (arrow mark in the figure). Pronepheric and gill plate bulgings (arrow mark in the figure) are more conspicuous. About three somites can be recognized. Stomodeal cleft is started to appear as an area slightly depressed in vertical direction, between the sides of adhesive organ. By this time, the head portion can be recognized.

**Stage (17):** Early tail bud; **43 hrs; TL - 2.6 mm.** Four hours later after the previous stage, he hypophysis can be seen, as a transverse, darkly pigmented bar above the stomodeal cleft. The anlages of olfactory pits (arrow mark in the figure) are visible at the both ends of this hypophysis, as darkly pigmented round spots. The gill plates (arrow mark in the figure) are very prominent, and visceral arches can be easily recognized. Optic bulge is faintly visible. Hatching takes place. The embryo responds to strong mechanical stimulus, by lateral bending of the body. The olfactory pit analge (arrow mark in the figure) is seen but still the pit is not formed. The tail bud is grown slightly beyond the trunk region.
Stage (18): Late tail bud; 51 hrs; TL 3.1 mm. The tail bud length equal or smaller than one third of snout-vent length. Optic and pronepheric bulgings are clearly visible. Eighteen to twenty somites are seen. The embryos show more muscular response to mechanical stimulus. The olfactory analges have developed slight concavities.

Stage (19+20); 59 hrs; TL 3.8 mm. Tail length is equal or smaller than half of the snout-vent length. Gill buds (arrow mark in the figure) start branching. Olfactory pits are formed. Stomodeal cleft is deep. Some larvae show fast lateral bending of the body towards the tail side. When the larvae were put into the fixative all show such type of lateral undulations of the body. Now the larvae show a strong tendency to adhere to horizontal surfaces. Hypophysis has disappeared from the body surface. Olfactory pits are well developed.

Stage (20+): 63 hrs; TL 4 mm. Tail length is equal or larger than half of the body length. Gill ramification is advanced.

Stage (21); 78 hrs; TL 5 mm. The gills become elongated. The tail length is equal to body length. About 6 hrs. after the previous stage the gill circulation is seen. About 8 to 9 hrs. after the previous stage the larvae show a tendency to attach to vertical walls of the container, becoming completely separated by the loosened jelly mass to which they were attached for sometime after hatching. Now the eye lens and
auditory vesicles are clearly visible. Opening of the mouth takes place. Mouth is of rhomboid shape, wider than high. Oral sucker splits into two lateral oval shaped, grooved bulgings.

**Stage (22+23): 97 hrs; TL 5.3 mm.** The tail length is larger than body length. Digestive track is slightly bent in “S” shape. Four hours back, before this stage, concentration of black pigment in the dorsal region of the eye was started. Now the pigment fully covers the dorsal region of the eye. The opercular folds (arrow mark in the figure) have started to develop, just covering the base of the gill stems.

**Stage (24): 124 hrs; TL 6.5 mm.** The right gills are completely covered by opercular fold, the left are not. The eye lens is completely surrounded by black pigment, except little gap in its ventral side. About 16 hrs. later after the previous stage, the labial teeth start to appear, when the opercular fold has covered half of the right gills. By this stage, inner tooth rows are well developed, and intestine is seen in three coils. Hind limb anlage (arrow mark in the figure) can be recognized as slightly whitish round area. Adhesive organs have started to reduce. Cornea becomes transparent. Faecal matter appears in the container indicating the starting of the feeding.

**Stage (25): 151 hrs; TL 6.6 mm.** Left gill is completely covered by the opercular fold to form the spiracle (arrow mark in the figure). The outer tooth rows are well developed. The hind limb bud becomes more
conspicuous. Oral suckers have left very little markings. Vent tube is tapered.

**Stage (26); 7 days; TL 7.1 mm.** The hind limb bud length is smaller than half of its basal width. The vent tube becomes tubular with a wide opening. Visceral organs are clearly seen through the transparent ventral side of the larvae.

**Stage (27); 10 days; TL 7.5 mm.** The hind limb bud length is larger or equal to half of its basal width, but smaller than at stage 28.

**Stage (28); 12 days; TL 10 mm.** The hind limb bud length is larger or equal to its basal width, with conic shape, but smaller than at stage 29.

**Stage (29); 18 days; TL 11.5 mm.** The hind limb bud length is larger or equal to one and half of its basal width, but smaller than at stage 30.

**Stage (30); 22 days; TL 15 mm.** The hind limb bud length is equal to double of its basal width. The lateral line sense organs are clearly visible on the dorsal side of the body, although they had started to appear at stage 24.

**Stage (31); 24 days; TL 16 mm.** The distal end of the hind limb bud is oar-shaped, no indentations visible.
Stage (32); 26 days: TL 16.5 mm. The indentation (arrow mark in the figure) between fourth and fifth toes appears.

Stage (33); 28 days: TL 17 mm. The indentation (arrow mark in the figure) between third and fourth toes appears.

Stage (33+); 29 days; TL 16 mm. The thigh region is more distinct.

Stage (34+35); 33 days; TL 16 mm. The indentations between the third and second toes, and second and first toes appear.

Stage (36); 35 days; TL 17.5 mm. The primordia of second, third, fourth and fifth toes are clearly recognized. Thigh, shank and sole can be distinguished in the hind limb.

Stage (37); 37 days; TL 19 mm. The primordia of five toes are definitely separated. The ventro-lateral margins of the trunk portion start to become opaque.

Stage (38); 38 days; TL 20 mm. The metatarsal tubercle appears.

Stage (39); 39 days; TL 20 mm. The subarticular tubercles appear.
**Stage (40): 40 days; TL 22 mm.** The vent-tube (arrow mark in the figure) is present.

**Stage (41): 41 days; TL 22 mm.** The vent-tube is lost. The ventral side of the larva has become completely opaque. The palm of the fore limb can be slightly seen through the transparent gill chamber on the ventral side. Teeth on the lower lip get lost and upper ones are disorganised by clumping together. The labial extensions are much reduced.

**Stage (42): 43 days; TL 19 mm.** The fore limbs emerge outside by rupturing the skin of gill chamber. The labial teeth are completely lost, but the horny beak (arrow mark in the figure) on the lower jaw is still present. The angle of the mouth does not reach beyond the level of the nostrils. The nictitating membrane is formed.

**Stage (43): 45 days; TL 13 mm.** The tail is diminishing and the tail fin is greatly reduced. The horny beak is completely lost and the angles of the mouth reach beyond nostrils to the level of the anterior end of the eyeball. Remodelling of the head begins.

**Stage (44): 46 days; TL 10 mm.** The body length is larger than tail length. The angles of the mouth reach the level of the center of the eye.
Stage (45): 47 days; TL 7.5 mm. The shape of the tail is stub-like. The angles of the mouth reach the level of the posterior margin of the eyeball.

Stage (46): 48 days; TL 8 mm. The tail has almost disappeared. Metamorphosis is completed.
Explanation to the figures in plate - 1

Embryos and larvae of the toad *Bufo melanostictus* (schn.), of stages 3 to 20 in series of Gosner stages of development. Numbers given to the figures directly refer to the number of the stage in Gosner series.

Stage 13. Figures drawn from lateral and dorsal sides of the embryo.
Stage 14 and 15. Figures drawn from lateral, dorsal and anterior sides of the embryo.
Stage 16 and 16+ Figures drawn from lateral, dorsal and ventral sides of the embryo.
Stage 17 to 20. Figures drawn from lateral side of the embryo.

Regions marked by the arrow are referred in the text.
Explanation to the figures in plate - 2

Larvae of the toad *Bufo melanostictus* (Schn.) of stages 21 to 33 in series of Gosner stages of development. Numbers given to the figures directly refer to the number of stage, in Gosner series. All figures drawn from lateral sides of the larvae.

*Regions marked by the arrow are referred in the text.*
Explanation to the figures in plate - 3

Larvae of the toad *Bufo melanostictus* (Schn.) of stages 33+ to 46 in series of Gosner stages of development. Numbers given to the figures directly refer to the number of stage, in Gosner series. All figures drawn from lateral sides of the larvae.
Explanation to the figures in plate - 4

Embryos and larvae of the toad *Bufo melanostictus* (Schn.) of stages 17 to 29 in series of Gosner stages of development. Numbers given to the figures directly refer to the number of stage, in Gosner series. All figures drawn from ventral sides of the embryos and larvae.

Regions marked by the arrow are referred in the text.
Explanation to the figures in plate - 5

Larvae of the toad *Bufo melanostictus* (Schn.) of stages 30 to 41 in series of Gosner stages of development. Numbers given to the figures directly refer to the number of stage, in Gosner series. All figures drawn from ventral sides of the larvae.

*Regions marked by the arrow are referred in the text.*
Explanation to the figures in plate - 6

Larvae of the toad *Bufo melanostictus* (Schn.) of stages 42 to 46 in series of Gosner stages of development. Numbers given to the figures directly refer to the number of stage, in Gosner series. All figures drawn from ventral sides of the larvae.

Regions marked by the arrow are referred in the text.
Explanation to the figures in plate - 7

Embryos and larvae of the toad *Bufo melanostictus* (Schn.) of stages 17 to 29 in series of Gosner stages of development. Numbers given to the figures directly refer to the number of stage, in Gosner series. All figures drawn from dorsal sides of the embryos and larvae.
Explanation to the figures in plate - 8

Larvae of the toad *Bufo melanostictus* (Schn.) of stages 30 to 41 in series of Gosner stages of development. Numbers given to the figures directly refer to the number of stage, in Gosner series. All figures drawn from dorsal sides of the larvae.
Explanation to the figures in plate - 9

Larvae of the toad *Bufo melanostictus* (Schn.) of stages 42 to 46 in series of Gosner stages of development. Numbers given to the figures directly refer to the number of stage, in Gosner series. All figures drawn from dorsal sides of the larvae.
Observations:
Part (B).
External and internal features of the embryos of the toad, *Bufo melanostictus* Schn.), from two-cell stage to advanced neural tube stage.
Two-cell stage; (stage 3); embryo diameter, 1.3mm.; age, 2hrs.;

External features

1. Polar pit is visible as a concave surface. About 20 mins. later, after the eggs are laid, the concave surface of the pit of the polar body is observed. At the time of the appearance of the first cleavage furrow, the surface of the animal hemisphere is dark-brown in color, and that of vegetal hemisphere is brownish white. The dark-brown pigment covers about 1/3\textsuperscript{rd} of the total egg surface, in the form of a dome on the animal hemisphere. The intensity of the brownish tint of the vegetal hemisphere gradually decreases towards the vegetal pole. In between animal and vegetal hemispheres, no marginal zone or the region of gray-crescent can be so clearly recognized, as a different region, in its coloration.

2. The portion of gray crescent becomes clearly recognized at the end of two-cell stage. The first cleavage furrow, which is vertical, appears in the animal pole, at about 1.40 hrs. later, after the eggs are laid. In next 10 mins. the first cleavage furrow is extended beyond the area of the marginal zone, but not reached the vegetal pole.

At about 2 hrs. later, after the eggs are laid, two blastomeres those are completely divided from each other, are visible. At this
stage, on one side of the egg, the region of chorda-mesoderm, in the portion of gray-crescent, can be distinguished easily, as a dorso-ventrally widened region, with pale brown color. This broad end of the gray crescent is raised up from its narrower end on other side, to an angle of about 45°.

The first cleavage is holoblastic and passes through the mid-line of the gray-crescent.

**Internal features**

1. **Two blastomeres are not completely separated.** Although the two blastomeres are visible externally, to be completely divided from each other, they are partly separated internally. At the animal pole the cleavage furrow is extended inside the egg only up to 1/10th of the egg diameter, at equatorial region up to 1/20th of the egg diameter, and only up to egg cortex level, at the vegetal pole region.

2. **At this stage itself the cleavage cavity or the anlage of blastocoel is appeared.** It is at about the centre of the egg. In the plane, at right angles to the division plane, and passing along the AV axis, the cleavage cavity is visible as a narrow cavity spread along the AV axis. It measures about 1/10th of the egg radius, in its length, along the AV axis. Along the plane of division it is spread laterally, in a distance of about 20% of the egg diameter.
3. **Two blastomeres are completely separated from each other in the animal hemisphere by formation of a thin partition wall in between, but not by complete inward penetration of the cleavage furrow.** The separation of the two blastomeres, in animal hemisphere, is by collection of cortex granules all along the plane of formation of the partition walls of the daughter blastomeres. In 8μ thick sections, at 10 x 40 magnification, it can be recognized that this region consists of two separate walls for the two blastomeres. This is very clear at the ends, i.e., inner and outer margins of this partition plane. These two thin layers are continuous with the thin pigment lining of the cleavage cavity. Also, pigmentation is more denser along the margins of the division plane.

4. **Simultaneous beginning of formation of partition walls, from the surface of the cleavage cavity, outwards, towards the outer surface of the egg, and vice versa, is visible in vegetal hemisphere.** Indication of beginning of the growth of the partition wall, in vegetal hemisphere, along the first division plane, can be recognized by dense collection of dark-brown pigment on the outer surface of the cleavage cavity and on the inner surface of cleavage furrow.

Thus, two blastomeres in vegetal hemisphere, are separated from each other for about 10% (on average) of the egg diameter, in outer side of the egg. Separation from inside to outside of the egg is not progressed much; beginning is indicated by dense collection of pigment
granules on the surface of the cleavage cavity. On the outer side of the egg, separation is maximum at the equatorial plane. It gradually decreases towards the vegetal pole.

5. Sperm entry point, penetration path and copulation path are still visible. A wider, darkly colored region is spread along the partition wall in the animal hemisphere. It starts from the outer surface of the animal hemisphere in one lateral side of the animal hemisphere, but more nearer to animal pole, and is extended inwards. The lateral side of the egg in which it originates is opposite to the side that has wide portion of gray-crescent. It originates from a point that is just along the first cleavage plane, and remains in the same plane, as it is extended inwards. It extends inwards up to a level, nearer to the centre of the animal hemisphere. As it originates from the surface of the egg, it is wider. Gradually, it narrows and disappears, as it is extended inwards. As it starts to extend inwards, it is extended in downward direction, for some distance. But, again at its inner end, it is bent in upwards direction.
Four-cell stage; (stage 4); embryo diameter, 1.3 mm.; age 2.25hrs.;

External features

1. Second cleavage furrow, like first one, extends slowly in the vegetal hemisphere. The second cleavage furrow, which is also vertical appears at right angles to the first, and divides the egg into four cells. In 10 mins after the completion of the previous stage, the second cleavage furrow covers about 1/3rd of the egg circumference. In next 15 mins four cells are formed.

2. Third cleavage furrow also is faintly visible. Formation of third cleavage furrow can also be recognized on all four cells. The third cleavage furrow is at right angles to the first two cleavage furrows, and is above the equatorial plane, more or less parallel to the equatorial plane. The third cleavage furrows still being incomplete, cover about 2/3rd of the distance between first and second cleavage furrows.

Internal features

1. The partition wall along the second plane, still is incomplete in animal hemisphere itself. Only about 3% of the egg diameter is
penetrated by the second cleavage furrow, at the equatorial plane. The penetration gradually increases towards the animal pole and decreases towards the vegetal pole.

The blastomeres, which are to be separated by second cleavage division, are separated from each other, for about 15% (on average), of the egg diameter. This separation is in outer side of the egg. More separation is towards animal pole and gradually less towards vegetal pole. This separation is by formation of thin partition wall inner to the second cleavage furrow.

Almost to the same extent, the separation can be recognized in outward direction, starting from the surface of the anlage of the blastocoel, in animal hemisphere. But still, there is no indication of development of the partition wall, along the second division plane, from inside of the egg to outside, in vegetal hemisphere.

2. The partition wall along the third cleavage plane is formed only on the side of the partition wall of the first cleavage plane. Adjacent to the anlage of blastocoel, the partition wall of the third cleavage plane, is completely extended in between those of the first and second, as much as the partition wall of the second cleavage plane is developed here, partly. Further outer to this, the third partition wall is developed in outward direction, till it reaches the outer surface of the egg. But it is developed only alongside the partition wall of the first cleavage plane, and gradually narrows in its lateral width, as it reaches the outer surface of the egg.
3. Eight blastomeres incompletely separated from each other, are formed in this stage. Extent of development of partition walls in vegetal hemisphere is very less, either along first division plane or second division plane. Thus first two blastomeres that were to be separated by first division plane, are separated completely from each other, in animal hemisphere, in previous stage only. Each of which should have divided again by second cleavage plane, into two smaller blastomeres have partly done so, in animal hemisphere itself. Thus first and second cleavage planes, in this stage, produce four blastomeres still which are not completely separated from each other. The incompletely developed third partition wall separates partly, the four smaller micromeres, from four larger, macromeres. The micromeres are more separated from each other than the macromeres.

4. The cleavage cavity (anlage of blastocoel) is furthermore enlarged. It measures about 15% of the egg diameter, along the AV axis, and is situated in the centre of the animal hemisphere, more towards the equatorial plane.
Eight-cell stage; (stage 5); embryo diameter, 1.3 mm.; age 2.35 hrs.;

External features

1. Four micromeres and four macromeres are formed. The third cleavage furrow, which was already appeared in previous stage, is completely developed, dividing the egg into four smaller micromeres, which form the upper tier, and four larger macromeres, which form the lower tier.

Usually, the micromeres are dark-brown, and macromeres are pale-brown in their colors. The micromeres are not exactly on the macromeres below it. They are situated slightly towards the corner of the two macromeres.

Internal features

1. Still, the micromeres are not completely separated from the macromeres. The micromeres are almost separated from each other by first and second cleavage divisions. The macromeres also seem to be separated from each other, to some extent by first and second cleavage division planes. But the extent of separation of blastomeres cannot be decided easily, by evidence of spreading of pigment along the division planes, in vegetal hemisphere, as it is possible in animal hemisphere.
The micromeres are separated from the macromeres from outside to inside of the egg, up to half of the distance to be separated.

The anlage of blastocoel is much enlarged in this stage, compared to that of the previous stage, and measures about 20% of the egg diameter, along the AV axis.
Sixteen-cell stage; (stage 6); embryo diameter, 1.3mm.; age 3.35 hrs.;

External features

1. The fourth and fifth cleavage planes loose their symmetry, sometimes. After 25 mins. of the previous stage, the four micromeres of the upper tier divide into eight, by two, synchronous vertical cleavage furrows, at right angles to each other. In next 5 mins, the cleavage furrows are visible to be extended up to the half of the length of the macromeres in the lower tier of cells. In next 30 mins the macromeres are also divided into eight smaller macromeres. Within this last 30 mins. time, the fifth cleavage, which is horizontal also appears in the upper tier of cells dividing the eight micromeres into sixteen smaller micromeres. Rarely, the fourth and fifth cleavage planes loose their symmetry, and because of which, distorted blastomeres are resulted, sometimes. It happens more often in micromeres than in macromeres.

2. About nine micromeres are present along the marginal zone. Although micromeres appear to be slightly irregularly divided, they are still almost equal sized. Rarely two to three exceptionally small ones may be found among them. Such cells remain internally, as large as other micromeres, but their major portion becomes overlapped by the neighbor micromeres. Of the sixteen micromeres nine are along the
marginal zone, above macromeres. And other seven are around the animal pole. The position of the micromeres bordering the marginal zone appear to be shifted in clockwise direction with respect to the position of macromeres below them.

**Internal features**

1. The macromeres are separated from each other, only about half of their length, along the AV axis. Still, the micromeres are not completely separated from each other. Some are separated completely, and some only by cleavage furrow, from the outside of the egg towards inside. The micromeres appear to have distorted more. The macromeres are separated only about half of their length along the AV axis. The anlage of blastocoel is not much enlarged than it was in the previous stage.
Thirty two-cell stage (morula); (stage 7); embryo diameter, 1.3 mm.; age 3.55 hrs.;

External features

1. **The macromeres become divided horizontally.** Till 10 mins. after the previous stage, the micromeres are not divided further. But in next 10 mins., both micromeres and macromeres start to divide simultaneously.

   The micromeres start to divide more speedily and irregularly, the synchrony and symmetry of cleavage divisions being lost in animal hemisphere especially towards animal pole. But in vegetal hemisphere, the eight elongated macromeres divide horizontally, giving rise to eight smaller macromeres towards the marginal zone. These are only as large as the micromeres bordering the marginal zone. Often these small eight macromeres are in rectangular shape, with their long axis parallel to the equatorial plane of the egg. But the next cleavage furrows in vegetal hemisphere start only after the next 20 mins of time but in an irregular manner.

Internal features

1. **The cleavage cavity is much enlarged.** At the end of this stage, the embryo enters in the morula stage, where the cleavage cavity is much
enlarged and it can be called as very early blastocoel. The blastocoel being situated at the central portion of the embryo is covered by the blastoderm, which is almost of same thickness in all sides.

2. **Blastoderm remains no longer single-celled, in its thickness.** The blastocoel measures about 30% of the embryo diameter, along the AV axis, in animal vegetal section. The blastoderm, at the marginal zone, is slightly narrower and one cell in thickness, with larger cells. At the vegetal pole region also it is one cell in thickness, but is with larger pear-shaped cells. At the region of animal pole, it is with smaller cells, and is with two to three cells in thickness.

The inner surface of the blastocoel is smooth in the animal hemisphere, but still, in vegetal hemisphere the blastocoel is much radiated being spread in wide intercellular spaces of the vegetal cells.
Early blastula stage; (stage 8); embryo diameter, 1.3 mm.;
age 5.35 hrs.;

External features

1. Still, micromeres can be easily recognised. After 40 mins of the previous stage, there appear no much difference between the size of the micromeres and macromeres. But after that the speed of cell division rapidly increases in animal hemisphere forming very smaller cells compared to larger cells in the vegetal hemisphere. At this stage, although the vegetal blastomeres are still larger, the surface of the vegetal hemisphere appears more smooth, than that of the animal hemisphere, because of gradual reduction in penetration of the cleavage furrows, as the cleavage is continued. But in animal hemisphere the micromeres are smaller but still they can be easily observed, and surface of the animal hemisphere appears to be rough.

Internal features

1. The blastoderm becomes thinner towards the animal pole, indicating the start of epibolic process. The blastocoel is slightly expanded, and it measures about 44% of the embryo diameter, along the AV axis. The wall of the blastocoel is thinner towards animal pole, and greatly thicker towards vegetal pole. The thickness of the blastocoel
wall at the animal pole is about 12%, and at vegetal pole is about 44% of the embryo diameter. The thickness of the blastocoel wall is about two cells towards animal pole, and about three cells towards the marginal zone. But there is not so much difference in thickness of the cells of the two regions.

2. Cortical layer is distinguishable in the blastoderm. At this stage, the cells, which constitute the blastocoel wall, at the animal hemisphere, appear to be cuboidal in their shape, especially the single layer of cells called the cortical layer, on the surface of the embryo. The boundaries of these surface cells are very distinct because of their dark-brown coloration along the cell borders.
Late blastula stage, just before appearance of dorsal blastoporic lip; (stage 9+); embryo diameter, 1.3 mm.; age 3.55 hrs.;

**External features**

1. **The outer surface of the egg is very smooth, due to smaller blastomeres.** As the cleavage divisions are continued, the cells appear gradually smaller in both animal and vegetal hemisphere. Although the cells of the vegetal hemisphere are all of the equal size, they are larger than the micromeres, and they remain very large especially around the vegetal pole.

   At this stage, the outer surface of the egg appears very smooth, because of very small micromeres and macromeres. The micromeres can be recognized as dark-brown colored very small dots, throughout the animal hemisphere. The macromeres are also greatly reduced in their size especially towards the marginal zone.

**Internal features**

1. **The blastocoel is shifted towards the animal hemisphere, with continued epibolic process.** The epibolic movement of the cells of the animal hemisphere, which was started in the animal pole region, in the previous stage, gradually spreads towards the vegetal pole, as the
cleavage proceeds. Because of this, the wall of the blastocoel gradually becomes more and more thinner towards the animal pole, and thicker towards the vegetal pole. The size of the cells is decreased, with continued cleavage. As a result of this, the blastocoel is gradually shifted towards animal hemisphere, and its floor is flattened more and more.
Gastrula stage with appearance of dorsal blastoporic lip;
(stage 10⁻, one hr. later); embryo diameter, 1.4mm.; age 12.35 hrs.

**External features**

1. **Postero – dorsal end of the embryo becomes distinguished.** At this stage, blastulation ends and gastrulation is started. About 15 min later, after the previous stage, a dense collection of dark-brown pigment is observed in vegetal hemisphere, at about lower margin of the broad portion of the gray crescent. This is the region where the dorsal lip of the blastopore is to be formed and it marks the posterior end of the dorso median line of the future embryo. This pigmented region is slightly concave and slightly bent on both sides, towards posterior ends of the latero median lines of the embryo. In next 1.45 hrs. with its increased concavity, and its increased lateral extension, that area appears as slightly curved line, with dark brown pigment. Now it can be said that the embryo is in the early stage of crescent-shaped dorsal blastoporic lip.

2. **Epibolic extension of blastoderm is visible towards the dorsal blastoporic lip.** The dorsal lip is formed at a distance of about 1/6th of the embryo circumference from the vegetal pole. It extends on both sides parallel to the equatorial plane. At this stage, although the
micromeres and macromeres are very small, still they can be recognised under low magnification. But there are some larger macromeres around the vegetal axis. A patch of dark-brown pigmented cells moving towards dorsal blastoporic lip can be perceived. This indicates the beginning of the increased epibolic movements on dorsal side of the embryo.

**Internal features**

1. **The blastoderm becomes thinner near to the dorsal blastoporic lip.** The thickness of the blastoderm in this stage is not same all over the animal hemisphere. Near to the dorsal lip and in lateral sides of the embryo, it is thinner and appears to be more or less equally thin in both regions.

2. **The cells of the embryo become split into presumptive epithelial layer and inner cell mass.** Throughout the entire surface of the embryo the single layer of cells of the presumptive epithelial layer is separated from the inner cell mass.

3. **Region of dorsal blastoporic lip appears to be more compact.** Now the involuting region of the marginal zone is clearly distinguishable from inner nutritive yolk mass, which appears as a loose mass of large cells in contrast to compact mass of cells of the marginal zone, especially in the region of dorsal blastoporic lip.
The separation gap between presumptive epithelial layer and inner cell mass is more towards the animal pole and it gradually decreases towards the vegetal pole. In animal hemisphere, it is less towards the ventral side of the embryo, and gradually it increases towards the dorsal side.

4. **Presumptive epithelial layer of the ectoderm and presumptive entodermal epithelial layer of the future archenteron can be distinguished, in the presumptive epithelial layer.** The single outer layer of cells, all over the animal hemisphere and marginal zone, where it is called as presumptive epithelial layer of the ectoderm, is made up of regular cubodial cells. The dark-brown pigment of the egg cortex is restricted only to this presumptive ectoderm and it is towards the outer surface of the layer. This presumptive ectoderm is thin towards animal pole and slightly thicker towards marginal zone. On vegetal hemisphere, where it is called as presumptive entodermal epithelial layer of the archenteron it is about three times thicker than it is in animal pole, being made up of larger cells.

5. **Involuting portion of inner cell mass can be recognized in the marginal zone.** The inner layer of cells lining the blastocoel roof, which is called as sensorial layer, is about two cells in its thickness at the animal pole. The number and size of cells of the sensorial layer gradually increase and the sensorial layer gradually thickens towards marginal zone; the involuting marginal zone esp. towards the dorsal
blastoporic lip side, is about three times thicker than the sensorial layer at the animal pole. But the non-involuting marginal zone, on dorsal lip side is slightly thinner than it is on opposite side. The inner vegetal cell mass along the AV axis measures about 55 % of the embryo diameter.

6. Invagination pit and gastrular slit are appeared, as an initiation of involution process, in the region of dorsal blastoporic lip.

Along the median plane of the embryo the dorsal blastoporal groove is invaginated up to the level that equals the thickness of the involuting marginal zone, of that region. The stretched marginal zone below which the invagination groove is formed, is clearly visible. Because, the marginal zone is separated from the inner nutritive yolk cell mass, by formation of the gastrular slit. At this region, because of formation of the gastrular slit, the floor of the blastocoel from its opposite side, is gradually raised up dorsally [i.e., moved towards anterior end of the embryo] to an angle of about 12°, in the median plane. The gastrular slit along the median plane measures for about 9 % of the outer circumference of the embryo, from blastoporal pit to its dorsal end.

7. Floor of the blastocoel is moved towards the anterior end of the embryo, in the region of dorsal blastoporal groove. The depth of the blastoporal groove gradually reduces as it spreads on lateral sides. At this stage, the straight distance between both lateral ends, up to
where the groove is formed, measures for about 15% of the embryo diameter. As the depth of the groove decreases, the uplift of the floor of the blastocoel and extent of the formation of the gastrular slit also decreases gradually towards the lateral sides of the embryo. The straight distance between two ends, up to where the gastrular slit is formed, measures for about 65% of the embryo diameter. Thus the floor of the blastocoel is slightly pushed, by the dorsal blastoporal groove, towards the anterior end of the embryo, as an indication of the beginning of the involution of the marginal zone, and formation of the archenteron on dorsal side of the embryo.

8. Bottle-shaped cells have appeared, but, still the involution process is not started. Although gastrulation is started at this stage, still there is no sign of involution. In the median plane of the embryo four to five bottle cells can be recognised. The dense collection of the dark-brown pigment visible externally in blastoporal groove is mainly concentrated in the posterior cell borders of these bottle cells. No sign of archenteron formation is there. The inner surface of the marginal zone, where the involution is to be started, still, appears plane and the marginal zone of this region is more or less as thick as it is in ventral side. The posterior end of the marginal zone bordering the blastoporal lip still is not considerably rounded. If the involution is started the posterior end of the marginal zone, i.e., the surface of the dorsal lip would have appeared rounded, because of inturning of the marginal zone.
Gastrula stage with crescent-shaped dorsal blastoporic lip;
(stage 10); embryo diameter, 1.4 mm.; age 13.35 hrs.;

**External features**

1. **The macromeres are more or less of the same size throughout the vegetal hemisphere.** The dorsal blastoporic lip is further extended more towards posterior ends of the latero-median lines of the embryo. Now it is in perfect crescent shape. The dark-brown pigment of the dorsal hemisphere is spread over more than half of the egg surface. It indicates, the epibolic extension of the wall of the animal hemisphere is gradually spread towards ventral side also. Still the individual cells can be recognised in animal hemisphere, under low magnification. The larger macromeres are more or less of the same size throughout the vegetal hemisphere.

**Internal features**

1. **The marginal zone of the dorsal side becomes thicker.** At this stage, the separation gap appeared in previous stage, between presumptive epithelial layer and inner cell mass of the embryo is disappeared except in the marginal zone area. The dark-brown pigment of the presumptive epithelial layer of the ectoderm, which was mainly concentrated in outer surface of this layer, in the previous stage is now
spread inwards in the layer. The blastular wall of the animal hemisphere appears more thin than it is in the previous stage. But the marginal zone, especially in dorsal side of the embryo, appears more thicker than it was in previous stage. Along the median plane, the ratio of thickness of blastocoel wall of the animal hemisphere, to marginal zone of the dorsal side is 1:3. This increased difference between the two layers seems to be due to disappearance of the gap between epithelial layer and sensorial layer, due to more thinning of the epithelial layer and due to increase of cell number in marginal zone. Along median plane, there are about eight compactly arranged smaller cells in its thickness, in marginal zone, in dorsal lip region. Where there were about four larger loosely arranged cells, in the previous stage.

2. Involution process is started, in dorsal blastoporic lip; but involuted layer still cannot be easily recognized. At this stage, the involution process is started in dorsal blastoporic lip as indicated by the rounded smooth posterior end of the marginal zone, along the median plane of the embryo. Although, the involuted inner layer is started to develop, it is not thickened to a considerable degree. Still the inner surface of the marginal zone appears slightly concave, and double layers cannot be perceived easily. It seems involuted inner layer is about half of the thickness of the outer layer, at marginal zone, and it gradually tapers dorsally [i.e., towards anterior end of the
3. **Presence of mitotic activity can be recognized, in the dorsal blastoporic lip.** In this stage, the region where the involution process is started, the mitotic figures can be seen, mainly in both layers of the marginal zone, and in blastular wall of that region; to a lesser degree, also in yolk cell mass of that side. In histological sections, stained with eosine and hematoxylin, this region appears more blue than the other region.

4. **Archenteron is appeared, and bottle-shaped cells appear to be pulling the archenteron towards dorsal side of the embryo.** In postero-dorsal side of the embryo, along the median line the narrow slit-like archenteron, which is about two times larger than it was in the previous stage, is developed. It is turned dorsally inner to the marginal zone, and because of this, the marginal zone appears like a club-shaped structure in lateral sections. There are about six to seven bottle shaped cells, bordered with dark-brown pigment, and they are visible as radiating away from the inwardly extending tip of the archenteron. Although the archenteron is narrow anteriorly, it is gradually enlarged below the dorsal lip. Now the blastopore being formed in the dorsal side of the embryo, it is present only as a narrow pore. The archenteron is heavily lined with dark-brown pigment at its advancing end i.e., the posterior end of the bottle cells.
Still the gastrular slit is prominently seen in this stage. It is bent towards the marginal zone and occupies about 11% of the total egg circumference, in the median plane.

5. **Invagination is more advanced only in dorso-median region of the embryo.** More advanced invagination, as it is indicated by the bulb-like appearance of the marginal zone, by the extension of archenteron towards dorsal side, and by the presence of bottle-shaped cells, is taken place only in the dorso-median region. The straight distance between lateral ends of such region is for about 25% of the embryo diameter. Within this 25% of the region, maximum advancement is along the median line, gradually decreasing in lateral direction. As moved, towards dorso-median line, more anteriorly progressed inner layer is closely apposed to the outer layer, so that they cannot be distinguished easily from each other. The anterior extension of the archenteron also decreases, as moved towards lateral sides.

6. **The involution process, still, is not reached the latero-median level of the embryo.** In a distance of about 7% of the embryo diameter, on each lateral side, involution is started as indicated only by the blastoporal groove, and bulb-like appearance of the blastoporal lip. Next to this, for about 5% of the embryo diameter on each lateral side, only blastoporal pit is observed, but no sign of invagination is observed. For about 25% of the embryo diameter on each lateral side,
beyond this, only gastrular slit, gradually decreasing in its antero-posterior width, is observed. Still, beyond this, towards mid-lateral line, no sign of beginning of gastrulation is seen.

7. **Endodermal cells in dorsal portion of the vegetal hemisphere, seem to have started migration toward anterior end of the embryo.**

Along median plane, small vacant spaces are found in middle of the dorsal region of the vegetal hemisphere, between A-V axis and dorsal blastoporic lip. They are found along 25% of the egg diameter, on each lateral side starting from the dorso-median line of the embryo. Such vacant spaces gradually decrease and their position shifted slightly towards the posterior side of the embryo, as moved towards the lateral sides of the embryo. Thus they appear in the postero-dorsal region of the embryo, where the major portion of involution is progressed, i.e., where the floor of the blastocoel is raised up dorsally. But nowhere, separate mesodermal and endodermal layers can be recognized in the inner layer.
Gastrula stage with horse shoe-shaped blastoporic lip; 
(stage 10 +); embryo diameter, 1.4mm.; age 15.35 hrs.

External features

1. The surface of the embryo becomes further more smoothened. The dorsal blastoporic lip further extends in lateral sides, now appearing in the shape of the horse-shoe. The area where the ventral blastoporic lip is to be formed is clearly marked by the dense pigmentation. The increased epibolic extension being spread throughout the egg surface, the surface of the embryo [except yolk plug] is becoming more smoothened. Individual cells are no longer conspicuous.

Internal features

1. Involution is much progressed, in dorso-median portion of the embryo. The involution of the marginal zone and formation of inner layer is reached the ventro-median line of the embryo. It is much progressed near the dorso-median line, where development of the inner layer is progressed anteriorly, to a level beyond the equatorial plane of the egg. In lateral and ventral sides of the embryo, development of the inner layer is progressed anteriorly, but, only to a level of equatorial plane of the egg.
2. The inner layer can be clearly recognized in dorso-median and ventro-median regions of the embryo, but not in lateral sides of the embryo. The outer and inner layers are closely apposed to each other. However, the extent of development of inner layer, can be easily judged near to the dorso-median and ventro-median lines of the embryo, but not in lateral sides. Near to the dorso-median line, where the archenteron is well developed, the extent of development of inner layer, anteriorly, can be easily judged by the extent of archenteron, in the anterior direction. Near to the ventro-median line, the cells of involuted inner layer can be easily distinguished from the yolk-endodermal cells, which are larger here. Thus, near to ventro-median line, portion of inner layer can be easily recognized in spite of absence of archenteron. In lateral sides of the embryo, neither archenteron is well developed nor cells of inner layer can be distinguished so easily, from yolk-endodermal cells. Inner layer in lateral sides somehow can be perceived.

3. The thickness of inner layer is same, both in its dorso-median and ventro-median regions. The blastoderm in animal hemisphere appears furthermore thin and smooth, over its surfaces. Around the animal pole, it consists of single layer of cuboidal cells, which appear to be much spread, over the surface. But towards the marginal zone, gradually it is of many cells in its thickness, where also, the outer layer is of cuboidal cells. The inner layer at the half of its antero-posterior length, is as thick as the outer wall is at the region, in dorso-median
and ventro-median sides of the embryo. Also thickness of itself in both of these regions is same.

4. The loose mass of vegetal cells, seemingly to be of endodermal layer, constitutes the anterior portion of the inner layer, as the inner layer is extended towards dorsal side of the embryo. Along the median plane, the inner layer covers about 16% of the total embryo circumference, in dorsal side. In front of it, for about in next 4.5% of the total embryo circumference, attached along the outer wall [blastoderm], is loose mass of vegetal cells, which is moved along with the anterior growing end of the inner layer. The inner layer, from dorso-median line, as moved towards lateral sides, in a distance, for about 30% of the embryo diameter, on each lateral side, suddenly decreases in its antero-posterior extension. So also, extension of the vegetal cell mass in front of it. But the proportions of both cannot be judged properly, as the tissue of both layers cannot be differentiated easily, in this lateral portion. At this region, the anterior extension of the inner layer is very little, it is being extended just beyond the level of the floor of the blastocoel. And from this point anterior extension of the inner layer, gradually decreases again towards the latero-median line of the embryo.

5. Development of inner layer is not progressed much, in ventral half portion of the embryo, and its anterior extension remains, more or less to be the same level all over, in ventral half portion of the
embryo. Along the ventro-median line, the inner layer, in its antero-posterior length, covers about 11% of the total embryo circumference. From ventral to lateral side also, it is difficult to differentiate the tissue layers as mentioned before. If the formation of loose mass of vegetal cells, along the margin of the floor of the blastocoel is considered as an indication of involution, it can be imagined that the involution is taken place, from latero-median line to ventro-median line of the embryo, more or less to the same degree, i.e., just beyond the level of the equatorial plane of the embryo; although, it is slightly more towards ventral side.

6. Anterior extension of the archenteron, in dorsal side of the embryo, follows the anterior extension of loose mass of vegetal cells, which is moved more anterior to the inner layer. In dorsal side, along the median plane, the archenteron is progressed anteriorly more than half of the length of the inner layer. Its anterior end is situated below the place where the tip of the inner layer, and loosened mass of the vegetal cells meet each other. Still, the archenteron is not expanded, although it appeared as it is started to expand below the dorsal lip, in the previous stage. All over, it appears as a double row of dark-brown pigment, and at its anterior end, the dark-brown pigmentation pattern of bottle cells can be recognized. But the bottle cells are not clearly visible. The archenteron gradually decreases towards lateral side, in a distance of about 25% of the embryo diameter on each side. The bottle cells are prominently seen along 9% of it,
from the median position, gradually reducing lateral to this, as the archenteron reduces. Further lateral to this, only the invagination pit is seen.

7. Development of the inner layer in ventral side of the embryo, is less dependent on formation of the archenteron. In ventral side, although the involution is much progressed and inner layer is clearly visible, involution is not indicated externally by the formation of the well developed invagination pit, but only by slight depression, along the pigment collected in presumptive blastoporal lip towards ventral side. This is in contrast with what happens when the blastoporic lip towards dorsal side, was not visible externally, in the previous stage.

8. Much of the vegetal cell mass of the dorsal portion of the blastocoel floor, has been moved to the dorso-median portion of the embryo, with continued process of involution. In stage 10\(^{-}\) (gastrula with appearance of dorsal blastoporic lip), the floor of the blastocoel was slightly moved anteriorly, in the dorsal side, where the inner layer is to be developed, resulting in the appearance of the gastrular slit. The inner vegetal cell mass beside the gastrular slit, appeared of slightly loose texture. The whole of the blastocoel floor appears to be raised up in a straight slope, from its opposite end. In stage 10 (gastrula with crescent-shaped blastoporic lip), with development of the
inner layer and archenteron, the floor was moved further anteriorly. And it appeared, that the loose vegetal cell mass especially adjacent to the archenteron, was moving anteriorly with the anterior tip of the inner layer. The loose appearance of the surface of the blastocoel floor, and its height was more in dorsal side. It gradually decreased in a curved slope towards the opposite side, i.e., near to the ventral side. Thus, the blastocoel floor was slightly raised up in dorsal side and slightly depressed in ventral side.

In this stage, much of the vegetal cell mass in dorsal half of the vegetal hemisphere, which is in the form of loose cell mass, is moved further anteriorly, next to the growing anterior end of the inner layer. The floor of the blastocoel in dorsal side is depressed more than it is in opposite side. This anteriorly moving layer of loosened vegetal cell mass, is almost in an angle of 90° to the blastocoel floor, in the dorsal side. Unlike in previous stage, now the cell mass of the rest of the blastocoel floor is also loosened. This loosened region is continuous with the anteriorly moved vegetal cell mass of the dorsal side. This continuous region of loosened cell mass, is more thicker at the anterior end, in front of the dorsal inner wall, and thinner in front of the inner wall of the ventral side.

9. The vacant spaces (seemingly to be formed by migration of endodermal cells) are found in the floor of the blastocoel, but in different places, other than in the previous stage. The vacant spaces as found in the previous stage, are found here also. Along the median
plane, they are situated between the archenteron and the floor of the blatocoel, being more towards the floor of the blastocoel, in dorsal side of the embryo. At the median plane they are few in number and small in their size. Towards each lateral side, they are spread for about 7-8% of the total embryo diameter. But they are more prominent in middle of this 7-8% region, in their number and size.
Gastrula stage with middle yolk-plug; (stage 12 -); embryo diameter, 1.4mm.; age 17.35 hrs.

External features

1. Epibolic movements in ventral side of the embryo, still can be recognized. One hr. later, after the previous stage, the blastoporal lip reached the ventral region, forming a circular ring of blastoporic lip. Although, the invagination groove was perfectly visible all along the ring, it was more sharp in dorsal half of it. But in its ventral half, it appeared slightly dull, due to dark-brown pigment of the groove, which was still visible externally. Also the flow of brown pigment towards the blastoporal lip could be perceived as an indication of epibolic extension of the surface of the blastoderm, especially towards ventral side.

Two hrs. later, i.e., at this stage also, the epibolic movements in ventral side of the embryo, still can be perceived externally. But the yolk plug is decreased in its size. The diameter of the yolk plug measures about 50% of the total embryo diameter, in this stage.

Internal features

1. More than half of the blastocoel, in posterior side of the embryo, is lined with a layer of loose mass of vegetal cells. In the previous stage, the inner layer was formed mainly in the median region of the
dorsal side of the embryo. Thus the posterior portion of the presumptive notochord was clearly formed. In this stage, the inner layer continues to be formed in dorso-lateral regions, beside the still more anteriorly progressed presumptive notochordal portion, and also towards ventral region of the embryo. The inner layer is considerably progressed anteriorly in all sides of the embryo. Its anterior extension is maximum in dorsal side, and it decreases towards the ventral side. Along with the furthermore anteriorly progressed inner layer, much of the vegetal cell mass of the blastocoel floor is also moved anteriorly. It is spread more anterior to the inner layer. These two layers are separated by formation of archenteron, which is also extended further more anteriorly, but still not expanded. Thus in this stage, more than half of the blastocoel, in posterior side of the embryo is lined with a layer of loose mass of vegetal cells.

2. The portion of loose vegetal cell mass which is spread infront of the inner layer, is extended furthermore anteriorly, in dorsal side of the embryo. Along the dorso-median line of the embryo, the inner layer, including the more anteriorly progressed anterior portion of the layer of the vegetal cell mass, totally covers an area of about 32% of the embryo circumference. The archenteron covers a distance of about 28% of the embryo circumference. The portion of the layer of vegetal cell mass is spread anteriorly, furthermore beyond the level of the tapered anterior end of the inner layer, and in front of the inner layer it
is attached to the outer layer, constituting about $\frac{1}{10}$th part of the total length of the inner layer, along the dorso-median line.

3. The archenteron still is slit-shaped all over, except the dorso-median region of the embryo, where it is slightly expanded at its anterior end. Along the dorso-median line of the embryo, the archenteron is clearly visible from its anterior to posterior end, as a line of vacant space separating the inner layer and the layer of vegetal cell mass lining the blastocoel. The dark-brown pigment, lining the archenteron is faintly visible, and is visible towards posterior side only. The archenteron is very slightly expanded at its anterior end, but only in dorso-median region of the embryo.

4. Outer and inner layers have become more distinct, from each other in dorso-median region of the embryo. The outer and inner layers of the gastrula, along dorso-median line of the embryo can be differentiated from each other easily, because of their slight separation from each other, and slight difference in the appearance of both of the tissues. They are more or less of the same thickness to each other, and each throughout their antero-posterior length. But the presumptive notochordal portion [chordamesoderm] of the inner layer [in front of which the vegetal cell mass layer is extended] is slightly thicker and tapered at its anterior end.

5. The layer of vegetal cell mass also, has become much thinner in dorso-median region of the embryo. Along the dorso-median line of
the embryo, the layer of loose vegetal cell mass, in its posterior half is as thick as the inner layer is at that region. It is gradually narrow towards the anterior end of the archenteron. But from here, again it continues anteriorly, overlapping the anterior portion of the inner layer and reach the outer wall, in front of the inner layer. As it reaches the outer wall, the layer of vegetal cell mass widens as much as the inner layer is at its anterior end.

6. The inner layer has become thicker, towards ventral side of the embryo.
Along the ventro-median line of the embryo, the inner layer [including the more anteriorly progressed portion of the vegetal cell mass layer] covers a distance of about 22% of the embryo circumference. Of this, 1/7th portion, at its anterior end, is the portion of the vegetal cell mass layer. The inner layer along ventro-median line of the embryo, can be differentiated from the outer wall of the region, but not clearly from inner most layer of the vegetal cell mass. The maximum thickness of the outer and inner layers together [except the portion of the innermost layer of the vegetal cell mass] in their posterior half, along ventro-median line, measures the double of the total thickness of the two layers, in their posterior half, along dorso-median line. In its anterior half, along ventro-median line, the inner layer tapers as it is extended anteriorly. Along ventro-median line of the embryo, where also the archenteron is in the form of a double line of dark-brown pigment, is progressed inside, as much as only to reach the inner surface of the inner layer. It is not extended further anteriorly separating the inner
most layer of the vegetal cell mass from the inner layer. Anteriorly, no separation can be easily done between the inner layer and the layer of vegetal cell mass. The layer of vegetal cell mass, in its posterior region seems to be as thick as the inner layer is, posteriorly. Gradually it becomes more thicker than it, anteriorly. As it moves anteriorly, it becomes tapered, and extends anteriorly beyond the level of the inner layer.

7. Many other changes can be recognized in the layers of the embryo, as the layers are extended laterally from the dorso-median and ventro-median regions of the embryo. As the inner layer is extended laterally, from the dorso-median line of the embryo, gradually it remains thin towards animal pole and thick towards the blastoporal lip. Also it gradually decreases, in its anterior extension. The total anterior extension of the layer of the vegetal cell mass also decreases, but, its thickness in its posterior side, gradually increases.

In the direction from ventro-median line of the embryo, towards its lateral sides, the inner layer gradually becomes thin and decreased in its anterior extension; and it can be easily distinguished from the outer layer. Likewise, the layer of vegetal cell mass extends anteriorly. It gradually decreases in its thickness towards its posterior side.

8. The inner layer and layer of vegetal cell mass are at their minimum anterior extension, along the ventro-median region. The inner layer is very thick, but is at its least anterior extension, along the
ventro-median line of the embryo. It gradually extends more and more anteriorly, in dorsal side of the embryo but it gradually is thinned, as it moves towards the dorsal side. The layer of vegetal cell mass is at its minimum anterior extension along ventro-median line of the embryo, and it gradually extends more anteriorly in dorsal side of the embryo. Along the ventro-median line its anterior end is very narrow, and it gradually thickens towards its posterior end. In dorsal side of the embryo, its anterior end shows a tendency to become wider gradually and its posterior end to become narrow.

9. The anterior extension of the archenteron gradually increases as it is extended towards dorsal side of the embryo. The archenteron slit formed is very less along ventro-median line of the embryo, and it gradually deepens more and more in anterior direction, in dorsal side of the embryo. Along, dorso-median line, where it is in its maximum depth, it becomes slightly expanded in its anterior end.

10. Floor of the blastocoel is become furthermore thinner. The floor of the blastocoel which was slightly thin towards dorsal side, in the previous stage, becomes more thin in this stage, and continues with the thinner layer of vegetal cell mass in dorsal side of the embryo. Towards ventral side, the floor of the blastocoel becomes gradually thick, and with its almost flat central portion, continues smoothly with the thicker layer of vegetal cell mass in ventral side, of the embryo.
11. A circular patch of blastoderm still is to be covered by inner layer, in antero-ventral side of the embryo. In this stage, the length of the layer of the vegetal cell mass which is extended in front of the inner layer, along dorso-median line of the embryo, still is to cover a distance of about 4% of the total embryo circumference, to reach the animal pole; and in ventral side, that distance is about 11% of the circumference. The straight distance between these two anterior ends, along median plane, measures about 45% of the embryo diameter. The maximum distance from lateral sides also measures roughly the same. Thus a circular patch of blastoderm at antero-ventral side of the embryo, still remains uncovered by the inner layer, [i.e., by the patch of vegetal cell mass which is ahead in the anteriorly extending inner layer].
Gastrula stage with late middle yolk-plug; (stage 12, one hr. later); embryo diameter, 1.4mm.; age 18.35 hrs.

External features

1. The yolk plug is further reduced in its size and its diameter measures about 35% of the embryo diameter.

Internal features

1. **Entodermal layer is distinguished.** The inner layer and archenteron are extended further, anteriorly; but still not completely covered the blastoderm. The archenteron is enlarged greatly. The blastocoel is reduced and is separated from the archenteron by the layer of vegetal cell mass, which was lining the blastocoel in the previous stage. Now, the layer of vegetal cell mass can be called as entodermal layer.

2. **The inner layer is started to be separated into mesoderm and endoderm.** This separation starts in the posterior region of the embryo, and separated region gradually extends into the anterior side, as moved towards dorsal side of the embryo. Thus in this stage, the outer layer can be called as ectoderm, and mesodermal and endodermal layers are started to appear separated from each other. Inner and outer layers are clearly separated from each other. Towards postero-ventral region,
where the inner layer was very thick in the previous stage, becomes thinner and extends further anteriorly. The outer layer is thicker in postero-dorsal region.

3. **Inner layer is thicker towards posterior end of the embryo, and the layer of vegetal cell mass is becoming more thicker.** Along the ventro-median line of the embryo, the archenteron is extended anteriorly only up to $1/4$th of the egg diameter. In front of it, although the inner layer is not separated from the thick layer of vegetal cell mass, lining the blastocoel, its anterior extension and thickness can be perceived. It gradually tapers anteriorly along with the narrow anterior end of the vegetal cell mass. At the level of the equatorial plane it is three times thicker than the ectoderm of that region. At this place the ectoderm and mesoderm layers appear to be of same thickness, i.e., about 4% of the embryo diameter. The layer of endoderm seems to be two times thicker. The inner layer from this level of equatorial plane, gradually is more thicker towards posterior side and thinner towards anterior side. Above the inner layer, the layer of vegetal cell mass, becomes more thicker than it was in the previous stage, as more and more of the vegetal cell mass of the blastocoel floor is moved in anterior direction.

4. **In ventral side of the embryo, mesoderm is clearly separated from the endoderm, but endoderm is not separated from the yolk-endodermal cells.** The archenteron in ventro-median region is clearly
spread up to the inner side of the ventral blastoporic lip. It is widened posteriorly as wide as it is on dorsal side. Along the archenteron, at medio-ventral line, the endodermal layer is clearly separated from its mesodermal portion of the inner layer. At this region the endodermal and ectodermal layers appear to be of same thickness, and mesoderm slightly thicker. Mesoderm continues anteriorly as a separated layer, gradually decreasing in its thickness. Up to its anterior end it is separated from thicker inner layer of vegetal cell mass [called as entoderm]. This mesodermal layer is not compact with smoother surface, in medio-ventral region of the embryo. In its posterior side, it appears slightly compact and becomes narrow anteriorly with fewer and fewer number of dispersed cells which are elongated in anterior direction. The portion of endodermal layer in front of the archenteron is not separated from the inner layer of yolk cells. But its continuity towards anterior end can be perceived as a region of more compactly arranged smaller vegetal cells slightly elongated in anterior direction. This region gradually tapers anteriorly, along with very thick layer of vegetal cell mass of the blastocoel floor of the ventral side.

5. The archenteron is much expanded at its anterior end. As the archenteron is extended from the ventro-median line, towards the dorso-median line of the embryo, it is penetrated more and more anteriorly. Along dorso-median line, it reaches the animal pole. Simultaneously, it gradually enlarges more and more towards its
anterior end. The ratio of the archenteron to blastocoel at the level of the equatorial plane is about 1:1.5.

6. The mesodermal and endodermal portions are very thin in mid-dorsal region of the embryo. The mesodermal layer, as it continues towards the dorsal side, it gradually becomes thinner and compact, especially in the region between its middle and its posterior end. At the mid-dorsal region, it is almost of a layer of single cell in its thickness, cells being elongated antero-posteriorly. Towards its posterior side, it gradually thickens and at its posterior end, along the dorso-median line, it remains of the same thickness as it is towards ventro-median line. But towards its anterior side, again it becomes more and more wider near the animal pole, as it moves towards the dorsal side.

The endodermal layer, as it moves towards the dorsal side, from ventro-median line, it gradually starts to appear as a separate layer of cells, distinct from the inner thick layer of vegetal cell mass. At the beginning, it also appears, especially towards its anterior portion, as a layer of loose cells elongated in antero-posterior axis. But gradually it becomes compact, and thinner towards its middle portion and wider towards its anterior and posterior ends, like the mesodermal layer.

7. Mesoderm and endoderm remains closely attached to each other in mid-dorsal region of the embryo. Towards the ventro-median line, the mesoderm and endoderm are separate layers. But as they are moved
towards the dorsal side, they are more and more closely attached to each other, in their middle portion, except towards their anterior and posterior ends. Along the dorso-median line, both layers look same, in the nature of their tissue, and their thickness, all along their length. Also they are closely attached to each other, except at anterior and posterior ends. But still they can be clearly recognized into two thin layers. The combined thickness of both of them, in mid-dorsal region, at the level of the equatorial plane, is about half of the thickness of the ectoderm in that region. At their ends, both together are about 1.5 times thicker than the ectoderm is at the region.

8. Much of the vegetal cell mass of the blastocoel floor is moved into the blastocoel. The layer of vegetal cells [entodermal layer] towards ventro-median side, is become much thicker and its anterior border is much widened. Much of the vegetal cell mass is moved into this layer, reducing the blastocoel. Its anterior end is further more thickened in its thickness. The mass of vegetal cells, at the anterior end along AV axis occupy about 1/7th part of the embryo diameter. This layer of vegetal cell mass being continued with endodermal layer, is more advanced anteriorly, than the layer of the mesoderm.
Gastrula stage with small yolk-plug; (stage 12); embryo diameter, 1.4mm.; age 19.35 hrs.

External features

1. Yolk-plug is slightly oval-shaped. The yolk plug is further reduced in its size and appears oval shaped, being very slightly elongated along the dorso-ventral axis. Along its elongated axis, diameter of the yolk-plug measures about 13 % of the embryo diameter.

Internal features

1. The archenteron is enlarged further. The endodermal layer [vegetal cell mass layer] is progressed further anteriorly, but still its margin from all sides, is not met at the point; only, its portion that is extending from the dorsal side, is crossed the level of the animal pole. The blastocoel is reduced and the archenteron is enlarged further.

2. Blastocoel becomes reduced more. Towards the ventro-median line of the embryo, the floor of the blastocoel at its anterior end is as thick as it is towards its posterior end. Thus, further more cell mass of the floor of the blastocoel, i.e., yolk plug is moved anteriorly, reducing the blastocoel.
3. **Chorda-mesodermal mantle, can be demarcated, more clearly.**

Like in the previous stage, towards ventral side, the mesodermal layer appears thick, and is of slightly looser texture. The endodermal layer can be more clearly distinguished from the mesodermal layer, especially towards ventral side. Like in previous stage, towards dorsal side the mesodermal and endodermal layers are thin, and show a tendency to be closely apposed to each other. In this stage, they are closely apposed to each other in antero-dorsal side. Also, above the level of the latero-median line, they are closely apposed to each other. Thus, in this stage, the median region on dorsal side of the embryo, where the mesoderm and endoderm are not separated from each other, can be called as chorda-mesodermal mantle.

4. **Neural plate has made its appearance in the postero-dorsal region of the embryo.** Near to the dorsal blastoporic lip the ectoderm is become slightly thinner. Here, in its lateral regions, the presumptive epithelial layer of ectoderm is slightly raised up from the sensorial layer. Thus, in postero-dorsal side of the embryo, the medullary [neural] plate has made its appearance. And as an indication of beginning of formation of the lateral ridges, the presumptive epithelial layer of dorso-lateral region, at the posterior side of the embryo, is loosened.
Gastrula stage with late small yolk-plug; (stage 12, one hr. later); embryo diameter, 1.4mm.; age 20.35 hrs.

External features

1. The yolk plug is very small and appears oval-shaped.

Internal features

1. Blastocoel becomes completely covered by the layer of vegetal cell mass, i.e., entodermal layer. More anteriorly progressed broad, front edge of the layer of the vegetal cell mass from the dorsal side, and more anteriorly moving front edge of the floor of the blastocoel meet each other in anterior region of the embryo. Thus, the blastocoel is completely covered by the layer of vegetal cell mass. Now this layer towards dorsal side can be termed as the 'completion bridge'. Much of the vegetal cell mass of the yolk plug is moved into the blastocoel with further more reduction of the blastocoel. The median portion of floor of the archenteron gradually is raised up in postero-dorsal side of the embryo. And it is gradually depressed downwards in lateral sides, as it becomes continued with the ventral blastoporic lip.

2. Three germ layers are furthermore clearly demarcated. In this stage, in antero-ventral region of the embryo, thick mass of vegetal cells [endodermal cells] is formed. The tapered front edge of the mesodermal layer towards this side of the embryo, can be easily
distinguished from this endodermal cell mass. Still it is not progressed anteriorly as much as the endodermal cell mass. But towards antero-dorsal side, the mesodermal cell layer cannot be distinguished easily from this antero-ventral thick endodermal cell mass as both layers gradually become more closure to each other towards antero-dorsal side.

Except in the dorso-median region, the mesodermal and endodermal layers are widely separated from each other, especially in dorso-lateral regions of the embryo. Towards ventral side, although they are not clearly separated from each other, they can be easily demarcated from each other.

That median area on dorsal side where the mesodermal and endodermal layers are not separated from each other, measures along the equatorial plane, as wide as about 18% of the total circumference of the mesodermal layer.

The mesodermal and endodermal layers [except in antero-ventral region], as in the previous stage, are thicker towards the posterior side and gradually thin towards the anterior side. Along the median line, on dorsal side, where the chordamesodermal mantle is not separated into layers, the mesoderm and endoderm together form a thin median region. From the dorso-lateral sides where they separate from each other, the mesoderm gradually thickens in lateral and ventro-lateral regions. The endoderm in dorso-lateral region is as thin as the mesodermal layer is. But it suddenly ends in yolk endodermal cell mass in lateral sides.
3. **Medullary plate formation is advanced further.** The medullary plate thickening is further extended more anteriorly. So also, the thickening of lateral ridges is in progress, as indicated by the more anterior extension of the loosening of the presumptive epithelial layer. Sensorial layer of ectoderm is more thickened towards dorsal side to form the medullary plate. In the medullary plate the columnar nature of the cells is slightly visible.
Neural plate stage; (stage 13); total length of the embryo 1.4mm.; age 24.35 hrs.

External features

1. The embryo appears to be slightly elongated. At this stage, the neurulation is started. The embryo appears oval shaped, being slightly elongated along the antero-posterior axis. The dorso median region of the embryo being slightly raised up from the surface of the embryo, and also being slightly flattened, the neural plate has made its clear appearance. The neural crest thickenings are appeared, but are visible very faintly. The anlage of oral sucker is clearly visible.

2. Regions of lateral ridges and neural groove are being marked by the pigmentation lines. About 2.30 hours later, after the previous stage, the formation of the neural plate was started in postero-dorsal region of the embryo, as a slightly flattened area adjacent to the small yolk plug, where the yolk plug was still visible from outside. After 1 hour of this, the area of the complete neural plate is visible as a very pale coloured area on dorsal side, in contrast to the darker coloration of the remaining surface of the embryo. Also appear in marginal regions of the neural plate, faint pigmentation lines along the inner and outer borders of the region where the lateral ridges are to be formed. The whole area of the neural plate can be perceived as it is slightly
flattened and slightly raised up from the surface of the embryo. The yolk plug disappears leaving a vertical blastoporic slit.

In next 30 minutes i.e., at the end of this stage, the neural plate becomes more conspicuous, and also a median line of dark pigmentation appears in postero-dorsal region, where the neural groove is to be formed.

3. Neural plate is wider anteriorly and narrower posteriorly. The lateral margins of the neural plate start from the lower end of the slit shaped blastopore. For a short distance upwards, it suddenly widens laterally. In postero-dorsal region, although the neural plate gradually and slightly widens laterally, it appears more or less of equal width up to the middle portion of it. From here, it widens laterally more and more, as it extends anteriorly. But it suddenly narrows in its downward portion, in antero-dorsal region. Thus the portion of the neural plate in antero-dorsal region, compared to its portion in posterio-dorsal region, is more wider and appears loop shaped, with its curved, transverse anterior margin.

4. Posterior part of neural plate is pale in its coloration. From its middle region, towards its posterior end, the neural plate appears more and more pale in its coloration, and the lateral ridges neither raised up above the level of the medullary plate nor pigmentation lines are formed along the borders. Also this postero-dorsal portion of the neural plate cannot be distinguished clearly from the lateral region of
the embryo. But along the median line, starting from the upper end of the blastoporic slit, a line of pigment gradually fading towards middle portion of the neural plate, is clearly visible. Towards this median line of pigment, the medullary plate is very slightly depressed.

5. Anterior part of neural plate is more darker in its coloration.
From its middle region, towards it anterior end the neural plate appears more darker than its more paler posterior region, and its lateral margins which appear still more darker, can be distinguished from the medullary plate, by faint line of pigmentation. This region of lateral margins appears more widened and thickened towards its anterior side, being in its maximum width and thickness at its anterior end, in lateral sides of the antero-dorsal region. Also, up to this region, from its middle portion the lateral margins of the neural plate, can be easily distinguished from the lateral side of the embryo, as this lateral margin is slightly raised upwards. But still it is not raised up from the level of the medullary plate. The wider anterior end of these lateral margins continue into the remaining transverse, downward anterior margin of the neural plate. But, this anterior margin of the neural plate appears very slightly thickened and very narrow especially towards its median portion. However, it can be distinguished very faintly from the antero-ventral region, i.e., the region of the sense plate. Also it can be faintly distinguished from the medullary plate, as this region of the medullary plate appears more darker than elsewhere of it.
6. **Two bulgings of the neural crest material are faintly visible.** In lateral sides of the antero-dorsal region, where the lateral margins of the neural plate appear more wider and thicker, it can be perceived by careful observation, that along with the portion of the lateral ridge, lateral to it, it is made up of two, very slight bulgings of the neural crest material.

   The antero-posteriorly thickened bulging of neural crest cell mass is situated more closely with the lateral ridge, and it blindly ends posteriorly in lateral ridge. It appears slightly wider than the lateral ridge. In front of this posterior bulging of neural crest cell mass, more wider and more or less round-shaped bulging of the anterior neutral crest cell mass is situated. This anterior bulging also is visible as separated from the neural ridge region, by slight depression.

7. **The anlage of oral sucker is distinctly visible.** The anlage of the oral sucker is appeared in antero-ventral region of the embryo, more anteriorly and more or less parallel to the loop shaped anterior, transverse margin of the neural plate. It appears as a crescent-shaped, dark colored, transversely elongated band with glossy appearance. It is very clearly distinguished from the surrounding region of the embryo. It is equally wide all over its length and its lateral ends extend upwards, nearer to the broad anterior ends of the lateral margins of the neural plate. All over its length, it appears to be very slightly bulged, and a groove along its length, is slightly visible, in its median portion.
No changes in the surface of the region of the sense plate, i.e., the region between anterior transverse border of the neural plate, and the anlage of the oral sucker, can be recognised, other than that it is very slightly depressed and more darker near the transverse border of the neural plate.

**Internal features**

1. **The mesoderm is extended further anteriorly.** The mesodermal layer in ventral region, of the embryo, is extended further anteriorly nearer to the anterior border of the archenteron floor i.e., below the animal pole. Mesodermal layer is gradually thicker towards lateral and posterior sides of the embryo, where it is made up of about three cells in its thickness. From this lateral and posterior region, towards postero-dorsal side, it gradually is slightly more ticker, being made up of about four cells in its thickness. Towards anterodorsal side, it gradually becomes thin, and towards centre of the dorsal side it gradually becomes very thin. As it is extended towards median line, in antero-dorsal region, the mesodermal layer is closely attached to the endodermal layer of that region. There is no continuity between mesodermal layer from antero-dorsal side and that from antero-ventral side, in the antero - median region of the embryo.

2. **The archenteron in ventral side of the embryo is enlarged furthermore.** The archenteron in ventral side is extended slightly more
anteriorly than in the previous stage, and it is enlarged completely from its posterior end to anterior end, more than it was in any of the previous stages. The blastocoel is much reduced.

3. Anterior half portion of the floor of the archenteron is plane all over it. As in the previous stage, the floor of the archenteron is raised up in posterior side, i.e., towards dorsal side of the yolk plug. And simultaneously, its lateral portions, are gradually depressed towards ventral side, i.e., towards the ventral side of the yolk plug. This raised up feature of the archenteron floor remains in the posterior half of the embryo. But in its anterior half portion, the floor of the archenteron is almost plane all over.

4. Lateral width of the chorda-mesoderm is decreased and its thickness is increased. Along the dorsal median line, the thickening of notochordal anlage and spreading of prechordal plate is started. Here the endodermal layer is not separated from the mesoderm. This unseparated median band of chorda-mesoderm is more narrow in its lateral width than in the previous stage. The chorda-mesoderm is much thickened in notochordal portion, and also appears to be slightly more thickened all over along its median region. From lateral sides of this unseparated median portion, the separation of the two layers is visible gradually more and more towards lateral sides.
5. **The portion of endoderm remains thinner than the mesoderm as both become separated in the region of chordamesoderm.** From dorso-lateral region, towards dorsal-medial line, the endodermal layer is more thin than the mesodermal layer of this region. Here, it is almost of single layer of cells in its thickness. In lateral sides of the embryo, the endodermal layer ends up in yolk endodermal cells. But its continuity towards ventral side of the embryo, as a thick separate portion of the endodermal cell mass of yolk, somehow can be perceived. In the same manner, the cells lining the archenteron floor also can be perceived as a separate portion of yolk endodermal cell mass. The cells lining the archenteron floor appear somehow different from the central yolk endodermal cells.

6. **Endodermal cells cannot be distinguished clearly, along chordamesoderm.** Along the median ventral line, where the endodermal layer can be perceived as it is in its maximum thickness, it appears to be compact and narrow towards posterior side, especially along the archenteron. It is narrow anteriorly and unites in front of the blastocoel, with the endodermal layer of the archenteron floor, and with the cells of chorda-mesoderm of the antero-dorsal side. The endodermal layer is gradually thin towards dorsal side. All along the dorsal median line, where the endoderm and mesoderm are closely attached to each other it is of almost of single layer of cells, except at the anterior and posterior ends of this region, where it is difficult to discern the cell layers. In dorso-lateral sides, it is very thin, slightly
more thicker in antero-dorsal region, and still more thicker in postero-dorsal region. In antero-dorsal region, it is slightly more thicker than the mesoderm is in that region, but it is thinner than the mesoderm is, in postero-dorsal region.

7. **Neural plate is clearly demarcated, by its thickening.** The boundary of the area of the neural plate is more or less completely demarcated, by thickening of its borders to form the lateral and transverse ridges, and the thickenings of neural crest cell masses, although the neural ridges are very faintly visible externally. As it is visible externally, the lateral extension of the neural plate is maximum in antero-dorsal region and narrow in postero-dorsal region. At its maximum lateral extension, it measures about 42% of the embryo circumference. This region is at a distance of about 42% of the AV diameter, from the animal pole.

8. **The inner surface of the neural plate appears flat, as the transverse and lateral ridges meet each other.** For a short distance posteriorly, in the region, where the transverse ridge, meets the lateral ridges, the inner surface of the lateral ridges appears flat, and lateral ridges from both sides appear to meet in the median line, without being separated by formation of thinner portion of medullary plate inbetween. Their outer surface being convex, smoothly continues with the convex outer surface of the embryo. But as moved towards posterior side, the
inner surface also appears convex, and thinner medullary plate appears in between the two ridges, separating the lateral ridges.

9. **Lateral ridges are much thickened in the region where they are widely separated from each other.** As the lateral ridges from the transverse ridge continue posteriorly, they continue to become gradually more and more thickened. They attain their maximum thickness in the region, where the lateral ridges are widely separated from each other in antero-dorsal region, as it is visible externally. That is behind the thickenings of neural crest cell masses. Also from the level of neural crest thickenings, the thinner medullary plate starts to appear between the thicker lateral ridges, and it gradually becomes more thin towards posterior side. Relatively the lateral ridges are also gradually thin towards posterior side after they attain the maximum thickness in antero-dorsal side.

10. **Lateral ridges and neural crest thickenings originate from a single strip of thickened ectoderm.** Towards antero-dorsal side the outer surface of the lateral ridges is slightly raised up from the surface of the embryo. But it is not easily perceivable externally. Towards antero-dorsal side, still, the surface of the embryo appears circular, but towards postero-dorsal side, it is slightly flat towards the neural plate. In antero-dorsal side, the posterior bulging of neural crest cell mass, and lateral ridges appear externally, as separate bulgings of the ectoderm. But, internally the region between the two bulgings is more
thickened, and both bulgings appear to be originated from a single strip of thickened ectoderm.

11. Medullary plate is slightly depressed inwards along the pigmentation line of the neural groove. In postero-dorsal side, at a distance of about 68% of the AV diameter, from animal pole, the surface of the thinner medullary plate, where median line of pigmentation of the neural groove is visible externally, start to appear slightly depressed towards the median line. This depressed region continues posteriorly for a distance of about 22% of the AV diameter. Beyond this posteriorly, the lateral ridges become more thin, so that no distinction between neural ridge and medullary plate can be done.

11. Posterior part of anlage of notochord is distinguished. Along 1/3rd of the dorso-median line, the notochord is gradually thickened posteriorly. As it moves towards posterior side, the notochord can be perceived that it is separated from the paraxial mesoderm, almost up to the posterior end; but it appears to be not separated from the endodermal layer. Towards its anterior side, the portion of notochord appears to be slightly separated from the endodermal layer.

12. Mesoderm is formed of double layers. In lateral region of the embryo, and in its upper and lower portion, where the mesodermal layer is more than one cell in its thickness, the cells of the mesodermal
layer are arranged in double layers. It is more clear towards anterior side than towards the posterior side of the embryo.

13. Somitogenic portion is started to be thickened. In postero-dorsal region, the paraxial (i.e., somitogenic) mesoderm is started to thicken, gradually more and more towards posterior side, with its simultaneous gradual increase in its lateral extension. In the posterior end of the embryo, at its paraxial end it is about 3 times thicker than its narrower portion towards the lateral side of the embryo.

14. The sensorial layer of the epidermal ectoderm is very much stretched and notoplate is appeared along the pigmentation line of the neural groove. The sensorial layer of ectoderm has become very thin being stretched into a very thin layer of single cell thickness, up to the margin of the neural plate. In ventral side of the embryo it is very closely attached to the epithelial layer, so that both cannot be distinguished easily. In postero-dorsal region of the medullary plate, exactly where the pigment line of neural groove is formed, in thinner portion of medullary plate, both sensorial and epithelial layers are of single cell in their thickness, and the neurectoderm is very thin, both layers being very closely attached to each other. Towards lateral sides, they gradually thicken into the lateral ridges.

15. Columnrization of cells of the neurectoderm is more towards the pigmentation line of the neural groove. Towards the median line,
of the neural plate, especially in postero-dorsal region where pigmentation line of neural groove is appeared, the epithelial layer of the neurectoderm tends to attach more closely with the sensorial layer. But away from this, towards margin of the neural plate, over the region of sense plate, over bulgings of the neural crest, it tends to be loose. It appears very loose especially over neural ridge thickenings, towards anterior side, and over the thickening of the posterior neural crest bulging. The columnization of cells of the neurectoderm seems to be more towards the median region, where pigmentation line of the neural groove is visible.

16. **Portion of oral sucker still is not clearly distinguished.** In antero-ventral region, no significant changes are recognizable along the oral sucker, except slight depression of the ectoderm, i.e., too towards median portion of the oral sucker.

17. **Much of the antero-dorsal portion of medullary plate is closely attached to the prechordal plate mesoderm.** Towards posterior side of the embryo, up to the blastoporal lip, the ectoderm and mesoderm are separate from each other. Also they are separate from each other in lateral sides of the embryo. In antero-dorsal region, above the animal pole, the mesodermal portion of the prechordal plate is separate from the ectoderm. But in middle portion of the dorsal side, the mesoderm is very closely apposed to the medullary plate, along its median line.
18. **Endodermal cells can be recognized in middle portion of the chordamesoderm.** The lateral width of the chordamesoderm is more towards the anterior side. The mesoderm and endodermal cells are not discerned in antero-dorsal region. But towards the middle portion of the chordomesoderm, and in the anterior half of the notochordal region, thinner prechordal portion, and single layered endoderm can be distinguished, although they are closely attached to each other. This anterior portion of the notochord appears to be with cells of the stacked nature. Below the notochord the single layered endoderm is distinctly visible as its cells appeared to be arranged in dorso-ventral direction. Up to the dorsal lip it is of single layer of cells and appears to be dorso-ventrally arranged. At the dorsal lip, it cannot be distinguished from the thicker cell mass of the mesoderm.

19. **Three germ layers cannot be distinguished from each other, in dorsal blastoporic lip.** In the rim of the blastoporic lip, towards ventral side, the mesodermal and endodermal layers are thinner than in the previous stage. The mesodermal layer at its posterior end appears to be separated from both the ectodermal and endodermal layers, and both the mesodermal and endodermal layers appear to be slightly disorganized; but still they are separated from each other. As move towards the dorsal side of this blastoporic lip, mesoderm endoderm and ectoderm cannot be distinguished, and appear more compact.
Explanation to the figures in plate - 10

Fig. (1) Two-cell stage, section nearer to the AV axis parallel to it, and at right angles to the first cleavage plane.

Fig. (2) Upper portion of the same section of Fig. (1) at higher (10 x 10) magnification.

Fig. (3) Two-cell stage; section along the plane at right angles to the first cleavage plane, parallel to AV axis, in the region of penetration path of the sperm.

Fig. (4) Four-cell stage; section nearer to the AV axis, parallel to it, and at right angles to the second cleavage plane.

Fig. (5) Eight-cell stage; section nearer to the AV axis, parallel to it.

Fig. (6) Sixteen-cell stage; section nearer to the AV axis, parallel to it.

Fig. (7) Thirty-two cell stage (morula); section nearer to the AV axis, parallel to it.

Fig. (8) Early blastula; section nearer to the AV axis, parallel to it.

Fig. (9) Late blastula just before appearance of dorsal blastoporic lip; section nearer to the AV axis, parallel to it.

(a) First cleavage furrow; (b) cleavage cavity (anlage of blastocoel); (c) blastocoel; (x) artifact; (d) partition wall along the first cleavage plane; (e) second cleavage furrow; (f) penetration path; (g) partition wall developed in outward direction along the second cleavage plane; (h) third cleavage furrow; (n) partition wall along the third cleavage plane, developed along side the partition wall of the first cleavage plane; (o) macromere; (r) micromere; (s) blastomere formed in the marginal zone by first horizontal division in the vegetal hemisphere.
Explanation to the figures in plate - 11

Figs (1-6) lateral sections nearer to the median plane of the embryos at different stages of gastrula.

Fig (1) Gastrula with dorsal blastoporic lip just appeared
Fig (2) Gastrula with crescent-shaped dorsal blastoporic lip
Fig (3) Gastrula with advanced, crescent-shaped dorsal blastoporic lip
   (not described in the text)
Fig (4) Gastrula with horse-shoe-shaped blastoporic lip
Fig (5) Gastrula with middle yolk-plug
Fig (6) Gastrula with late, middle yolk-plug

(b) (in fig 1), invagination groove, (in fig 5), portion of layer of loose mass of vegetal cells, which forms the floor of the archenteron (d) epithelial layer of presumptive ectoderm, (e) sensorial layer of presumptive ectoderm, (f) presumptive entodermal epithelial layer, (i) gastrular slit, (x) artifact, (g) vacant spaces in yolk endoderm, (q)(in figs 2, 3 & 4), anterior end of the archenteron, (in figs 5 & 6), archenteron, (k) dorsal blastoporic lip, (c) blastocoel, (j) anterior portion of inner layer which is formed of loose mass of vegetal cells, (l) pigmented groove in the region of ventral blastoporic lip, (m) inner layer, (n) outer layer, (la) ventral blastoporic lip, (ad) endoderm, (ea) ectoderm, (fa) mesoderm, (ga) endodermal cells
Explanation to the figures in plate - 11
(continued from the back page)

Figs. (7 to 9); Neural plate stage, lateral sections; 61\textsuperscript{st} (Fig. 7) 81\textsuperscript{st} (Fig. 8) and 111\textsuperscript{th} (Fig. 9) of totally 276 serial lateral sections, into which whole embryo was cut  Cutting started from left side of the embryo

(e) vestige of blastocoel, (b) lateral neural ridge, (e) medullary plate, (g) paraxial mesoderm, (x) artifact, (l) endoderm, (f) mesoderm, (j) neural crest cell mass, (q) archenteron, (l) ectoderm, (d) epithelial layer of neural ectoderm

Figs. (10 to 12); Neural plate stage; cross sections; 134\textsuperscript{th} (Fig. 10), 179\textsuperscript{th} (Fig. 11), and 239\textsuperscript{th} (Fig. 12), of totally 266 serial cross sections, into which whole embryo was cut  Cutting started from anterior end of the embryo

(m) floor of the archenteron, (h) anlage of notochord, (b) portion of lateral neural ridge, (n) blastopore, (j) portion of neural crest cell mass, (q) archenteron, (d) epithelial layer of neural ectoderm,
Neural fold stage; (stage 14); total length of the embryo 1.5mm.; age 26.35 hrs.

External features

1. Neural plate is clearly distinguished. The bulgings and depressions of the neural plate and other features of the embryo present in the previous stage are well developed and clearly visible. The contour of the neural plate also remains same, and the neural plate appears to be of key-hole shape. Towards its margin the neural plate is raised up, more clearly from the surface of the embryo. Medullary plate is more depressed along its median line, and the neural plate is slightly more narrower laterally. Inner portion of the neural plate, being more depressed medially, and its borders being more raised up from the surface of the embryo, neural plate, in this stage, appears to be folded all along its margin. Thus, regions of transverse and lateral neural ridges now can be called as transverse and lateral neural folds.

2. Transverse and lateral neural folds appear to be of same size. The inner border of the neural fold is clearly visible by development of the pigmentation line all along the inner border. The thickening of the transverse neural fold in its median portion is less and it gradually increases in its width and thickness towards its lateral sides, i.e., towards the corners, where it unites with the lateral folds and also with
the anterior thickening of the neural crest material. Both, the transverse neural fold [except towards its median portion] and lateral ones appear to be more are less same in their thickness and width.

The outer surface of the transverse neural fold and that of antero-dorsal portion of lateral neural fold is concave and inner surface more flat. In antero-dorsal region, the neural fold becomes more angular, towards median region of the transverse neural fold.

3. **Bulgings of the neural crest material appear to be more larger than the neural ridges.** Both neural crest bulgings appear more wider and thicker than the neural ridges. The anterior neural crest thickening appears more thicker than the posterior one. The anterior neural crest bulging appears as a postero-laterally extended portion of the transverse neural fold. It is situated in the anterior region of the embryo at a level of the latero-median line of the embryo. Behind it the posterior one also is situated in antero-dorsal region, but in the region from where the neural plate bends antero-ventrally.

4. **The neural groove is extended furthermore anteriorly and the neural plate is depressed towards the neural groove.** The pigmentation line of the neural groove is extended beyond the middle portion of the neural plate, i.e., nearer to the transverse neural fold. Towards this pigmentation line, the concavity of the median region of the medullary plate is clearly visible all along the medullary plate. In postero-dorsal part, the neural groove is a concave depression along the
median region of the medullary plate. As the neural groove extends anteriorly, in antero-dorsal region, the concavity of the medullary plate, widens and deepens more. For some distance, anteriorly, the neural groove is not concave but with angular depression. Still anteriorly i.e., behind the transverse neural fold, the whole, laterally wider, median portion of the medullary plate appears to be depressed inwards, only the transverse neural ridge being raised upwards.

5. **Anlage of oral sucker and neural crest thickenings are positioned in a line that is somewhat concentric to the transverse neural ridge.**

In antero-ventral side, the anlage of the oral sucker is recognizably, raised up from the surface of the embryo. Its width all along its length, is decreased towards its median portion. Its wider, end portions are situated below the anterior thickenings of the neural crest. In these end portions, presence of a groove is faintly visible.

In a narrow median region of sense plate, that is at the level of lateral end of the oral sucker, there is a slight depression. It is bordered in its lateral sides by thickening that is branched from the transverse neural ridge. Thus the lateral portions of sense plate being separated by this median groove, appear distinctly from the more thicker lateral portions of transverse neural ridge, and oral sucker.

The outer borders of areas of neural crest thickenings and oral sucker are smoothly continuous to each other, along a imaginary loop-shaped line. This line is somewhat spread concentric to the loop-shaped antero-dorsal portion of neural plate. It is joined posteriorly
with the middle portion of the neural plate, as the posterior neural
crest thickening ends posteriorly. As it is moved antero-ventrally it is
gradually more away from the neural ridge.

Internal features

1. **The ectoderm is become very thin.** All over the embryo, in portion
other than the portion of neural plate, blastopore, and anlage of the oral
sucker, the ectoderm is of more or less equal thickness. Also, it is
become very thin, compared to other germ layers. All over the embryo,
the epithelial layer of the ectoderm is of single layer of cells. The
sensorial layer of the epidermal ectoderm, up to the margin of the
neural plate, also is of single layer of cells and is very closely attached
to the epithelial layer.

   Up to the margin of the neural plate, the ectodermal layer, except
the region nearer to the blastopore, the cells of the sensorial layer
being very much spread and being closely attached to the epithelial
layer, the ectoderm is become very thin.

2. **Palely pigmented cells have been appeared inbetween the densely
pigmented cells of the epithelial layer of the ectoderm.** Till this
stage, epithelial layer of ectoderm was formed of cells, only which
were pigmented, all over the ectoderm. In this stage, very palely
pigmented cells have appeared in between the pigmented cells. This can
be easily recognized in one or two sections, at the beginning or end of a
series of serial sections, of the embryo, where those sections becoming
cut more parallel to the surface of the embryo, contain only the portion of epithelial layer. Moreover, it is possible to get such sections, only if the surface of the embryo is convex, in its outline. Because of this their presence in the epithelial layer of neural ectoderm could not be confirmed, as the neural plate has slightly concave surface. In spite of this, the neural plate appears to be of pale coloration, in contrast to epidermal ectoderm. (But, change in coloration of epidermal ectoderm, although it might have been there, in between previous stage and this stage, it could not be recognized easily, in observations on external features of the embryo). In section of this stage, it can be recognized that they are spread all over the epidermal ectoderm, they are as many as pigmented ones, and are equally distributed all over, in between the pigmented cells. Oral sucker seems to be an exception, by having more number of pigmented cells.

3. Anlage of oral sucker is formed of elongated larger cells of epithelial layer of ectoderm. The anlage of the oral sucker is thinner and narrower towards its median portion, and gradually wider and slightly thicker towards its lateral ends. All along its length, it is made up of single layer of epithelial layer cells and single layer of sensorial layer cells. It is constituted of about four to six cells in its width, externally from epithelial layer and same number of cells internally from sensorial layer. The cells become larger, and elongated antero-posteriorly, as the oral sucker thickens in its lateral sides. Towards its lateral ends, in the region of the groove, cells of epithelial layer are
slightly smaller and shorter. And those of sensorial layer, relatively are slightly larger. But all along its length, the region of oral sucker internally is plane, it is being thickened externally only. The epithelial layer cells forming the oral sucker are heavily pigmented with dark-brown pigment, towards their outer surface.

4. Neural plate is much thickened all over; medullary plate and neural ridges have no clearcut borders. The neural plate is much thickened. Its lateral extent is decreased. Thickening of median region of neural plate is progressed more anteriorly, up to the transverse neural ridge, and it is more thicker behind the transverse neural ridge. There it is thicker than the transverse neural ridge itself. In this more thickened anterior region of the medullary plate, the neural plate appears externally to be much depressed, inwards. The transverse and lateral ridges being much thickened, the margin of the neural plate is distinctly raised up vertically, from the surface of the embryo. The marginal thickening of the neural ridges is gradually reduced towards the median line of the neural plate. No median portion of the neural plate can be differentiated, so clearly, as medullary plate, as it was possible in the previous stage.

5. Thickness of the neural plate along the lateral margin is much increased, relative to its thickness, along its median line. Although, the transverse and lateral ridges of the neural plate, externally appear to be of more or less equal thickness and width, all over the margin,
the margin of the neural plate, internally is very much thickened and is more wider towards the anterior end of the lateral neural ridges. It gradually is thin and gradually narrows in posterior side, and in the median region of the transverse neural ridge.

Just posterior to the transverse neural ridge, for a short distance posteriorly, the region of medullary plate can be distinguished, from the lateral and transverse ridges, which are raised up vertically from the surface of the medullary plate. Here, external surface of the region of the medullary plate is laterally flat. But posterior to this, in most of the anterior part of the antero-dorsal region, where the lateral ridges are very much thickened, and gradually narrow towards median line, both the external and internal surfaces of the neural plate are in the form of more deepened angular groove with laterally flat lateral sides. As moved towards posterior side, the lateral neural ridges become thinner, and the surface of the neural plate on both sides remains as a shallow, concave surface. Thus in this stage, along the median line, the neural plate is very thin, relative to the much thickened lateral ridges.

6. **Inward folding of the neural plate is started, nearer to the blastopore.** The neural plate is not folded inwards medially, except in its region nearer to the blastopore. Thickened lateral regions and thinner median region of the neural plate, are almost in a straight line laterally all along the length of the neural plate. But only nearer to the blastopore, the neural plate is slightly folded inwards, medially. Here,
outer surface of the neural plate is an angular depression, medially, and the inner surface is a median ridge.

7. Neural crest material is clearly segregated into mesencephalic, rhombencephalic and trunk neural crest portions. The portion of the neural crest, in the thickened lateral margin of the neural plate, is clearly separated from the portion of the lateral ridge. In antero-dorsal portion two pairs of neural crest cell masses (mesencephalic and rhombencephalic portions) occupy the region that is visible externally. As it can be recognised from the surface of the embryo, not only both pairs are separated from each other, but also from portion of neural crest of the transverse neural ridge and from the portion of neural crest (trunk portion) that is extended posteriorly. Posterior extension of trunk neural crest can be recognized, up to the anterior region of the postero-dorsal part of the neural plate. The extension of neural crest portion along the transverse neural ridge can be recognized, although it is not separated clearly from the portion of neural ridge, as it is also in postero-dorsal side of the embryo, in case of trunk neural crest portion.

8. Segregated portions of neural crest material are overlapped each other, as they are separated. The mesencephalic portion of neural crest material appears to be overlapped at its anterior end, by the neural crest portion from the transverse neural ridge. At its posterior end it overlaps the anterior end of the rhombencephalic portion.
9. Portions of neural crest towards their outer side, are formed of loose mass of cells. Portion of the neural crest material is separated from the portion of the neural ridge, more clearly towards the inner surface of the neural plate. Towards this surface, neural crest material appears more compact with closely packed cells. But towards their outer (dorsal) side, the neural crest material is formed of loose mass of neural crest cells, which becomes spread between the neural crest and portion of neural ridge.

10. Neural crest material constitutes the outer half portion of the neural fold. The portion of neural crest, constitutes the outer half portion of the neural fold, all along it. In width on each side, the neural crest, roughly is as wide as about 1/3rd of the width of the half of the neural plate of that region. In antero-dorsal region, the position of neural crest material is slightly towards the lower portion of the thickened lateral ridge. Posterior to this, the neural crest portion occupies a position more lateral to the neural ridge.

11. Neural crest cells are easily distinguishable. The neural crest cells are larger, more rounded and slightly more darker, compared to the cells of neural ridge. Texture of neural crest appears loose than the compact texture of the neural ridges. The mesencephalic portion of
neural crest cell mass is with more densely pigmented cells in its dorsal portion [but not in loose layer of neural crest-cells spread between it and neural ridge].

12. Neural crest cells start to appear from the median portion of the transverse neural fold. From the median region of the transverse neural ridge, the sensorial layer starts to thicken into the cell mass of neural crest. It gradually increases posteriorly, and gets constricted into mesencephalic and rhombencephalic neural crest masses in lateral sides of the antero-dorsal region. Towards its median region, the transverse neural ridge, in its upper portion of the outer surface, and also in most of its inner surface, is formed of neural crest cell mass. From this median region, as the neural ridge proceeds posteriorly, the neural crest cell mass becomes confined only to the outer half portion of the neural ridge. In antero-dorsal region, when mesencephalic and rhombencephalic neural crest portions become segregated, the neural crest cell portion from antero-median region, continues posteriorly as a loose dispersed layer of neural crest cells, between the neural ridge and the segregated portions of neural crest masses. This loose cell layer of neural crest is situated along the dorso-lateral region. This layer continues posteriorly, and gradually ends in the more compact neural crest portion, that is spread towards the postero-dorsal side.

13. Sensorial layer of the epidermal ectoderm extends dorsally over the epithelial layer of neural ectoderm, as the sensorial layer of
neural ectoderm becomes loosened from the epithelial layer of the neural ectoderm. Both in antero-dorsal region, and in the region posterior to it, the neural crest is separated both from the neural ridge, and epithelial layer of the ectoderm. As it becomes separated, the sensorial layer of epidermal ectoderm extends dorsally, along the separated portion of epithelial layer of neurectoderm, as a single layer of cells and is closely attached to the epithelial layer. These cells being very small, are clearly distinguished from the much larger cells of the neural crest. This thin sensorial layer is clearly visible along the outer surface of the neural ridge. But still posteriorly, the neural crest cell mass is not separated from the epithelial layer, and sensorial layer appears to be ended in cell mass of the neural crest, and further posteriorly it appears to be ended into the sensorial layer of the neural ridge.

14. The attachment of epithelial and sensorial layers of ectoderm becomes changed, as they form the neural plate. The epidermal ectoderm gradually remains thinner towards the apex of the neural fold. Towards the median line of neural plate, the epithelial and sensorial layers are closely attached to each other. Here, cells of epithelial layer tend to be columnar

In antero-dorsal region, where the sensorial layer is much thickened, columnar cells are present at the lower surface of the neural plate, and at the lower surface of the neural crest.
15. *Prechordal plate mesoderm is spread into a single layer of cells.*
Like in the previous stage, the mesodermal layer is thinner towards chorda-mesodermal portion in antero-dorsal region, thicker towards postero-ventral region, and more thicker in postero-dorsal region, i.e., towards notochord. The prechordal plate mesoderm is spread into a single layer of cells, almost from the anterior end of the notochord up to the anterior end of the archenteron floor. The thickness of mesoderm in antero-ventral region, relative to its thickness in postero-ventral region is increased. Portions of both in this stage, appear more or less same in thickness. Also, the thickening is increased ventrally.

Except the portion of prechordal plate and in small portions beside it, two layered nature of mesoderm in its lateral portions is still visible all over, except in the more posterior part of the postero-dorsal region. Although, arrangement of cells in two layers is spread more towards ventrally, no two layers are clearly visible.

16. *Anlage of notochord is elongated, but, still, is not clearly distinguished at its anterior end.* In about posterior half of the embryo, the notochordal and somitogenic portions are gradually thickened posteriorly, and the prechordal portion is spread into single layer of cells, in anterior half portion. In between, the portion of notochord, although it appears compact, is not clearly separated from dispersed loose cells, which constitute the somitogenic and prechordal portions at the beginning. Thickening of notochord in this stage, is progressed more anteriorly, and is more thickened further. It is of
almost of equal thickness all over, although appears slightly thin at anterior end and thick at posterior end. The notochord all over its length, is clearly separated from the lateral somitogenic portion. In most of its middle portion it is made up of about four cells across, in thickness. Nearer to the blastopore, cell number increases to five to six. For 1/3 \(^{rd}\) of its length, in the middle, the cells appear to be of stacked nature.

17. The regions of dermatome and myotome can be recognized in the somitogenic portion. The somitogenic portion, all along the length of the notochord, is thickened towards the notochord. The lateral extent of the somitogenic thickening is confined to the lateral border of the neural plate. In the more anterior region, where the somitogenic cells are more dispersed and smaller, the thickness of somitogenic portion is of about three cells. Along most of the middle portion of the notochord, the thickness of somitogenic portion is about of two cells in thickness. Here, the cells are compactly arranged. Again towards posterior side, up to the blastopore, four to five compactly arranged cells are present in the thickened somitogenic portion. Towards the anterior side, the future myocoelic space in the thickened somitogenic portion is visible as a more wider space between the cell layers. Nearer to the blastopore, no sign of myocoel or double layers, in the much thickened somitogenic portion can be recognized. But, between these anterior and posterior ends, the outer thinner layer of dermatome can be
easily recognized from more thicker inner myotomal layer. But no myocoel is visible.

18. **Segmentation of the somitogenic portion is started at anterior end.** In antero-dorsal region, where the thickening of somitogenic portion still is with dispersed cell mass, thinning of mesodermal layer towards lateral plate mesoderm is not gradual, from the thicker somitogenic portion. Both portions are separated by more thinner region. But posteriorly, all over it is gradual. The beginning of segmentation of somitogenic region is very slightly visible along the anterior 1/3rd length of the notochord. Three to four segments are very faintly appeared.

19. **The archenteron is extended medio-ventrally, at its anterior end.** The yolk plug is moved inside from the blastopore. The yolk plug being moved towards the centre of the egg, the floor of the archenteron is more or less in the form of a rounded mass. It is continued with the remaining portion of the endodermal layer, through its wider, shallow, margin. The mark of median ridge of the archenteron floor, still remains as a slight median ridge posteriorly. But in antero-ventral region, the anterior margin of the archenteron floor is extended downwards, medio-ventrally.

20. **The endoderm except in the floor of the archenteron is of single layer of cells, in its thickness.** All over, except the floor of the archenteron, the endoderm is of single layer of cells in its thickness.
Except in the margin of the archenteron floor, except in the narrower, dorso-median, chorda-mesodermal portion and except in the surrounding area of the blastopore, the endoderm is of more or less equal thickness, all over. But in narrow dorso-median region, where the chorda-mesoderm still is not clearly separated, the portion of endoderm appears thinner. This region is below the notochord and prechorlal plate. Around the blastopore and along the margin of the archenteron floor, the thickness is due to two to three cells in thickness.

21. The cells of endoderm are distinctly visible, in chorda-mesodermal mantle. The separation of mesoderm and endoderm in dorsal chorda-mesodermal layer, is progressed more towards medio-dorsal line. The endoderm is attached to mesoderm only along the single layered prechordal plate, notochord, and nearer to the blastopore, also with thickened somitogenic portion only in its part that is more nearer to the notochord. Along anterior 1/3rd of the notochord, it is almost separate from the notochord. In middle portion of the notochord, although it is clearly separated, it is closely attached to it. Along posterior 1/3rd of the notochord and along part of more posterior somitogenic portion, although it is unseparated, cells of endoderm are clearly visible.

22. Transverse neural ridge remains more closer to the prechordal plate mesoderm. In dorso-median region, the neural plate and mesoderm are slightly more widely separated from each other than in
previous stage. But, they remain more closer, only in antero-dorsal region, between the transverse neural ridge and dorsal half portion of the prechordal plate. As the lower half, antero-ventral portion of the prechordal plate extends towards medially depressed archenteron floor, it is widely separated from ectoderm as wide as it is in dorso-median region. In this lower portion the cells are gradually larger, downwards.

23. Three germ layers cannot be distinguished from each other, as they are extended, towards dorsal side, in the blastoporic rim. In blastoporal rim, as moved towards the ventral median line, mesodermal portion becomes separated from the involuting portion of the blastoporal rim, and the mesodermal cells in the portion of rim become dispersed, loose cell mass. But, as moved towards dorsal median line, three germ layers cannot be distinguished from each other. Along ventral median line, in the rim of blastopore, the endoderm is about three times thicker than it is in dorso-median axis of the rim. But, in this much thickened portion of it, cells cannot be distinguished, it is being more compact. The ectodermal layer along the ventral median line of the rim is also more thickened. It is as thick as it is in same position in dorsal side. Here also, cells cannot be distinguished easily.
Neural tube stage; (stage 16); total length of the embryo 1.8mm.; age 30.35 hrs.

At this stage the embryo becomes much different, in its appearance. The changes are more in number. The external features of the embryo two hours later after the previous stage are also added in this stage, for easiness in relating the features of present and previous stages.

**External features: two hours later, after the previous stage**

1. **The lateral width of the neural plate is reduced.** The lateral width of the neural plate is reduced, as the neural folds are moved more towards dorso-median line. This movement is taken very fastly in antero-dorsal side, so that the lateral ridges in this region are more closer than in previous stage. But, still they are slightly more widely separated than in the postero-dorsal side, in this stage also. The transverse portion of neural fold cannot be distinguished from its lateral portion. The lateral folds are slightly more closer in middle of the neural plate, than in postero-dorsal region.

2. **The medullary plate appears to be more concave.** The reduction in lateral width of the neural plate is compensated in the inward movement of the neural plate, along the dorso-median line. Thus all along the neural plate, the lateral width of the medullary plate is greatly reduced, and it appears to be very much depressed ventrally.
Still the line of the neural groove can be recognised, but the region of the medullary plate, appears to be more concave.

3. Ingression of epithelial layer along the neural groove, is more advanced above the blastopore. Dark pigmentation line of neural groove, along small portion of dorso-median line, just above the blastopore appears to be more darker and more anteriorly progressed. Thus, ingression of epithelial layer is more advanced here, than in the previous stage. Below the blastopore, the body wall adjacent to the lateral lips tends to be folded laterally, as indicated by dark colored, small median depression.

4. Portion of prosocoel can be demarcated. Behind the transverse neural fold, the transverse depression of the medullary plate is further depressed very much forming the anlage of the prosocoel. In this stage, the lateral width of the transverse depression being reduced greatly, and being depressed very much it appears roughly as a pear-shaped cavity. This cavity is wide anteriorly and narrow posteriorly with its long axis along the anteroposterior axis of the embryo. Formation of the future optic vesicles is started in both sides of the broad anterior end of the prosocoel. Its corners appear to be slightly more deeper in lateral direction, and appear to be an area of more darker pigmentation. The anlage of prosocoel is situated in about anterior half of the broader antero-dorsal portion of the neural plate.
5. **The neural fold is become more distinct.** The neural fold is prominently visible, all over and even more in antero-dorsal side, except along the median line in antero-dorsal side, where both lateral portions of it are clearly separated, from each other. Also, the neural fold is more clearly raised up from the surface of the embryo, in postero-dorsal side. The apex of the neural fold, in antero-dorsal side is slightly more angular and appears to be more bent towards dorso-median line. All over the neural plate, the neural fold is more clearly raised up from the surface of the medullary plate.

6. **The border of the oral-sucker is raised up from the surface of the embryo.** The oral sucker has become more wider towards its ventro-median portion, than it was there, in previous stage. Relative to its lateral portion, it is slightly thinner towards its median portion. It appears as a very much darkly pigmented 'U' shaped band, with clear cut borders and is of more or less equal width all over it, across the embryo. All along its length its inner portion is depressed inwards, and its border is raised up from the surface of the embryo.

**External features : four hours later, after the previous stage**

1. **Neural folds approach each other.** 3 hours later, after the previous stage, both neural folds touch each other at the same time, leaving a little gap at the anterior and posterior ends. In next one hour, these gaps also disappear and complete neural tube is formed. At this time,
jelly coverings become almost dissolved and the embryo covered only by the chorion becomes free.

2. **The embryo appears oblong in its shape.** Along the dorso-median line of the embryo, the neural folds are met and are closely attached to each other. When the embryo is viewed from the dorsal side, it is oblong in shape, being elongated antero-posteriorly. At its anterior end it appears more blunt because of the lateral ends of the oral sucker, which are more raised up from the surface of the embryo. The neural folds are closely attached to each other all over the length of the embryo. Angular appearance along their apex, in their antero-dorsal part being disappeared they have rounded surface all over. But they are separated from each other by a prominent groove.

3. **Anlage of optic vesicle is clearly visible and neuromeres are faintly visible.** In antero-dorsal side, the neural folds, gradually, are much thickened anteriorly, as they are turned antero-ventrally. Anlages of the optic vesicle can be clearly recognised in the place inner to the lateral ends of the oral sucker, but above their level. These are more clearly visible as they are slightly protruded anteriorly. Behind the optic bulgings, the neural fold on each side, appears to be constricted into four divisions, indicating the divisions [neuromeres] of the brain internally. Anterior three are slightly smaller than the optic bulgings. And the fourth one is slightly more elongated, i.e., up to the place where posterior thickening of neural crest material (rhombencephalic
portion) meets the neural ridge. These divisions of neural fold cannot be recognised easily. Segmental nature towards posterior part of neural ridge can be recognised, but they are further more faintly visible.

4. **Posterior neuropore can be recognized.** The neural folds are more closely attached to each other, all over, the embryo. But, below the postero-dorsal bent corner of the neural tube, very thin slit, in the form of a dark line is still visible in the blastoporal region.

5. **Mesencephalic portion of neural crest is migrated ventrally, and rhombencephalic portion of it is started its migration.** Posterior thickening of neural crest material (rhombencephalic portion) appears to be more spread and more elongated antero ventrally. Also it appears to be slightly more thickened toward its posterior end. But anterior thickening of neural crest material (mesencephalic portion) cannot be distinguished from the neural ridge. Thus antero-dorsal part of the embryo, in front of the posterior thickening of the neural crest appears smooth. The anterior end of the rhombencephalic portion is become slightly more nearer to the lateral end of the oral sucker.

6. **Border of the oral sucker is much raised up from the surface of the embryo, and is turned over the grooved portion of oral sucker.** In antero-ventral region, the oral sucker appears in ‘V’ shape. All over its length, its inner portion appears to be much depressed. The depressed appearance is mainly due to its border region which is more
thickened further and appears to be raised up more from the surface of the embryo. Also, its thickened border shows a tendency to bend towards the depressed inner portion. At its lateral ends, the oral sucker being more widened further, each lateral portion of oral sucker appears like a spoon in its shape. But, towards its median portion, the oral sucker has become more narrow. Towards its median portion, thickening of its border and raised up appearance of its borders, from the surface of the embryo is also very less. The thickened border of oral sucker appears very pale in color in contrast to its darkly pigmented inner depressed portion.

7. **Neural tube remains more straightened antero-posteriorly.**

Postero-ventral side of the embryo, is become more thicker than its antero-ventral side. Along dorso-median line, neural tube portion is straightened antero-posteriorly. The postero-dorsal portion of the embryo is more narrow than its bulky postero-ventral region or wider antero-dorsal region or narrower antero-ventral regions. Also, postero-dorsal region of the embryo, as it is extended dorsally, it becomes situated gradually more and more posterior to the level of the postero-ventral region.

Internal features: four hours later, after the previous stage

1. **The epidermal ectoderm and the neural ectoderm cut off from each other, along the dorso-median line of the neural tube.** The median portion of medullary plate is further moved ventrally along the
median plane, and the apexes of the neural folds are moved towards dorso-median line, approaching each other along the dorso-median line. Thus neural tube is formed all along the dorso-median region of the embryo, but still imperfectly.

The neural folds from both sides seem to be met each other along the pigmentation lines from which the region of medullary plate and neural ridges were separated from each other, in the previous stages. At this point of contact the epidermal ectoderm and neural ectoderm are cut off from each other, all along the dorso-median line. But still in this stage, cut ends of both sides are not completely fused each other, both being held just side by side. The epidermal ectoderm is clearly separated from the neural tube, all over the length of the embryo. But it is not completely raised up from the place where it becomes cut, so that dorso-median surface of the embryo would have become smooth; Still, epidermal ectoderm of both sides is separated by a prominent groove along the dorso-median region of the embryo, as it is visible externally.

2. Sensorial layer of the neural ectoderm, still, is not fused along the dorso-median line of the neural tube. An impression of separation groove is also present along dorso-median portion of neural tube. Portions of neural tube from both sides are attached to each other along the dorso-median line, only through the epithelial layer of the medullary plate, i.e., the ependymal layer of the neural tube. But the sensorial layer portions from both sides are still separated from each
other by an impression of the groove. Attachment of both of its lateral portions of the neural tube is more clear in antero-dorsal side. But in postero-dorsal side, still the neural tube is 'U' shaped.

3. **Anterior neuropore is in existence, in dorsal surface of the optic vesicles.** In antero-dorsal surface of the optic vesicles, the sensorial and epithelial layers of the neurectoderm are very closely attached to each other. Here, lateral portions of the neural tube i.e., portions of optic vesicles from both sides, appear to be separated from each other, just by penetration of the epithelial layer, neither neurocoel is formed, nor portion of penetrated ependymal layer is not broken from portion of epidermal ectoderm, both being continuous. Thus, this small region between optic bulgings represents the anterior neuropore. But suddenly posterior to this small region of neuropore, the neurocoel lined by ependymal layer starts to appear.

4. **The blastopore is become moved inside the embryo, forming there the neurenteric canal.** As the portions of neural fold around the slit-shaped blastopore become approached dorso medially, the blastopore becomes shifted inside the embryo, to a position that is anterior and dorsal to its position in previous stage. Now its orientation is neither vertical nor horizontal, with its upper end being situated more anteriorly than its lower end. Thus it is situated in between posterior end of the neurocoel and that of the archenteron [now can be called as gut cavity], making the two cavities continuous, inside the embryo.
Thus the neurenteric canal is clearly formed. In postero-dorsal side of the embryo, as in previous stage, the dorso-median region of the gut roof is closely placed with thick somitogenic portion, nearer to the neurenteric canal. This portion of gut roof is narrow laterally. At its posterior end, this laterally narrower endodermal portion joins the neurenteric canal.

5. The neurenteric canal opens outside, in the postero-dorsal direction, forming the posterior neuropore. The neurenteric canal instead of being slit-shaped as the blastopore was in previous stage, it is become opened again. But adjoining portion of neurocoel, immediately is a narrow space in contrast to more spacious neurenteric canal. More or less up to the anterior end of the neurenteric canal, the epidermal ectoderm is cut off from the neural tube and remain attached, as it is described above. Posterior to this, above the space of the neurenteric canal, lateral sides of the neural groove, have become more closer. But epidermal ectoderm without being cut, this portion of neurenteric canal opens outside, in postero-dorsal direction. This posterior neuropore, is clearly present as a slit-shaped opening in this stage. Again at the posterior tip of the posterior neuropore, the epidermal ectoderm is cut off from the neural tube, and forms a continuous layer.

6. Mesodermal and endodermal cells cannot be distinguished clearly from each other, in the region of neurenteric canal. Posterior to the
notochord [all over the border of the posterior neuropore], the portion of neurectoderm is suddenly more thicker posteriorly. In this portion, single layer of elongated, columnar, cells of neurectoderm, with more darker pigmentation is clearly visible. But mesodermal cells cannot be distinguished so clearly from endodermal cells which constitute the portion of neurenteric canal [i.e., posterior to the notochord in lateral sides of the neurenteric canal]. Here, cells of both layers are closely placed to each other and are more or less same in their size and shape.

7. Region of proctodeaum is distinguished, below the neurenteric canal. As the neurenteric canal is formed, the dorsal part of slit-shaped blastopore of the previous stage with thicker notochordal portion and thinner endodermal portion, forms the anterior wall of the neurenteric canal. The ventral part of the slit shaped blastopore which was with thicker endodermal layer and thinner mesodermal layer in previous stage, is moved more inside the archenteron [hindgut] in this stage, as the neural tube is formed at the posterior tip of the medullary plate. The median area of archenteron, ventral to and just adjacent to this inwardly moved thicker endoderm represents the area of future hindgut and proctodaeum. In the future region of proctodaeum, the endoderm unlike in the ventral lip of blastopore, is suddenly more thin. The ectoderm of this region is slightly more thicker, and it is broken in between lateral thickenings. The proctodaeal portion of endoderm is closely attached to the broken region of the ectoderm, the mesodermal portion being clearly away from this attachment.
8. **The neurocoel is slit-shaped.** As the neural tube is formed, it becomes elongated, antero-posteriorly. It further becomes more thick at its anterior end forming the optic vesicles. The cavity of the tube, i.e., neurocoel remains more or less slit-shaped. The neurocoel is slightly wider laterally and narrow in its dorsal and ventral sides. This shape of neurocoel varies slightly in different regions of the neural tube, all along the length of the neural tube.

9. **The wall of the neural tube is thinner in its ventral portion, and is thicker in its lateral portion.** The ependymal layer is very closely attached to the sensorial layer of the neural tube. The neural tube is thinner in its ventral side and is thicker in its lateral sides. Its lateral sides are more or less equally thickened all over, in dorso-ventral direction. At its anterior end, where the neural tube is very much thickened, a median groove is present along the ventro-median line of the outer surface of the neural tube. In this thickened, anterior portion, its ventro-lateral sides are slightly more thicker, than its lateral sides. This ventro-lateral portion forms the lower surface of the optic bulgings and short adjacent portion, posterior to it. This region is situated more anterior to the anlage of notochord and is above the anterior tip of the archenteron.

10. **Neural tube formation towards its anterior end seems to be due to three simultaneous actions.** In the transverse region of the
medullary plate, just posterior to the transverse neural ridge, the formation of neural tube appears to be due to three simultaneous actions, in the region. First one is the thickening of the region of the optic vesicle, at the antero-lateral ends of the medullary plate, as the thickened walls of the optic vesicles are formed in this stage. Second one is that the triangular shaped region [with its broader ends towards the optic vesicles] of medullary plate is more actively depressed medio-ventrally, simultaneously extending, laterally forming the anlages of optic vesicles. This feature is externally visible, two hrs. later after the previous stage, as described in external features of the embryo. The third action is the completion of the neural tube formation by approach of neural folds, as it happens in posterior region of the medullary plate.

11. **Main divisions of central nervous system are distinguished in the neural tube.** As it is externally indicated by slight segmentations of the neural ridge, the neural tube is clearly separated by constrictions into three main regions of the future brain, i.e., prosencephalon, mesencephalon and diencephalon, and into the region of spinal cord. Prosencephalon is the region that is internal to the externally visible, optic bulgings. The mesencephalon at the antero-dorsal bent region of the neural tube [now called as cephalic flexure], seems to be formed of three segments, posterior to the optic bulgings. Posterior to this is situated, the rhombencephalon, which is visible externally as a longer segment, posterior to the segment of the mesencephalon. Remaining all
of the posterior portion of the neural tube posterior to rhombencephalon represents the region of spinal cord.

12. **Rhombencephalon and anlage of spinal chord are not clearly distinguished from each other.** Because of very much thickened walls of the optic vesicles the prosencephalon is the thickest region of the neural tube. The constriction between prosencephalon and mesencephalon being more narrow, the mesencephalon is narrow at its anterior end and wider at its posterior end, where it meets the rhombencephalon. The anterior end of the rhombencephalon is slightly more wider than the mesencephalon, as the mesencephalon meets rhombencephalon. The rhombencephalon being more larger of the three portions of the brain, continues posteriorly into the anlage of spinal cord without much demarcation from the spinal cord.

13. **Cavities of divisions of the anlage of brain, still are not clearly distinguished from each other.** The regions of brain are clearly distinguished from each other, on the surface of the neural tube. But in neurocoel not so much distinct separation can be done. As the slit-shaped neurocoel enters the portion of neural tube, anterior to cephalic flexure, the ventral portion of neurocoel widens laterally into the anlages of optic vesicles. Also, it enlarges posteriorly and widens at its ventral aspect, as it enters the neurenteric canal.

14. **Ventral portion of prosocoel is slightly extended laterally, to form the anlage of optic vesicles.** In this stage, it is just represented
by prosocoel bounded by thickened wall of the optic vesicles. The prosocoel is more wider in its ventral side, as it is derived from the transverse anterior portion of the medullary plate. It is narrow along its antero-dorsal side, as the neurocoel is all over the neural tube. Simultaneously the prosocoel from its ventral side is slightly extended laterally towards the thickened wall of the neural tube, forming the anlage of the optic vesicle.

15. **Wall of the prosencephalon is equally thickened in its lateral and ventro-lateral portions.** The prosencephalon is slightly larger than the mesencephalon in its antero-posterior length. Along, about posterior half of its length, the prosocoel is gradually narrow, but not as much as it is along the dorso-median line. In more than half of its length, from its anterior end to posterior end, mainly in its lateral sides, the prosencephalon is bounded by thickened wall of anlage of optic vesicles. The prosencephalon is almost equally thickened in lateral and ventro-lateral portions. But it is slightly more thicker in its antero-lateral portions. In its ventral side, it is thinner because of ventro-median groove, in its outer surface.

16. **The neurocoel is slit-shaped and neural tube is of circular contour, from mesencephalon onwards posteriorly.** As the lower and posterior portion of prosocoel, which is slightly wider, enters the mesencephalon, the mesocoel becomes completely slit-shaped, i.e., the typical shape of the neurocoel. Thus the mesocoel and rhombocoel
cannot be distinguished from each other by the shape of their ventricles. So also, not by the shape of the wall of the neural tube constituting them. From mesencephalon onwards, posteriorly, neurocoel is slit-shaped and neural tube has circular contour generally, up to the region nearer to the neurenteric canal. Nearer to the neurenteric canal lower portion of neurocoel slightly widens again and wall thickness increases in ventral side of the region of the spinal cord.

17. Rhombencephalon and anlage of spinal cord are also segmented, but very faintly. The rhombencephalon and mesencephalon are clearly separated from each other. But the portion of rhombencephalon can be recognised to be divided into two portions, although it is very difficult to perceive it from the surface of the embryo. Internally, it is formed of two equal segments, by two, shallow faintly visible constrictions, one in its middle and the other at its posterior end, i.e., the region where the rhombencephalic neural crest material meets the neural tube. Posterior to this, segmentation of region of spinal cord can be perceived internally, but not so clearly. The rhombencephalic segments are larger than the segments of the spinal cord. Thus portion of rhombencephalon can be recognized as a segment distinguished from the portion of spinal cord, although not as much clearly as it is from mesencephalon.

18. Cells of ependymal layer appear to be elongated towards outer surface of the neural tube. Cells of the ependymal layer, being very tightly and closely attached to the remaining cell mass of the neural
tube, they appear to be arranged in a radiating manner, from the surface of the neurocoel to outer surface of the neural tube. They appear to be elongated radially, at right angles to the surface of the neurocoel. The inner and lateral borders of the cells are pigmented. The inner end of the cells appear to be slightly narrower, and pigmented lateral borders appear to be radiating away from the surface of the neurocoel. This elongation can be perceived just on the side of the neurocoel only.

19. **Neural crest material is started to migrate ventrally.** Dorso-laterally situated ridges of mesencephalic and rhombencephalic neural crest material are split along their length into separate segments. Cells of these segments are started to migrate ventrally spreading over the epidermal ectoderm. As they are moved downwards, they become spread dorso-ventrally, forming the anlages of visceral arches. Formation of anlages of visceral arches is more advanced towards anterior end. Thus, anlages of anterior portion of Meckel’s cartilage, mandibular arch, and hyoid arch are clearly developed. Anlage of first branchial arch is distinguishably appeared.

20. **Mesencephalic neural crest material is segregated into two portions.** The mesencephalic neural crest material becomes divided into two segments, anterior smaller and posterior larger. They are migrated to the ventro-lateral portion of the embryo, and anlages of anterior portion of Meckel’s cartilage and mandibular arch are formed.
21. **Anterior portion of mesencephalic neural crest forms the anlage of anterior portion of Meckel's cartilage.** The neural crest material of anterior smaller segment, is migrated to the postero-ventral portion of the optic vesicle, forming the anlage of the anterior portion of Meckel's cartilage. The anlage of Meckel's cartilage is situated along antero-posterior axis, with its thicker posterior end and narrower anterior end. Towards its anterior end the cells are more dispersed. More of the much darkly pigmented cells of mesencephalic neural crest are migrated with the anlage of the anterior portion of Meckel's cartilage. These cells constitute the antero-lateral side of the anlage of the Meckel's cartilage.

22. **The anlage of anterior part of Meckel's cartilage occupies the position between the anterior end of the optic bulging and lateral end of the anlage of oral sucker.** The thicker and more compact posterior portion of the anlage of anterior part of Meckel’s cartilage is situated on the inwardly thickened, wider, lateral portion of the oral sucker. From here, its narrow anterior portion of loose cells extends anteriorly. This extension is mainly on ventro-lateral sides of the optic bulging. These anteriorly extended cells show a tendency to attach to the epidermal ectoderm. These cells are spread anteriorly, covering a distance more than half of the optic bulging. Thus the anlage of anterior part of Meckel’s cartilage occupies the region of epidermal ectoderm of sense plate between the anterior end of the optic bulging and wider lateral end of the oral sucker. These anteriorly spread cells
almost reach gradually the anlage of hypophysis, but separated from it by spread of mesenchymal cells in between. Towards anterior end of the anlage of hypophysis, the number of cells of mesenchyme gradually increases, but that of anlage of Meckel's cartilage decreases.

23. The anlage of anterior part of Meckel's cartilage is very short segment. The anlage of anterior portion of Meckel's cartilage being very short segment, extending antero-posteriorly, its thicker, posterior portion is placed just lateral to the anterior most tip of endodermal layer. This portion of anlage of Meckel's cartilage extends upwards for a short distance, in postero-lateral side of the optic bulging. As it extends upwards it becomes narrow in its thickness.

24. Posterior portion of mesencephalic neural crest forms the anlage of mandibular arch. Posterior to the anlage of Meckel's cartilage, is formed the anlage of mandibular arch, by posterior larger segment of the mesencephalic neural crest portion. It is very narrow towards its dorsal end. But, much thickened towards its lower end. Major portion of its lower side is situated posterior to the optic vesicle and posterior to the posterior end of the anlage of anterior part of the Meckel's cartilage. Posterior to the anterior part of anlage of Meckel's cartilage, the anlage of mandibular arch is very closely placed to it and is almost at right angles to it. Mandibular arch is spread in dorso-ventral direction.
25. **Lower portion of anlage of mandibular arch is situated at the level of the anterior end of the gut cavity.** Being very much larger and thicker, the anlage of mandibular arch, at its postero-ventral side, is spread over inner bulging of the oral sucker, well below the level of posterior portion of the anlage of the anterior part of Meckel’s cartilage. But it gradually becomes narrow anteriorly, as it becomes closely apposed to the anlage of anterior part of Meckel’s cartilage.

Much of the larger mass of the anlage of mandibular arch is situated in ventro-lateral portion of the optic vesicle. About half of this thicker portion, in its anterior side, is extended inwards ventral to the optic vesicle. In remaining posterior half, it is spread along epidermal ectoderm. The large lower portion of the anlage of mandibular arch is situated at the level of anterior end of the gut cavity. As the anlage of mandibular arch extends upwards dorsally, it becomes narrow, with gradual decrease in its thickness, all along its length.

26. **Anlage of hyoid arch in its structure, resembles the anlage of mandibular arch.** This anlage seems to be formed from the anterior portion of the rhombencephalic neural crest material, as the rhombencephalic neural crest material appears externally to be more extended, towards wider lateral end of the oral sucker, simultaneously appearing more spread antero-posteriorly. It is also spread dorso-ventrally, posterior to the anlage of mandibular arch. But its lower end is above the level of lower end of the anlage of mandibular arch.
The anlage of hyoid arch is situated at the level of anterior portion of the notochord where it is bent downwards. The anlage of hyoid arch is wider at its ventral end and narrow at its posterior end, but not as contrastive as the anlage of mandibular arch, and is gradually reduced in its size in its dorsal part.

27. First to fourth branchial arches seem to be derived from the posterior part of the rhombencephalic neural crest. In the region of posterior part of rhombencephalic neural crest material, which is still very clearly visible externally, two streams of downwardly migrated neural crest material can be easily distinguished, although both streams are not progressed much downwards, and although both are not clearly separated from each other along antero-posterior axis of the embryo.

The anterior stream is slightly more advanced downwards thus, representing the anlage of first branchial arch. The posterior stream as it is indicated externally, is more thickened than the anlage of first branchial arch. It seems it is the mass of neural crest material, from which 2nd, 3rd, and 4th branchial arches are to be formed.

The anlage of branchial arches is situated well above the level of ventral end of the anlage of hyoid arch. The anlage of first branchial arch towards it ventral side is clearly separated from the anlage of hyoid arch, but its separation from posterior thicker stream can only be perceived. Towards their dorsal side these two streams of neural crest turn posteriorly, and still are connected to each other there. Because,
these two streams are much shorter, and are less migrated ventrally, they do not appear to be spread dorso-ventrally.

28. It is difficult to distinguish the cells of sensorial layer of epidermis, from the cells of head mesenchyme and dispersed cells of neural crest, in antero-dorsal side of the embryo. Along the dorsal portion of epidermal ectoderm, presence or absence of sensorial layer cells cannot be recognized clearly. This is furthermore difficult in antero-dorsal side, as the downwardly migrating neural crest cells and mesenchyme cells are dispersed in between epidermal ectoderm and neural tube. Again, only among the cells of neural crest material spread in between, it is difficult to judge whether they are of anlages of visceral arches, or are the loose cells of neural crest which were present, in previous stage in between compact neural crest material and thickened sensorial layer of medudllary plate. Also, above the neural tube, it is difficult to distinguish between cells of neural crest from cells of sensorial portion of the neural tube.

29. The anlage of oral sucker is thickened due to its furthermore elongated cells of the epithelial layer. The oral sucker is much more thickened towards its lateral, wider, spoon-shaped end. Its thickness gradually decreases towards its median portion. The increased thickness of oral sucker is due to larger, furthermore elongated cells of the epithelial layer. In its spoon-shaped lateral end, it is more thicker towards its posterior margin, than it is in its anterior margin. As this
spoon-shaped portion extends ventrally, both its anterior and posterior margins become more thicker, than the portion in between, thus forming a groove in between. In the groove, the epithelial cells are less elongated. As the oral sucker extends ventro-medially, the thickened borders tend to overlap the grooved median portion; the grooved median portion becomes slightly bulged inside the embryo. The thickness of the borders gradually decreases as the oral sucker extends ventrally.

30. Epithelial layer of anlage of oral sucker is formed of pigmented and non-pigmented cells, and pigmented cells are of bottle-shape. Formation of oral sucker by two types of elongated cells of epithelial layer, i.e., pigmented and non-pigmented is clearly visible in this stage. Former are prominently present in wider, lateral ends of the oral sucker, and less towards the median portion of the oral sucker. Number is more in depressed inner portion of the oral sucker, less along the thickened border.

Wherever, they may be along the oral sucker, the pigmented cells are bottle-shaped, and narrow neck portion of the cell is directed towards outer surface of the embryo, wider portion being inside. The non-pigmented cells are spread in between the pigmented cells, being elongated inside to outside. In the medio-lateral portion of the oral sucker, where the groove along the oral sucker appears deep, the groove also appears more darker than the wider lateral ends of the oral sucker, in spite of more number of pigmented cells in the lateral ends. This is because the outer surface of oral sucker in the groove being more
concave, the whole surface is lined with narrow necks of the pigmented cells: the outer surface of the non-pigmented cells appears to be placed inwards between these narrow outer ends of these pigmented cells. In the borders of oral sucker, narrow ends of pigmented cells, are separated from each other by wider outer portion of non-pigmented cells.

31. **Region of nasal placode can be recognized.** At the anterior most tip of the embryo, in between epidermal ectoderm, and anterior border of the prosencephalon, the sensorial layer cells become slightly elongated antero-posteriorly and appear larger also. Thus, the portion of the sensorial layer of this region becomes recognizably thicker indicating the region of nasal placodes. It is slightly narrow along the median line of the embryo.

32. **Epibranchial and dorso-lateral placodal regions are recognizably developed.** Like sensorial layer cells of nasal placodes, the sensorial layer cells of epidermal ectoderm are slightly larger and elongated laterally, in antero-lateral sides of the embryo, posterior to the anlage of mandibular arch. The thickening of the lateral placode gradually decreases dorsally and ventrally. In dorsal half portion the thickening extends more posteriorly, reaching the anlage of hyoid arch. The placodal portion, towards its dorsal portion becomes slightly bent inwards, along dorso-ventral axis, in between the anlages of quadrate cartilage [upper portion of mandibular arch] and hyoid arch, as the
epidermal ectoderm is bent inwards. Nearer to the anlage of hyoid arch it is more thickened further, so that this thickening appears to be much thicker than the nasal placode. Thus, in this stage, the region of dorso-lateral placode is more recognisably developed, than the ventral region of epibranchial placode.

33. **Anlage of epithelial hypophysis is appeared and Rathke's pocket in it can be recognized very slightly.** A mass of sensorial layer cells is projected inside the embryo, and is closely placed to the ventro-median portion of the prosencephalon. As this mass originates from the epithelial layer it is more wider. As it extends posteriorly, it suddenly becomes narrow. Its wider anterior portion can be faintly recognized to be somewhat porous, indicating the beginning of formation of Rathke's pocket. The cells of this anlage are also elongated antero-posteriorly. But it could not be recognized whether its maximum posterior extension is by [like in placodal regions] much elongated single cells or by many moderately elongated cells aligned in a row, antero-posteriorly.

The anlage of hypophysis is situated in between anterior most tip of the gut, and the mesenchyme cells that occupy ventro-lateral portion of the optic lobes, along the anterior portions of the anlages of Meckel's cartilage. Posteriorly, it is extended to a level not more than the anterior most tip of gut. Its wider anterior portion is almost above the level of the anterior end of the gut. Anteriorly, this anlage is continuous with the anlage of nasal placode.
34. **Prechordal plate mesoderm is distinguished.** In anterior side, the mesodermal portion of loosely arranged cells is disorganized, and cells are dispersed. In antero-dorsal side, these spread cells show a tendency to be attached to the anlagen of the viscesal arches and neural tube. In a narrow, dorso- median region, in front of the notochord, the single layered portion of mesoderm is not dispersed. Thus, prechordal plate mesoderm is distinguished. Nearer to the rhombencephalon, the dispersed mesenchyme starts to form somites in posterior direction. In antero-ventral side, it gradually becomes arranged in double layered lateral plate mesoderm, in posterior direction. Clear continuity between dorsally appeared somitogenic portion and ventrally appeared lateral plate mesoderm, starts to be visible, posteriorly, at the level of rhombencephalon.

35. **There remains a gap of dispersed mesenchyme in the region of stomodaeum.** Ventrally dispersed mesenchyme, as it starts to form the lateral plate mesoderm, portions of lateral plate mesoderm, from both sides of the embryo, become continuous to each other, below the antero-ventral portion of the gut wall. Thus, lateral plate mesoderm of both sides are met each other in the region of fore gut. But, before they meet, a median gap remains in mesodermal portion, in the antero-ventral region of the embryo. Here, the epidermal ectoderm and anterior part of the gut are more nearer to each other. Thus the region of stomodaeum can be recognized.
36. **Anlage of heart can be recognized.** Double layered structures of lateral plate mesoderm from both sides, before they meet below the wall of the fore-gut, they are more thickened towards their ventrolateral borders, than they are in their lateral sides. This thickened appearance is due to less dispersed, rounded cells. Two to three such cells are there across the thickened border. Such thickened portion of mesoderm, starts to appear from the level of the posterior end of the oral sucker. Thus, portion of anlage of heart can be recognized. It soonly ends posteriorly, as the lateral plate mesoderm of both sides meet below the fore-gut.

37. **Mesoderm becomes thicker in lateral sides of the anlage of proctodaeum.** Posterior to the anlage of heart, when the portions of mesoderm from both sides of the embryo become continuous ventrally, the ventro-median portion of mesoderm remains thinner than the lateral portion of it. Thus mesoderm in ventral portion remains thinner than the lateral portions, upto the posterior end of the embryo. But, nearer to the region of proctodaeum, both portions are more or less of the same thickness up to the lower border of the anlage of the proctodaeum. In postero-lateral side, nearer to the lateral sides of the anlage, it becomes slightly thicker, forming thicker lateral borders of the anlage of the proctodaeum.

38. **Anlage of notochord is clearly distinguished at its anterior portion, and stacked nature of its cells is more clearly visible.**
Anlage of notochord is much extended anteriorly, upto the posterior end of the prosencephalon. At its anterior end it is clearly distinguished from the cells of the adjacent germ layers, and appears perfectly compact, and instead of being narrow at its anterior most end, it appears blunt.

Cells appear to be more stacked, in middle portion. Also stacked nature of cells is clearly visible in anterior portion. In middle portion, stacked cells have more pigmented borders.

39. The mesodermal portion is more thickened, towards the somitogenic ridge. The somitogenic portion in anterior side, is formed of less compactly arranged cells, around relatively larger and imperfect myocoel. Gradually, in posterior direction, posterior to the level of rhombencephalon, it is formed of larger number of cells compactly arranged, around relatively a narrow myocoelic slit. In about anterior half of this compact portion, the mesodermal portion of lateral side, is gradually thicker posteriorly, and it gradually thickens furthermore dorsally into the somitogenic portion. In remaining posterior half of this region, the mesodermal portion of lateral side gradually becomes thinner posteriorly, than it is anteriorly. But the somitogenic portion is further more thickened posteriorly, than it is in anterior half portion; this thickening continues towards the tail-bud. Thus, the mesodermal portion is more thickened, towards somitogenic portion.
40. About four segments of somites are formed. The somitogenic portion is clearly divided across it, into about four segments of somites. Complete separation of first somite from the second one can be recognized by narrow slit, in between. Second, third and fourth somites are clearly separated from each other, but are closely attached to each other, without separation gap in between. The fourth somite is not clearly separated from the remaining posterior portion of the somitogenic ridge, into which somite formation is not progressed further posteriorly.

41. First somite onwards posteriorly, the mesodermal portion is split into two layers: an outer layer and an inner layer. First somite onwards posteriorly, more or less up to the neurenteric canal, the somites and posterior unsegmented portion of somitogenic mesoderm is split into two layers: thinner outer layer and very much thickened inner layer. Both layers are clearly separated all along the dorso-lateral region of the embryo. This split is extended more ventro-laterally, in postero-lateral side, where the somitogenic ridge is not segmented into somites. The outer layer remains uniformly thin all along the region of the split. But it is more thin in posterior side of the embryo. The inner layer is very much thickened into myotomal portion, along the lateral sides of the notochord. But it suddenly becomes as thin as the outer layer, all along the split. The myotomal portion is gradually more and more thickened posteriorly.
42. **Spaces of myocoel and coelom, and regions of myotome, dermatome, and pronephros are distinguished in the somites, first to third.** In somites first to third, the outer thinner layer is slightly projected inwards. The projection is at the level where the myotomal portion ends into the thinner, inner layer. Along their dorsal margin, the portions of outer layer of these somites tends to be attached to the myotomal region. Thus, in these somites, the slit-shaped space between outer and inner layers, slightly can be distinguished as myocoelic space and coelomic space, respectively above and below that inwardly projected portion of outer layer. Also, the portion of outer layer below that inwardly projected portion, appears to be very slightly thicker than the upper portion, indicating the region of pronephros in the future stage, and the upper part indicating the dermatome. The slit between two layers is not extended further downwards, below the portion of pronephros. Also, the myotome is not separated from the inner layer. Thus the parietal layer, visceral layer and coelom are not clearly formed below the level of these somites.

43. **Slit of coelomic space is extended more ventrally, posterior to the third somite, but regions of myotome, dermatome or pronephros cannot be distinguished.** In posterior part of the somitogenic ridge, where the somitogenic portion is not segmented into somites, the slit of coelomic space is extended more downwards. Outer layer from the much thickened inner layer of somitogenic portion is more widely separated towards dorso-lateral side of the embryo. But in outer layer,
no portions of dermatome or pronephros can be distinguished. In the much thickened somitogenic, inner portion, portions of inner layer [i.e., visceral layer] and myotome cannot be distinguished from each other. Because, towards posterior side, the thickening of myotomal portion extends more and more laterally, into the inner layer.

44. Endodermal layer, in posterior side, is clearly separated from the somitogenic ridge, but, still not from the notochord. The separation of endodermal layer from notochord is progressed furthermore posteriorly, and separation from somitogenic region more or less up to the anterior end of the neurenteric canal. Here the cells of posterior end of notochord and those of somitogenic portion are very compactly placed. The endodermal portion here can be distinguished by cuboidal nature of its cells. But this portion of endoderm is closely attached to the notochord and somitogenic cells.

45. Fore-gut, mid-gut and hind-gut can be easily differentiated. The archenteron is extended furthermore postero-ventrally, in the region of the liver diverticulum. The yolk endodermal portion is closely placed to the middle, straight region of the neural tube, with much reduction in archenteron space in the region. Three regions of archenteron which represent three main divisions of the alimentary tract of the future embryo, namely fore-gut, mid-gut and hind-gut can be easily differentiated. The middle region of narrow archenteron space represents the mid-gut. It is bounded ventrally by very much thickened
yolk endodermal layer. The anterior, much wider space of archenteron, i.e., below the brain and anterior tip of the notochord represents the fore-gut, and posterior end of the archenteron, ventral to the posterior end of the neurenteric canal represents the hind-gut.

46. **Anterior end of the fore-gut is more closer to the epidermal ectoderm indicating the region of stomodaeal cleft.** The anterior end of the fore-gut is situated almost below the optic thickenings, and is restricted to the region in between the optic thickenings, as the fore-gut narrows laterally. Along its ventro-median line, as the fore-gut extends posteriorly, it remains away from the body surface, in most of its anterior portion, in front of the liver diverticulum. As the endodermal layer extends more anteriorly along ventro-median line, it extends more and more closer to the ectodermal layer indicating the region of stomodaeal cleft. More closeness is below the level of optic bulging. Also, as the fore-gut is extended more and more anteriorly, it becomes more narrow laterally, Towards anterior side, the cavity of fore-gut is 'V' shaped, with narrow lower end and wider upper end. At the level of the bent anterior portion of the notochord, it widens laterally, as the fore-gut meets the mid-gut and liver diverticulum.

47. **Mid-gut is clearly separated from the anlage of notochord, but hind-gut, still is not.** Posterior to the bent anterior portion of notochord, loose mass of somitogenic mesoderm starts to appear. Here, more or less at the level of dorso-median, anterior end of the mid-gut,
endodermal layer is closely placed with notochord, with spread mesodermal cells in between. But, it becomes separated by notochord, posteriorly, more or less up to the region of hind-gut. Still it is slightly bent towards notochord, as an indication of its attachment to the notochord, in previous stages. Nearer to the hind-gut region, the endoderm is attached to the notochord medially. Still posteriorly, around anterior end of the neurenteric canal, the endodermal and mesodermal layers are very closely attached to each other, and cannot be distinguished easily from each other. But towards posterior end of the neurenteric canal, two portions have been separated clearly and thick endodermal layer is moved inside the archenteron of the portion of the hind-gut. Just below this, the proctodaeum region is clearly formed in median region, as described earlier. Here mesoderm is receded sideways, and ectoderm and endoderm are closely attached to each other.

48. The liver diverticulum is extended upto mid-ventral level of the embryo. Liver diverticulum is formed of two chambers, anterior and posterior. Posterior chamber is formed by evacuation of endodermal cells. Anterior chamber is formed by posterior extension of ventral portion of the fore-gut. Portion of liver diverticulum extends upto mid-ventral portion of the embryo, along ventro-median line, below the thick yolk endodermal portion of mid-gut. Upto its posterior end it is more or less equally wide, but is separated across it into two portions, by a thinner layer of endodermal cells spread dorso-ventrally, more or
less in its middle portion. This layer without being exactly along dorso-ventral axis, its dorsal portion extends antero-dorsally, and continues with the surface of the yolk endodermal cell mass of the floor of the mid-gut. Thus, the anterior cavity of the liver diverticulum suddenly opens in fore-gut.

49. The ventral part of the liver diverticulum is thicker in its anterior portion and thinner in its posterior portion. As the thinner medio-ventral portion of foregut extends towards the liver diverticulum, that thinner portion becomes gradually thickened, as much as the ventro-lateral portions of fore-gut becomes thickened posteriorly. Thus the thickening of endodermal layer bordering the ventral portion of liver diverticulum gradually increases towards the middle portion of the liver-diverticulum where the liver diverticulum becomes separated into two portions, by the spread of thin endodermal layer. Thus the cavity of the anterior portion of the liver diverticulum gradually narrows posteriorly by thickening along its ventral border. Posterior to this, the cavity of posterior portion of liver-diverticulum suddenly widens again. But the thickness of endodermal layer along mid-ventral portion of posterior portion of the liver diverticulum, gradually decrease posteriorly for some distance. But, again it thickens and ends in the yolk endodermal cells, as the cavity of the posterior part becomes narrow and disappear posteriorly.
50. All over the gut cavity, cells lining the cavity are elongated and columnar, at right angles to the surface of the endodermal layer. Towards ventro-lateral sides, below the anterior end of the notochord, and slightly posterior to this, towards ventral sides of the junctional area of liver-diverticulum and fore-gut, the endodermal layer is more thicker, so also, along the region where the endodermal layer meets the yolk endoderm. Except these two places, the remaining portion of endoderm all over the embryo, is formed of single layer of cells. Towards thicker portions, towards yolk endoderm, cells are larger, also more than one cell are present along the thickened region. All over the gut-cavity the cells lining the cavity are elongated and columnar, at right angles to the surface of the endodermal layer. This is prominently seen towards the thicker portions of the gut-wall. Single layer of yolk-endodermal cells, which line the floor of mid-gut constitutes the largest, and more elongated cells of the endodermal layer. Below this, the cell mass of yolk endoderm still appears more porous in the region of blastocoel. In postero-ventral region of yolk endoderm, smaller, dispersed, endodermal cells are present and can be distinguished easily from the larger yolk endodermal cells.
Explanations to the figures in plate - 12

Figs. (1 to 5) Neural fold stage; cross sections, 12th (Fig 1), 15th (Fig 2), 21st (Fig 3), 48th (Fig 4) and 122nd (Fig 5) of totally 148 serial cross sections, into which whole embryo was cut. Cutting started from the anterior end of the embryo.

(a) endoderm, (b) transverse neural ridge, (d) mesodermal cells, (g) mesencephalic neural crest cell mass, (i) anlage of notochord, (e) sensorial layer of epidermal ectoderm, (j) lateral neural ridge, (k) anlage of oral sucker, (l) thickest portion of lateral neural ridge, (q) archenteron, (r) mesoderm, (f) neural crest cells, (x) artifact, (t) rhombencephalic neural crest cell mass, (u) neural groove, (v) somitogenic mesoderm.

Figs. (6 to 7); Neural fold stage, lateral sections, 37th (Fig 6) and 62nd (Fig 7), of totally 135 serial lateral sections, into which whole embryo was cut. Cutting started from left side of the embryo.

(s) (in fig 6) mesencephalic neural crest cell mass, (in fig 7) ectoderm (t) rhombencephalic neural crest cell mass, (c) vestige of blastocoel, (m) portion of somitogenic ridge where somites are started to be formed, (v) somitogenic ridge, (a) endoderm, (r) mesoderm, (k) oral sucker.
Explanation to the figures in plate - 13

Figs. (1 to 7); Neural tube stage, cross sections, 60th (Fig 1), 70th (Fig 2) 77th (Fig 3) 107th (Fig 4), 121st (Fig 5), 151st (Fig 6) and 156th (Fig 7) Of totally 180 serial cross sections, into which whole embryo was cut Cutting started from the anterior end of the embryo

(s) endoderm, (t) rhombencephalon, (v) fore-gut, (w) anlage of notochord, (y) anlage of branchial arches, (d) anlage of spinal cord, (e) anlage of liver diverticulum, (f) region where anlage of pronephros is appeared in next stage (g) dermatome, (h) myocoel, (i) myotome, (x) artifact (j) parietal layer of mesoderm, (k) visceral layer of mesoderm, (l) mid-gut, (ab) hind-gut.
Explanation to the figures in plate - 14

Figs. (1 to 7); Neural tube stage, lateral sections, 9th (Fig 1), 12th (Fig 2), 15th (Fig 3), 17th (Fig 4), 23rd (Fig 5), 25th (Fig 6), 29th (Fig 7), of totally 91 lateral serial sections, into which whole embryo was cut. Cutting started from left side of the embryo. [Cut is not exactly parallel to the median plane of the embryo]

(r) anterior portion of anlage of Meckel’s cartilage, (p) anlage of mandibular arch, (z) anlage of hyoid arch, (It) anlage of 1st branchial arch, (la) anlage of 2nd to 4th branchial arches, (b) anterior somites, which are formed of loosely arranged cells, (h) myocoel, (i) myotome, (g) dermatome, (o) prosencephalon, (u) mesencephalon; (t) rhombencephalon, (f) region where anlage of pronephros is appeared in next stage, (or) oral sucker, (q) head mesenchyme
Explanation to the figures in plate - 15

Figs. (1 to 4); Neural tube stage, lateral sections; 32\textsuperscript{nd} (Fig 1), 34\textsuperscript{th} (Fig 2), 38\textsuperscript{th} (Fig 3) and 45\textsuperscript{th} (Fig 4), of totally 91, serial lateral sections into which whole embryo was cut. Cutting started from left side of the embryo. [But cut is not exactly parallel to the median plane of the embryo]

(t) rhombencephalon, (u) mesencephalon, (o) prosencephalon, (p) anlage of mandibular arch, (b) somites, (aa) somitogenic ridge, (q) head mesenchyme, (w) anlage of notochord, (me) rhombocoel, (af) neurenteric canal, (pp) proctodaeal plate, (ab) hind-gut, (l) mid-gut, (v) fore-gut, (e) anlage of liver diverticulum, (mc) mesocoel, (ma) prosocoel, (m) anlage of hypophysis, (s) endoderm, (or) oral sucker, (na) unspread head mesenchyme in prechordal plate
Explanation to the figures in plate - 16

Figs. (1 to 5) Neural tube stage; horizontal sections, 30th (Fig 1), 37th (Fig 2), 43rd (Fig 3), 50th (Fig 4), and 56th (Fig 5) of totally 125 serial horizontal sections, into which whole embryo was cut. Cutting started from ventral side of the embryo, [But, cut is not exactly at right angles to the median plane of the embryo]

(q) head mesenchyme, (m) anlage of hypophysis, (e) anlage of liver diverticulum, (s) endoderm, (v) fore-gut, (ab) hind-gut, (p) anlage of mandibular arch, (o) prosencephalon, (pp) proctodaeal plate, (or) oral sucker, (r) anterior portion of anlage of Meckel's cartilage (nl) mesoderm
Explanation to the figures in plate - 17

Figs. (1 to 6); Neural tube stage, horizontal sections 66th (Fig 1), 70th (Fig 2), 79th (Fig 3), 86th (Fig 4), 97th (Fig 5), and 111th (Fig 6) of totally 125 serial horizontal sections, into which whole embryo was cut. Cutting started from the ventral of the embryo. [But, cut is not exactly at right angles to the median plane of the embryo]

(l) mid-gut, (af) neurenteric canal, (nc) posterior neuropore, (ab) hind-gut, (v) fore-gut, (p) anlage of mandibular arch, (z) anlage of hyoid arch, (It) anlage of first branchial arch, (la) anlage of second to fourth branchial arches, (ma) prosocoel, (mc) mesocoel, (n) anlage of optic vesicle, (o) prosencephalon, (t) rhombencephalon, (nd) region of anterior neuropore, (mn) neurocoel of anlage of spinal cord, (w) anlage of notochord, (b) somites, (j) parietal layer of mesoderm, (k) visceral layer of mesoderm, (q) head mesenchyme
Advanced neural tube stage; (stage 16+, two hrs. later); total length of the embryo 2.0 mm.; age 34.35 hrs.

External features

1. Tail bud is extended further posteriorly, beyond the level of proctodaeum. The epidermal ectoderm is completely fused all along the antero-posterior axis in dorso-median line of the embryo. The impression of the median groove along it is disappeared. From stage (13) onwards, as the embryo reaches this stage, it gradually becomes more elongated antero-posteriorly, and is decreased in its width across. The head and trunk portions of the embryo, are distinctively separated, as the embryo across it, is become narrow in between the anlage of oral sucker and trunk portion. The tail-bud is extended further posteriorly in postero-dorsal direction of the embryo, beyond the level of the proctodaeum. The portion of trunk narrows in postero-dorsal direction and smoothly ends in the tail-bud.

2. Portion of brain is distinctly visible, as anlages of visceral arches are migrated furthermore downwards. The anlage of hyoid arch is elongated further, dorso-ventrally, and its lower end is migrated to a level below the lateral end of the oral sucker. Parallel to and very close to the anlage of hyoid arch, the anlage of branchial arches is also migrated furthermore ventrally, but not as much as the former. Dorsal end of the latter remains at a level above that of the former. Both anlages are migrated well below the level of anlage of brain. The anlage of brain
is clearly distinguished from these two anlages. Both anlages of visceral arches remain as two bulges that are elongated dorso-ventrally, below the level of portion of brain. The anlage of branchial arches is more wider along its antero-posterior axis, than the anlage of hyoid arch is, along that axis. Both, all along their dorso-ventral length, appear to be equally widened along their antero-posterior axis.

3. **First visceral pouch is clearly formed.** The anlage of quadrate cartilage can be perceived as a dorso-ventral thickening, in front of the anlage of hyoid arch, and more closer to the posterior margin of the lateral end of the anlage of the oral sucker. But, anlages of quadrate cartilage and hyoid arch are separated widely, as the embryo across it, is narrow in between these two anlages. Thus the anlage of first visceral pouch is clearly formed. The anlages of hyoid and branchial arches being closely attached to each other, the region of visceral pouch between them remains very shallow.

4. **A darkly pigmented line of hatching gland is developed along dorso-median line of the anlage of brain.** The dorsal portion of the embryo is much elongated antero-posteriorly, with further, posterior extension of the tail-bud. Postero-ventral thicker portion of yolk endoderm gradually becomes furthermore narrow as it extends posteriorly, and smoothly ends in the tail-bud. Dorso-median portion of embryo, appears more narrow, than it is in previous stage, with furthermore elongation of the neural tube.
With further ventral migration of the anlages of visceral arches portion of anlage of brain in this stage appears as a prominent portion in antero-dorsal side of the embryo. The portion of brain is rounded dorsally, as the impression of groove on it is disappeared. But, there is developed a darkly pigmented line all over its dorso-median line, posterior to the place of anterior neuropore.

5. Anlage of spinal chord is not as distinct as the anlage of brain is. The middle portion of the neural tube becomes clearly exposed outside, with ventral migration of the anlage of the branchial arches. But, this middle portion is not clearly distinguished from the surface of the body, like the portion of brain. Here, the dorso-lateral portion of the embryo, smoothly narrows towards the dorso-median line of the embryo, without distinct appearance of the portion of the neural tube. The portion of tail-bud gradually becomes more wider laterally than the embryo is in its middle dorso-lateral portion, and becomes smoothly narrowed towards dorso-median line of it. Here, also, portion of neural tube cannot be distinguished easily from the surface of the tail-bud.

6. The region of proctodaeum remains in a position that is ventral to the tail bud. Posterior to the dorso-median pigmentation line of the portion of the brain, the portion of the neural tube, along its dorso-median line is pale in its coloration. This coloration is extended posteriorly around the depression of the proctodaeum. The region of proctodaeum, which was in vertical position at the posterior end of the embryo, is now situated in the ventral region of the tail-bud, as the tail-bud is extended
slightly more posteriorly. The proctodaeum appears as a deeper, distinctly formed depression, and is narrow laterally. Also this region appears smaller and more rounded in its contour.

7. **Optic bulging is extended postero-laterally.** More anteriorly situated bulgings of the eye, are extended further more laterally up to the lateral end of the oral sucker, more closer to it. The optic bulgings are now in a position that is slightly lateral and slightly ventral and more posterior to their position in previous stage. Also, they are slightly more bulged, covering larger surface area.

8. **Anlage of nasal pits is appeared.** It is appeared as a darkly pigmented, slightly transversely elongated median region, just at the anterior end of the medio-dorsal pigmented line of the portion of the brain. By closer observation, it can be recognized that there is a dot-like, darkly pigmented, larger region at each lateral end of this transverse portion. These dot-like portions are slightly above the level of the inner transverse portion. Thus anlages of nasal pits are clearly appeared in this stage. The narrow transverse portion between these, seems to be the region of anlage of hypophysis.

The anlages of nasal pits are just adjacent to the optic bulgings, in the anterior side of the optic bulgings. The region of anlages of nasal pits and that of hypophysis somehow is glossy in its appearance.
9. **Anlage of pronephros is appeared.** Just posterior to the dorsal end of the anlage of branchial arches, a slight bulging of the anlage of the pronephros can be easily recognised.

10. **Anlage of tail-fin can be recognized.** Portion of tail-bud can be distinguished from the portion of postero-ventral trunk portion, by a slight depression, in between the two. This depression is extended further posteriorly in between the region of proctodaeum and tail-bud. Along the above mentioned pale colored, dorso-median region of the neural tube, a narrow ridge can be distinguished in the region of tail-bud. Its extension can be recognised upto the dorsal end of the proctodaeum. Thus the region of fin-fold is appeared. Towards the proctodaeum, gradually it reduces.

11. **The grooved portion of the oral sucker is not visible externally, and posterior part of oral sucker appears to be degenerating.** The borders of oral sucker appear to be much thickened further and grooved region along it, is completely overlapped by the borders. Thus the grooved region is not visible externally. The posterior border in its portion just adjacent to the spoon-shaped lateral ends of the oral sucker appears to be raised up furthermore from the surface of the embryo, The anterior border of the oral sucker also is much raised up from the surface of the sense plate. But the spoon-shaped lateral ends of the oral sucker remain more or less same, in their shape and size. The ventro-median ‘V’ shaped portion of the sense plate appears to be shrinked and degenerating.
12. The oral groove is more distinctly formed. The stomodeal invagination is clearly recognizable, as a median, dorso-ventrally elongated depression in the upper portion of the sense plate. This is the region in between the anlagen of nasal pits dorsally and lateral ends of the oral sucker ventro-laterally. Along median line, the groove disappears ventrally, in the shrinked portion of sense plate indistinguishably. Above the dorsal end of the stomodeal invagination, the portion of brain is extended slightly more anteriorly in relation to the dorsal end of the stomodeal invagination. By keen observation, it can be perceived that the lateral sides of stomodeal invagination are slightly depressed inwards. Thus portions of upper and lower jaws of the future mouth can be recognised in this stage.

Internal features

1. The epidermal ectoderm is become a continuous layer over the whole embryo, with disappearance of anterior and posterior neuropores. The cut margins of epidermal ectoderm from lateral sides of the embryo, are completely fused to each other, along the dorso-median line of the embryo. In the region of anterior neuropore the portion of epithelial layer of ectoderm which was just penetrated as ependymal layer, is cut off from its penetrated portion, and its cut lateral margins are fused along the dorso-median line of the optic thickening. Simultaneously, it is become continuous with margins of epidermal ectoderm adjacent to it, at its anterior and posterior sides, along the median line. Thus the anterior neuropore becomes disappeared.
The lateral sides of the neurenteric canal are completely fused along the median line. Thus the continuity between the neurocoel and gut cavity is disappeared in this stage. The epidermal ectoderm along the outer rim of the canal is broken and is fused along the median line. It is also become continuous with its anterior portion that is already fused along the dorso-median line.

2. The neural tube is completely formed into a tubular structure. Along the dorso-median line of the neural tube, portions of sensorial layer of it from both lateral sides, are completely fused with each other. Thus, the impression of the groove along the dorso-median line of it is almost disappeared. It has more or less smoother surface all along its dorsal surface, from anterior to posterior end of the neural tube.

3. A median patch of neural tissue is situated above the proctodaeum. As the epidermal ectoderm is cut off from its continuity with the portion of the medullary plate in the posterior rim of the neurenteric canal, lateral portions of the medullay plate, are fused to each other, along the median line of the region of the neurenteric canal. The posterior portion of the neural tube is thus extended up to the dorsal end of the proctodaeum, by an angular turn in the region of tail bud. This ventrally bent portion of neural tube, having no extension of neurocoel, just remains as a dorso-ventrally elongated median patch of neural tissue, dorsal to the proctodaeum.
4. **Region of posterior tubercle is appeared.** The anlage of infundibulum from prosencephalon is extended below the notochord. Thus the region of posterior tubercle along ventro-median portion of neural tube being clearly appeared, portions of mesencephalon and prosencephalon, are furthermore definitely distinguished from each other, along the ventro-median line of the neural tube. But in lateral sides of the neural tube, portions of both cannot be distinguished from each other.

5. **Anlage of infundibulum is appeared.** The prosencephalon occupies the anterior portion of the embryo. It is much increased both in its antero-posterior length and its width across it. The ventral portion of prosencephalon that was facing towards postero-ventral region of the embryo is extended further more posteriorly in postero-ventral direction of the embryo. It is well below the level of anterior end of the notochord. Thus the anlage of infundibulum is appeared.

6. **Region of optic stalk can be recognized.** The prosencephalon is further extended laterally in the region of optic vesicle. The prosocoel is gradually narrow for a longer distance, as it is extended like this, in lateral direction. But, for a very short length, as prosocoel is further extended laterally, this small portion of optic vesicle is become narrow across it. thus the portion of optic stalk is distinguished at lateral end of the optic vesicle.

7. **Anlage of optic cup is appeared.** The anlage of optic stalk is slightly turned in posterior direction of the embryo. At its lateral end, it touches
the epidermal ectoderm very closely. This whole portion of tip is closely attached to the epidermal ectoderm. Thus the anlage of optic cup is also started to appear in this stage. Outer retinal and inner pigment layer of the future eye can be easily distinguished in the portion of optic cup. The anlage of optic cup is situated in mid-lateral portion of the prosencephalon, but slightly nearer to the anterior wall of the prosencephalon.

8. Region of epiphysis can be recognized. In middle of the dorsal side of the prosencephalon, a smaller outwardly curved region is there, in the dorso-median portion of the prosencephalon. In this region the prosocoel, is slightly extended in outward direction. This region has slightly thinner wall. Thus the region of anlage of epiphysis can be recognized.

9. Region of optic chiasma can be recognized. In the middle of the anterior wall of the prosencephalon (i.e., the wall facing the ventral side of the embryo), the wall of prosencephalon is bulged inwards but very slightly (this can be easily recognized in lateral sections of the embryo), in median region of the prosencephalon. The bulg is at right angles to the median line of the prosencephalon. And it is at the level of the anlages of optic cups. Thus the region of anlage of optic chiasma can be recognized.

10. Mesencephalon is situated in antero-dorsal portion of the embryo. Along the dorso-median line of the neural tube, the wall of the neural tube is suddenly thickened into the region of mesencephalon. This dorso-median portion of mesencephalon is straightly above the anlage of optic
cup. It is situated in antero-dorsal portion of embryo, in antero-ventral to postero-dorsal direction.

Portions of mesencephalon and prosencephalon in lateral sides of the neural tube, cannot be distinguished. In this whole lateral portion, the wall of the neural tube remains as thick as the wall of the neural tube is in dorsal portion of mesencephalon. This is the region of wall of neural tube above and in front of the region of optic cup. Dorso-median portion of mesencephalon ends posteriorly at a level that is in between the region of posterior tubercle and anlage of optic cup.

Before appearance, along dorso-median line, the suddenly thinned portion of rhombencephalon, outer surface of mesencephalon appears slightly flat, in its dorso-median region. But whole dorso-median portion of it remains equally thickened all over it.

11. Lateral wall of rhombencephalon is slightly thinner than that of the mesencephalon. Just in front of the posterior tubercle, the prosencephalon and rhombencephalon become separated, along ventro-median line of the neural tube. More or less at the same level, anterior end of rhombencephalon is situated along the dorso-median line of the neural tube, in contrast to the thick dorso-median portion of the mesencephalon. But in lateral sides of the neural tube, regions of both portions cannot be distinguished from each other as they are met. Only it can be perceived that wall of rhombencephalon is slightly thinner and lateral width of the neural tube of its portion slightly larger.
12. **Anlage of spinal cord is narrow in its lateral width than the rhombencephalon is.** From the start of rhombencephalon, posteriorly all over (except the median patch of neural tissue in the region of neurenteric canal), the portion of neural tube appears to be a more homogeneous portion of neural tube, compared to the remaining anterior portion of the neural tube. Portions of rhombencephalon and spinal chord cannot be distinguished from each other definitely. But regions of both can be perceived mainly by the difference in width of both portions, former being wider and latter narrower.

As the portion of rhombencephalon is extended posteriorly, it is gradually raised up slightly upwards, in the upwardly raised up antero-dorsal region of the embryo. The portion of spinal cord appears to be started more or less at the place where the upwardly raised up dorso-median portion of neural tube, again is bent downwards. At this place a sudden decrease in lateral width of the neural tube can be recognized.

13. **Differences can be recognized in different regions of rhombocoel and wall of the rhombencephalon.** Before the start of portion of spinal cord, the portion of rhombencephalon remains more or less of equal width, all over, along its antero-posterior axis, but, slight differences can be recognised in different regions of it.

Just in front of the posterior tubercle, in very short region, rhombencephalon and prosencephalon remain unseparated, along the ventro-median line of the neural tube. Here, the portion of
rhombencephalon remains slightly longer dorso-ventrally, along the median plane. Here dorso-median portion of it remains slightly flatter, and ventro-median portion still is not appeared. The portion of rhombocoel, also is slightly longer dorso-ventrally and remains spindle shaped along the axis, although is slightly wider in its dorsal side.

Above the anterior end of the notochord, as the ventro-median portion of rhombencephalon appears, the rhombencephalon is circular in its shape across it, although is very slightly longer dorso-ventrally. Here the rhombocoel is spindle-shaped with its sharp dorsal and ventral ends. Lateral walls are more thickened in their middle and gradually narrow towards dorsal and ventral sides.

As the rhombencephalon is extended further posteriorly, dorso-lateral sides of it become slightly thicker, than the ventro-lateral sides. The spindle shaped rhombocoel appears blunt at its dorsal and ventral ends. Thus, dorso-median and ventro-median portions of rhombencephalon appear slightly flattened, with slightly more wider dorsal portion. As the rhombocoel is blunt dorsally and ventrally, a flattened appearance is perceptible along dorso-median and ventro-median regions of the rhombocoel, although, the wall of rhombencephalon along these median regions is very thin. Also, it is equally thin in both of these regions. In the posterior portion of the rhombencephalon, where it appears to be raised up dorsally, to its maximum height, again it becomes more rounded across it, like it is above the anterior end of the notochord. So, also, it remains rounded across it, as it is descended downwards. But in its
descended portion, it becomes gradually smaller in its diameter, as it is extended posteriorly, in the region.

14. **Dorsal portion of posterior part of rhombencephalon is covered by neural crest cells.** Nearer to its posterior end, the descended portion of rhombencephalon is slightly narrow towards its ventro-lateral side, but all over the length of this portion, the neurocoel remains spindle-shaped like it is in portion of rhombencephalon, anterior to this. But, some neural crest cells are migrated in between its dorsal portion and epidermal ectoderm. This region ends more or less at a level that is near to the middle region of the embryo.

15. **Dorsal portion of anterior part of anlage of spinal cord is formed of neural crest cells.** For a short distance, posteriorly, up to a level that is slightly posterior to the middle level of the embryo, neural tube in its wall on dorsal side is formed by more dispersed, slightly looser cell mass. The cells resemble the neural crest cells, being darkly pigmented and being round in their shape. Lateral walls of the neural tube appear to be separate, without being fused along the dorso-median line of the neural tube. Thus neurocoel appears to be opened into the neural crest cell mass. Also, discontinuity in pigmented lining of ependymal layer, is visible along the dorso-median line, confirming the incompleteness of the neural tube in this region.

16. **Portion of anlage of spinal cord is clearly distinguished from the surrounding tissues in the region of tail-bud.** As the neural tube is
extended in the tail-bud, and is then bent ventrally in the region of neurenteric canal, gradually, in posterior direction the neural tube is more and more closely apposed to the somitogenic portion in its ventro-lateral sides, and to the notochordal tissue in its ventral side. Inspite of their closer attachment portions of all can be distinguished from each other more or less upto the middle level of the closed neurenteric canal. This level is well below the level of the dorso-median line of the gut-cavity, i.e., slightly above the anal opening. Below this level, along the postero-median line of the embryo, cells of the neural tissue and mesodermal tissue cannot be distinguished, upto the dorsal border of the anal opening.

17. The groove of the oral sucker is much deepened. The thickened border surrounding the grooved portion of the oral sucker is extended outwards furthermore on the surface of the embryo. The grooved portion, is bent inwards furthermore, by folding of the oral sucker, all along its grooved region. Thus the groove along the oral sucker is deepened much, but shortened in its width across, bringing the outer (posterior) and inner (anterior) margins of oral sucker, more closer to each other, over the grooved portion. Thus groove becomes invisible externally.

In the spoon-shaped lateral ends of the oral sucker, in more anterior region, the outer margin of the oral sucker remains less changed. The inner border here, remains slightly raised up from the surface of the embryo. But, very soonly, posterior to this, the deepened groove with well developed margins appear and continue posteriorly.
18. The margin and the groove of the oral sucker are not developed in the ventro-median portion of the oral sucker. Posterior to the anterior, lateral end of the oral sucker, all over the length of each of lateral half portion of the oral sucker, outer and inner margins of the oral sucker are much extended outwards from the surface of the embryo. As they are extended outwards, they appear to be projected more straightly along dorso-ventral axis of the embryo, although are slightly bent over the groove. Both margins are more or less equal to each other in their dorso-ventral height and lateral width. Their dorso-ventral height is more than the lateral width. The margins appear, approximately, to be of same size up to the posterior end of the oral sucker. As both lateral portions of oral sucker meet posteriorly, suddenly the margins and groove of the oral sucker reduce in the ventro-median portion of the oral sucker, where both portions meet.

19. Posterior portion of sense plate, including its lateral part of oral sucker, is showing the signs of degeneration. The portions of lateral arms of oral sucker gradually are more closer to each other, as they are extending posteriorly to meet at the ventro-median line. As they become more and more closer, the lateral width of sense plate in-between gradually decreases. Thus, at one level posteriorly, the portion of sense plate completely disappears and lateral portions of oral sucker become so close that their inner margins are placed side by side, along the median line of the region of sense plate. Thus, posterior to this, only the portion
of oral sucker is extended, with disappearance of portion of sense plate, in between.

Further posterior to the region where the portion of sense plate disappears in between the inner margins of the oral sucker, inner margins gradually become so close that they cannot be distinguished from each other. As they become more and more closer, as oral sucker narrows laterally, an angular groove, (on the inner surface) in between them continues posteriorly at the start. But gradually, the angular groove disappears, and both portions of inner margin become indistinguishable from each other. This fused portion remains projected outwards, in between portions of outer margin, as much as outer margins are projected in the region. As the angular groove disappears the inner surface gradually becomes laterally flat. Further posterior to this, the fused portion of inner margins, gradually, but more suddenly, becomes thinner. This is the posterior most region of the oral sucker, being situated in-between the outer margins of the oral sucker which are still outwardly projected at this level of the oral sucker. In a shorter distance, posteriorly, projections of outer margin also, disappear, but inner area is continued posteriorly, with its same thickness, till the projections of outer margin become completely disappeared. This being the posterior most tip of the oral sucker this thicker portion soonly disappears and thinner epidermal ectoderm appears.

20. The region of oral sucker is more uniformly thickened all over.
The regions of sense plate and region of groove of oral sucker, that is
folded inwards, remain thinner, compared to the lateral or dorso-ventral width of the margins of the oral sucker. Both of the regions also are of same thickness, and like margins of the oral sucker, both of the regions appear to be equally thin all over, in spite of being slightly thicker towards anterior end.

21. **Cells forming the borders of the oral sucker tend to bend inwardly on themselves.** The cells of the borders of the oral sucker, as the borders overlap the grooved portion, show a tendency to bend inwards on themselves. Thus, borders of the oral sucker, in this stage, appear to be thick and appear to be raised up from the surface of the embryo, and become more distinctive. All over the portions of oral sucker and portion of sense plate in between its lateral arms, cells of the epithelial layer, (including both of the more elongated, pigmented bottle-shaped cells and unpigmented, but elongated cells), appear to be less in number in unit area, compared to the previous stage. The sensorial layer cells in the region of oral sucker are less spread.

22. **Pigmented bottle-shaped cells in the oral sucker are not evenly distributed, all over the region of oral sucker.** At anterior end of the oral sucker, where borders are not well developed, the pigmented cells relatively are more. The outer surface of its anterior end being slightly more depressed, in this stage, is completely lined by narrow, outer, darkly pigmented portions of bottle-shaped pigmented cells. More anteriorly in this region the pigmented cells are concentrated mainly towards outer, border. As the inner border starts to appear and groove becomes deeper,
the grooved surface remains darker. Posterior to this, when borders start to appear to overlap the groove, the pigmented cells become concentrated in between the groove and border. But the borders are formed only by non-pigmented cells. The region in between much thicker borders and thinner grooved surface, remains of medium thickness.

23. Cells are of unequal size, in shrunked portion of sense plate. In the portion of sense plate, in between the oral sucker, the bottle-shaped pigment cells are not found. This portion of sense plate is formed of epithelial layer cells which are unequal in their size. Thus, this portion appears to be wrinkled, externally.

24. Endodermal and epidermal layers are contacted each other, forming the oral plate. As the median portion of sense plate above the level of oral sucker, is furthermore bent inwards, the epidermal ectoderm, here, is more extended inwards, medially. Along this median region the sensorial layer cells of ectoderm are become more elongated antero-posteriorly. The epithelial layer is also more bent inwards, but not as much as the sensorial layer.

Simultaneously, along this median region the endodermal cells appear larger and are more elongated antero-posteriorly. As they are elongated, they appear to be of club-shaped, remaining wider anteriorly.

Thus by ectodermal invagination and endodermal evagination, both layers become closely apposed to each other along the median line, forming the oral plate, along the median depression which is visible
externally, in the region of stomodaeum. As the oral plate is formed, the endodermal and ectodermal layers are contacted all over, along the antero-median line of the embryo, between the level of the anlage of hypophysis and that of anterior end of the oral sucker.

25. The Rathke’s pocket in the anlage of hypophysis is become more spacious. Between dorsal end of the oral plate, and prosencephalon, the anlage of hypophysis is extended furthermore posteriorly. But it become slightly narrow in its lateral width, The Rathke’s pocket is become more spacious. The median depression of the epithelial layer of the oral plate, is extended up to the level of the anlage of hypophysis.

26. The nasal placode is furthermore thickened, but, below the level of externally visible nasal pit anlagen. Above the level of hypophysis, the sensorial layer along the median plane is suddenly narrow, But lateral to it, i.e., in whole portion, in front of the anlage of optic cup, it is furthermore thickened. The surface area of the thickening also is increased considerably. Majority of thickening and most portion of the nasal placode remain lower to and slightly lateral to the externally visible region of nasal pit. In the region of nasal pits, the epithelial layer is slightly flattened, but the sensorial layer is not as thick as it is in ventral side of the placodal region.

27. Anlage of anterior portion of Meckel’s cartilage cannot be distinguished from the lower portion of anlage of mandibular arch. Much of the lower portion of anlage of mandibular arch is migrated
furthermore anteriorly, below the optic portion of prosencephalon. Lower portion of anlage of mandibular arch and anlage of anterior part Meckel’s cartilage (so called in previous stage), below the optic portion cannot be distinguished, from each other, as it could be distinguished in previous stage. So also, the cells of mesenchyme between the endodermal portion and anlage of Meckel’s cartilage, below the optic portion cannot be easily distinguished from the cells of the anlage of the Meckel’s cartilage. The spread mesenchyme has become more compact and is closely attached with the cells of anlage of the Meckel’s cartilage.

28. Portion of anlage of quadrate cartilage is distinguished from the anlage of mandibular arch. In postero-lateral side of the anlage of optic cup, a portion of anlage of mandibular arch is remained. But it is not as thick as it was in previous stage, and also, it is slightly shorter, dorso-ventrally. Thus anlage of quadrate cartilage is slightly distinguished from the anlage of mandibular arch. The anlage of quadrate cartilage, is situated, slightly posterior to the level of anlage of Meckel’s cartilage, and remains just posterior to anlage of optic stalk.

29. Anlages of quadrate cartilage and hyoid arch are separated from each other dorsally. As it is visible externally, the anlage of hyoid arch is elongated dorso-ventrally. It also is become slightly narrower across it, but is of approximately uniform thickness all over its length dorso-ventrally, although is slightly thicker ventrally. In its dorsal side, it is clearly separated from that of the anlage of quadrate cartilage, and is extended beyond it dorsally.
30. **Anlage of 1st branchial arch is more clearly distinguished.** As it is visible externally, thicker neural crest material of branchial arches is spread much dorso-ventrally, so also, antero-posteriorly. This anlage also, in its dorsal side, is completely separated from that of hyoid arch. It is split into two separate streams of branchial anlagen. The anterior stream is more narrow than the posterior one, which is more spread antero-posteriorly, and in which one constriction can be recognized. Thus, anlage of first branchial arch is clearly formed in this stage.

31. **Anlagen of first and second gill-slits are clearly formed.** The endodermal layer in the region of anlagen of visceral arches, is evaginated in between the anlagen of visceral arches, and is closely attached to the ectodermal layer which is invaginated inwards in between the anlagen of visceral arches, as it is visible externally. Thus two visceral grooves and corresponding visceral pouches are appeared.

   In between the anlagen of mandibular arch and hyoid arch, ectoderm and endoderm are actually attached to each other. So also, in between the anlagen of hyoid and first branchial arches. Thus first and second visceral grooves and visceral pouches are clearly appeared. The attachment is complete in middle portions of the regions. At dorsal and ventral ends of these regions both layers gradually are separate from each other. As the anterior anlagen of visceral arches are situated more ventrally, the region of first visceral pouch and visceral groove is situated slightly ventral to the second one. The third visceral groove and visceral pouch are not clearly formed.
32. **Lateral placode, also ventro-lateral placode are furthermore thickened.** The ectodermal portion is clearly projected inwards, in the region of lateral placode. The sensorial layer of placodal region, is furthermore thickened, as the ectoderm becomes free from its attachment with endodermal layer and extends upwards. Whole sensorial layer up to the level of neural tube, is thickened, in between mandibular and hyoid arch anlages. This thickening is maximum along the inwardly folded region of ectoderm. It gradually narrows in anterior and posterior directions. Thus ventro-lateral placode is also well developed in this stage. That is the region just above the first visceral groove. The region of ventro-lateral placode remains narrow antero-posteriorly.

33. **Dorso-lateral placode is furthermore elongated antero-posteriorly, and otic vesicle can be recognized towards the posterior end of the dorso-lateral placode.** Above the region of ventro-lateral placode, i.e., dorsal to the anlages of quadrate bone and hyoid arch, whole portion of sensorial layer remains thicker. Thus dorso-lateral placode is extended much, antero-posteriorly, than in the previous stage. But both regions of ventral and dorsal placodes cannot be separated from each other. As the epidermal ectoderm extends upwards, the inwardly bent placodal region extends more and more towards a level, above the anlage of hyoid arch. Thus the dorso-lateral placode is more thickened above the level of hyoid arch. Here, it also, is slightly separated from the epithelial layer of the ectoderm, and this separated region is slightly bent inwards. Thus the region of otic vesicle is clearly formed.
34. **Hatching gland is developed in the region of brain anlage.** Along the dorso-median line, of the brain, the epidermal ectoderm is become much thickened. Across thicker region three to four bottle-shaped cells are present. These cells being narrow outside, a very shallow groove is formed all along the length of this region. This region is wider and thicker anteriorily, due to larger cells.

35. **Anlage of fin-fold is appeared in the tail bud.** In the region of tail-bud, the epidermal ectoderm towards the dorso-median line, remains relatively thicker. The thickened dorso-median portion becomes slightly distinguished, in its lateral sides, from the suddenly thinner epidermal ectoderm, by a slight inward depression in between. Thus anlage of region of fin-fold is distinguished in the dorso-median region of tail-bud.

36. **The sensorial layer of epidermal ectoderm becomes a continuous layer, all over the epidermal ectoderm.** The sensorial layer from lateral sides is extended to the dorso-median line of the embryo, thus becoming continuous all over the epidermal ectoderm. It is much spread and very closely attached to the epithelial layer. But nearer to the nasal placode, along the dorso-median region, presence or absence of cells of sensorial layer cannot be recognized but only a darkly pigmented region can be.

   The epidermal ectoderm is become thinner all over the embryo. Although it is slightly thicker towards anterior and posterior ends of the embryo, the epidermal ectoderm, in this stage appears to be more or less equally thickened all over.
37. **Sclerotomes are formed and somites have started rotation along their dorso-ventral axis.** In antero-dorsal side, mesenchymal portion is become more compact. The somite formation is extended further posteriorly, along the somitogenic ridge. Five more somites are constricted. The number of well developed somites is nine, where it was four in previous stage. Six of these nine somites, from second onwards posteriorly, up to the seventh one, have developed the sclerotome. The sclerotominal portions are well developed in the somites, third, fourth, fifth and sixth. The ninth somite, in its posterior side, is not separated from the somitogenic ridge. In about anterior five segments, rotation of segments, along dorso-ventral axis, is visible. In these somites cells appear to be elongated antero-posteriorly.

38. **The anlage of pronephros is furthermore thickened.** It is much thickened and thickened portion in its middle is slightly more bent outside, thus becoming clearly visible externally. It is extended posteriorly, approximately from the level of second somite. It becomes well developed at the level of third somite, again reduce much at the level of fourth somite. From here, it extends upto the level of sixth somite, but gradually, it completely disappears.

39. **The anlage of heart is situated between the first and second visceral pouches.** In antero-lateral side, the loosely arranged cells of the mesodermal layer, appear to be slightly more compactly arranged in double layers. In the region between the first and second visceral pouches, on both lateral sides, the double layers of mesoderm are slightly
apart from each other, and appear to be inflated. Thus the region of
anlages of heart is more clearly distinguished, from the remaining portion
of mesoderm. The anlage of heart of each side is situated over the
internally bent, grooved surface of the oral sucker, of its side. It is
extended antero-posteriorly, along it, in the region between the first and
second visceral pouches. This is approximately, the middle portion of the
oral sucker.

40. Anlage of notochord is furthermore elongated, more straightened,
more uniformly thickened and all of its cells are stacked. The
notochord is much elongated antero-posteriorly. All over its length, it is
further more straightened antero-posteriorly, and completely up to the
posterior end of the gut, it is clearly separated from the endoderm, so
also, from the somitogenic portion and neural tube. The notochord is
become more uniformly thickened all over its length. It is become more
closer to the mesencephalon and rhombencephalon, so also, with mid-gut
portion. But, it is clearly separated from hind-gut region. All over, its
cells are stacked. Also cells appear to be more separated from each other.
In the centre of the notochord, all over its length, a pigmented portion is
visible.

41. Subnotochordal rod is appeared and its cells resemble the cells of
endoderm, but are of smaller size. A thin strand of cells along dorso-
median line of the fore-gut and mid-gut, is attached to the endodermal
layer. This strand and endoderm are separated by a darkly pigmented
region. Here and there, it is separated from the endodermal layer. This
strand seems to be of single row of cells, which resemble more, the cells of endoderm, but much smaller than the endodermal cells of the mid-gut, that are along its dorso-median length.

42. Region of oesophageal plug is appeared. A small portion between portion of fore-gut and mid-gut, is become very narrow, and appears to be more rounded across it. This small portion of mid-gut is approximately nearer to the centre of the mid-gut. Thus the region of oesophageal plug is appeared, but still, the plug is not formed. From here, as the portion of fore-gut extends anteriorly, portion of mid-gut remains narrow laterally, for a shorter distance, and widens as it is extended into the sixth visceral pouch. In the region of hind-gut, anal opening is appeared.

43. Anlage of thyroid can be recognized, infront of the liver diverticulum. Liver diverticulum is reduced in its dorso-ventral and lateral width. Membrane like partition in its middle still is present. As liver diverticulum enters the portion of branchial region of fore-gut, the endodermal layer is thickened further. This is the region of endoderm, approximately just posterior to the posterior end of the oral sucker. Thus the region of anlage of thyroid gland is clearly appeared in this stage. It is at the level of the posterior visceral pouch, across the embryo, and above the levels of the lower end of oral plate and anlage of liver diverticulum. Just posterior to this, , the ventro-median wall of the anlage of liver diverticulum starts. As it starts, it is also, much thickened ventro-medially, as much as that of thyroid. But, the portion of thyroid is folded ventrally, and that of liver’s is not. Moreover, the portion of
liver diverticulum suddenly descends downwards, and soon it becomes narrow as it enters the portion of liver diverticulum.
Explanation to the figures in plate - 18

Figs. (1 to 7); Advanced neural tube stage, cross sections, 10th (Fig 1), 15th (Fig 2), 24th (Fig 3), 30th (Fig 4), 35th (Fig 5), 55th (Fig 6), and 65th (Fig 7) of totally 209 serial, cross sections, into which whole embryo was cut. Cutting started from anterior side of the embryo.

(o) prosencephalon, (or) inner and outer borders of the oral sucker, (u) mesencephalon, (t) rhombencephalon, (p) anlage of mandibular arch, (ma) prosocoel, (mc) mesocoel, (z) anlage of hyoid arch, (me) rhombocoel; (ap) first visceral pouch, (ne) anlage of optic cup, (a) anlage of heart, (ng) anlage of optic stalk, (lt) anterior portion of anlage of branchial arches, (nh) nasal placode, (no) region of epiphysis, (la) posterior portion of anlage of branchial arches, (m) anlage of hypophysis, (q) head mesenchyme, (og) oral groove, (s) endoderm, (ep) ear placode, (th) anlage of thyroid, (r) anterior portion of anlage of Meckel’s cartilage, (mk) middle portion of anlage of Meckel’s cartilage.
Explanation to the figures in plate - 19

Figs. (1 to 8), Advanced neural tube stage, cross sections, 89th (fig 1) 106th (Fig 2), 116th (Fig 3), 126 (Fig 4), 136th (Fig 5), 146th (Fig 6), 166th (Fig 7), and 176th (Fig 8) of totally 209 serial cross sections, into which whole embryo was cut. Cutting started from the anterior side of the embryo.

(d) anlage of spinal cord, (w) anlage of notochord, (aa) somitogenic ridge, (ab) hind-gut, (sl) sclerotome, (ta) portion of anlage of spinal cord where neural tube is not closed, in its dorsal side by interference of trunk neural crest cells, (tb) portion of anlage of spinal cord where neural tube is closed, in its dorsal side, without being interfered by trunk neural crest cells, (s) endoderm, (tf) anlage of fin-fold, (t) rhombencephalon, (sn) subnotochordal rod, (pa) region of oesophageal plug, (sa) trunk neural crest cell mass, (g) dermatome, (i) myotome, (f) anlage of pronephros, (e) anlage of liver diverticulum.
Explanation to the figures in plate - 20

Figs. (1 to 4); Advanced neural tube stage, lateral sections, 9th (Fig 1), 12th (Fig 2), 18th (Fig 3), and 28th (Fig 4), of totally 78, serial lateral sections, into which whole embryo was cut. Cutting started from left side of the embryo. [But, cut is not exactly parallel to the median plane of the embryo]

(p) anlage of mandibular arch, (vp) ventro-lateral placode, (z) anlage of hyoid arch, (nh) nasal placode, (lt) anterior portion of anlage of branchial arches, (la) posterior portion of anlage of branchial arches, (n) optic vesicle, (ap) first visceral pouch, (bp) second visceral pouch, (cp) third visceral pouch (f) anlage of pronephros, (dp) dorso-lateral placode, (r) anterior portion of anlage of Meckel's cartilage, (pk) posterior portion of anlage of Meckel's cartilage, (or) oral sucker, (q) head mesenchyme, (b) somites, (e) anlage of liver diverticulum, (th) anlage of thyroid
Explanation to the figures in plate - 21

Figs. (1 to 7); Advanced neural tube stage, horizontal sections, 25th (Fig 1), 36th (Fig 2), 40th (Fig 3), 92nd (Fig 4), 96th (Fig 5), 99th (Fig 6), and 100th (Fig 7), of totally 115 serial horizontal sections, into which whole embryo was cut. Cutting started from ventral side of the embryo [But, cut is not exactly at right angles to the median plane of the embryo]

(D) artifact, by detachment of mesoderm, (og) oral groove, (e) anlage of liver diverticulum, (th) anlage of thyroid, (r) anterior portion of anlage of Meckel's cartilage, (ap) first visceral pouch, (pk) posterior portion of analge of Meckel's cartilage, (bp) second visceral pouch, (q) head mesenchyme, (o) prosencephalon, (m) anlage of hypophysis; (pq) anal opening, (z) anlage of hyoid arch, (ab) hind-gut, (tf) anlage of tail-fin, (vg) first visceral groove, (vh) second visceral groove, (me) rhombocoel, (mc) mesocoel, (mn) neurocoel of spinal cord, (sa) trunk neural crest cells, (b) somites, (w) anlage of notochord