I. CHICK EMBRYOLOGY

Exercise 8 - Gross Morphology of the Chick Embryo (*Gallus gallus*)

Introduction

By tradition, the common domestic chick, *Gallus gallus*, is the popular choice in embryology laboratory because it is large, readily available, and useful in understanding the ontogenetic phases of amniotes. Likewise, the phylogenetic relationship among most animals can be inferred from the development of the structures of a chick embryo. A thorough learning of the chick's embryonic development is a must in the study of the mammalian embryo.

Since cleavage is over by the time the egg is laid, the chick embryo has already undergone the process of gastrulation. The primary organ rudiments are beginning to be laid down.

Objectives:

At the end of the exercise, the students are expected to:

1. Compare and contrast the general morphological features of the chick embryo in different stages of development.
2. Identify and characterize the diagnostic features of the chick embryo as development proceeds.
3. Extrapolate from chick to human development certain structures.
4. Appreciate the unfolding of body parts that are vital to the embryo in time and space.

Methodology

- Live chick eggs (19-24-hr old)
- Dissecting microscope
- Petri dish
- Chick's Ringer's solution (see appendix)
- Whole mount slides of embryo

Wet Method for Removing the Embryo from the Egg

1. Obtain a one-day old incubated egg from a hatchery and mark an X on its top. Be sure to hold it firmly and not rotate it to maintain the position of the blastodisc on top of the yolk.
2. Prepare a petri dish half-filled with warm (23^0-28^0 C) Ringer's solution for the egg.
3. Follow the illustrations given for this exercise. Open the egg carefully by tapping the bottom part of the shell (opposite the X-marked side) on the edge of the Petri dish. Make a transverse crack on the bottom side and slowly allow the egg to lay on the salt solution. A red discoidal area on top of the yolk will be seen if the egg is fertile. If

not, search on the sides or bottom of the yolk by holding the chalazae with forceps. Be sure not to puncture the membrane surrounding the yolk lest the yolk blurs your observation. You may open another one if the egg is infertile.

4. Once an embryo is found, study it using a dissecting microscope. Identify the following parts:

A. Area Pellucida – central clear region atop the massive yolk where the translucent embryo is located.
B. Area Vasculosa – peripheral mottled region surrounding the embryo.
C. Blood Islands – group of cells scattered in the area vasculosa.
What is the significance of these structures?

D. The embryo – a tiny structure at the center of area pellucida. It is composed of:

d.1. Primitive streak – thin dense line in the middle of area pellucida which represent the beginning of the embryo.
d.2. Head Process – represent the material for the developing notochord. It is located just above the Hensen's node.
d.3. Proamnion – a clear area on the cephalic region of the embryo. It is a misnomer. ***Why?***

d.4. Neural folds – paired, dark parallel "lines" flanking the lighter cavity, the ***neural groove***.
d.5. Head of the embryo – developing anterior region of the embryo visible as clear concave structure beneath the neural tube. It is composed of mesenchymal cells surrounded by the head ectoderm.
d.6. Foregut – the anterior part of the developing digestive tract located just below the head and opens via the *Anterior Intestinal Portal.*
d.7. Splanchnic Mesoderm – thick, dark crescentic structure which represent the rudiment of the heart on the posterior region of the foregut.
d.8. Somites – paired blocks of tissues on each side of the neural tube.
How many somites are there?

In between the somites are ***intersomitic grooves***.

d.9. Segmental Mesoderm- large mass of tissues that are still fused and located on each side of the somites.

Draw and label the parts of the chick embryo as described.

Questions:

1. Based on the over all appearance of the embryo, how can you tell the approximate age of the embryo?

2. What parts or structures serve as diagnostic features to identify the embryo?

3. The primitive streak appeared first among the structures of the embryo and yet it disappeared the earliest. What is the significance of the primitive streak?

4. Compare the embryo seen in the fresh state or live embryo from that of the whole mount embryo. What structures are clearly visible in the two preparations? What may account for the discrepancy?

The Chick Embryo – *Gallus gallus*

Exercise 9.1 – 18-Hour Chick Embryo (Whole Mount)

Introduction

The early stages of the chick embryos are commonly identified by the number of hours of incubation (at $38^0 – 39^0$C) needed to reach the point of development. Once the somites appeared, the number of somites may also be used.

At this stage, a definite embryo is starting to be formed and gradual unfolding of different parts occurs from the germ layers to the primary organ rudiments until definitive organs have developed. In chick, the appearance of a primitive streak initiates the series of organogenesis. The embryo resembles a straight line surrounded by dense staining masses.

Methodology

1. View CD of an 18-hour chick embryo. Take note of the most prominent features in this stage.
2. Study3-D models of a chick embryo and compare the organs you have seen.
3. Get prepared slides of whole mount of 18-hr chick embryo. Using LPO, scan the entire embryo and identify the following:

A. Area Pellucida – clear, pear-shaped central region where the embryo is found.
B. Area Opaca – blotchy and mottled region around the area pellucida.
C. Primitive Streak – elongated mass of cells in the center of area pellucida. It is in the process of regression. Its greatest length is attained at about the 19^{th} hour of incubation. It consists of the following:

c.1. Primitive Node – a round mass of cells at the tip of the primitive streak. It is also called the ***Primitive Knot*** **or the** ***Hensen's Node.***
c.2. Primitive Pit – a small and clear area beside the primitive knot. This and the primitive groove resulted from the sinking in of the cells.
c.3. Primitive Ridges or Folds – paired thickened "lines" or dense mass of cells that have migrated from the sides.
c.4. Primitive Groove – a long shallow canal that extend the full length of the primitive streak. It is bounded on both sides by the primitive folds.
c.5. Primitive Plate – the posterior end of the primitive streak seen as an area of diffused cells.

D. Head Process – dense mass of cells anterior to the Hensen's node. It foreshadows the notochord of the chick embryo.

E. Proamnion – a transluscent area on the anterior region of the head fold.
What accounts for its lucid appearance?

F. Head Fold – a thick dark crescentic structure anterior to the neural plate. This may not be seen in some whole mounts.

A. Somites—paired blocks of cells anterior to the primitive streak. These later expand to enclose the developing neural tube. These can only be observed in embryos of about 21 hours of incubation and onwards.

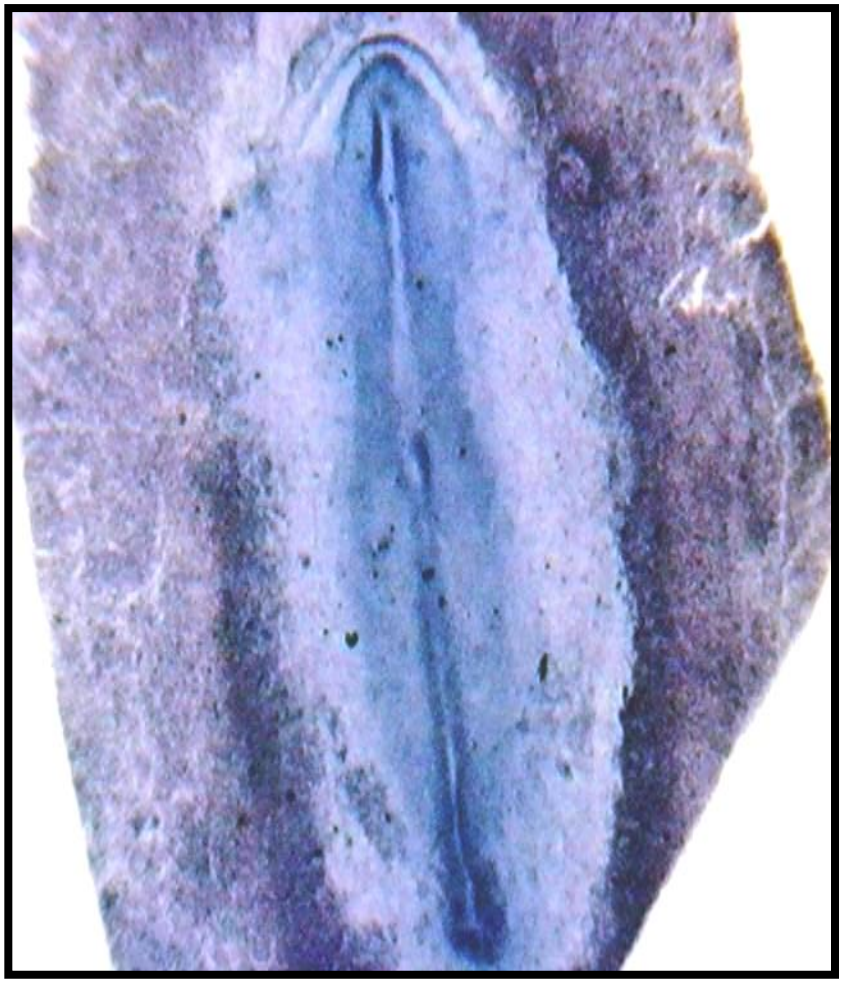
I. Head ectoderm – a single layer of cells which delineate the head region and foregut border.

J. Notochord – tubular supporting structure seen as a faint thin line beneath the primitive streak.

K. Blood Islands – a cluster of dense cells scattered in the area opaca vasculosa. They are more concentrated lateral and caudal to the body of embryo.

L. Area Opaca Vitellina – unmottled region beyond the area opaca vasculosa.

Label the parts of a whole mount 18-hr chick embryo.



Exercise 9.2 -- 18-Hour Chick Embryo (Serial Transverse Sections)

A few representative sections showing some prominent features of the embryo will be studied in detail. Bear in mind, however, that these are but small portions of an embryo that is in the actual process of active growth and differentiation. The embryo is a highly dynamic organism and these sections are simply "snapshots" of slices of parts of an embryo.

For simplicity, only those portions of the embryo that show distinct regions of the embryo will be highlighted. They will be referred to as "levels of _____, which means that the section pass through a particular region of the embryo. Refer to the whole mount figure for proper orientation of the different levels.

Descriptions here are mostly based on the appearance *per se* of the structures or organs from the observer's point of view. The terminologies on directions such as right and left are, likewise, according to how it is seen in the slide, hence, *opposite* to the real position in the embryo.

I. Level of Notochord or Head Process – the most anterior region of the embryo.

A. Notochord – rounded mass of cells below the neural plate.
B. Neural Plate – a band of cells in the middorsal region of the embryo. It is composed of tall columnar cells.
C. Epiblast – a thin layer of cells lateral to the neural plate.
D. Mesoderm – a diffused layer of cells seen as tiny dots below the ectoderm. They are more condensed in the center.
E. Hypoblast – a thin layer of cells below the mesoderm. Most cells on the extreme sides are in contact with the yolk.
F. Yolk – globular structures of varied sizes on both ends of the embryo. It is covered by the ectoderm.

2. Level of Primitive Pit this has been cut midway through the embryo.

A. Primitive Pit – V-shaped cavity at the middle of the developing embryo.
B. Primitive Ridge – dense mass of cells on each side of the primitive groove.
C. The three germ layers are still visible.

3. Level of the Primitive Groove – region between the middle and posterior region.

A. Primitive Gut - a rectangular space below the endoderm and flanked by the yolk.
B. Area Pellucida – a clear central region where the embryo is located.
C. Area Opaca – region lateral to the area pellucida composed of yolk and portion of the ectoderm, mesoderm and endoderm.

4. Level of Hensen's Node – the anterior region of the embryo just below the head process.

A. Hensen's Node – round mass of cells at the center and beside the primitive pit.
B. Neural Plate – elongated structure between the regressing primitive streak and the developing head fold.
C. Neural Folds – a pair of dark bands on each side of the neural groove.
D. Neural Groove – long narrow trough between the neural folds. This together with the neural plate and folds constitute the neural tube. Note that this extends toward the cephalic region of the embryo while the primitive streak starts and continuously decreases in length as it regresses.

Label the parts of an 18-hr chick embryo at different levels.

Level of the head process

Level of the Hensen’s node

Level of the primitive pit

Level of the primitive groove

Questions:

1. Why do you think the primitive streak must develop first and disappear soon?

2. To what organ is the primitive streak analogous in frog's embryo? Explain your answer.

3. Give the fates of the following :

A. Blood Islands
B. Head Process
C. Primitive streak
D. Head Fold

Exercise 10.1 – 24-Hour Chick Embryo (Whole Mount)

A one-day old chick embryo resembles a tall puppet with a veil, the *head fold*, and "swollen legs, the *somites*. These two parts are the most distinct features of the embryo in this stage due to its rapid growth and development. The head elongated anteriorly via continuous cell proliferation and became elevated above the yolk region. The four or five pairs of somites of the embryo serve as a benchmark of this stage.

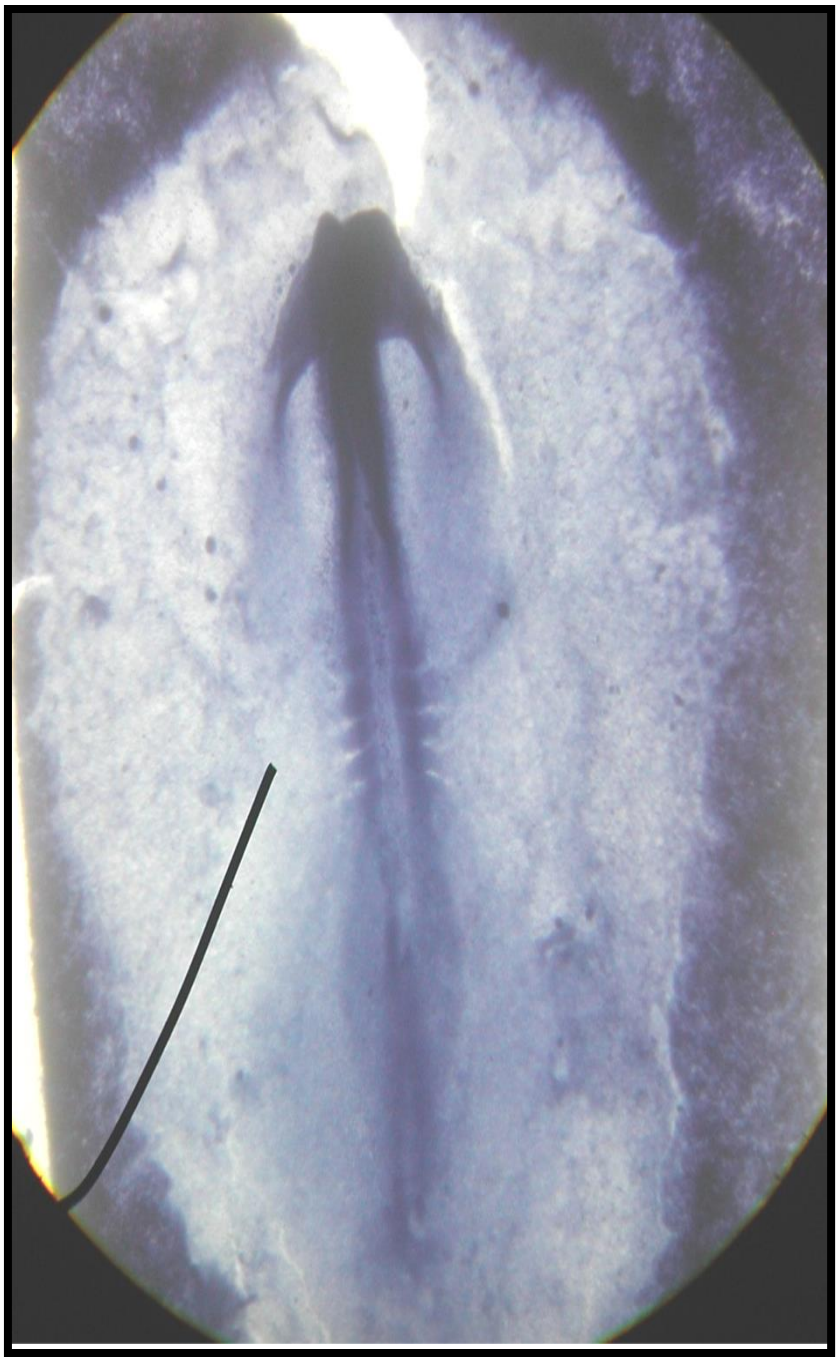
Study the models and whole mounted slides of a 24-hour chick embryo and identify the following parts:

A. Neural Folds – paired longitudinal mass of cells which started to fuse in the region of the future midbrain.
B. Anterior Neuropore --the anterior region of the developing neural tube which

remained open for a time.
How does the anterior neuropore differ from that of the caudal end?

C. Neural Groove – space which appeared like a letter V flanked by the neural folds.
D. Proamnion – a clear region above the head fold delineated by the lateral horn of the mesoderm.
E. Head Fold –a broad but short light "veil" on the anterior portion of the open neural tube. It is defined by a thick dark concave layer of head ectoderm.
F. Subcephalic Pocket –an external bay beneath the head of embryo. It resulted from the folding of somatopleure.
H. Thickened Splanchnic Mesoderm – unevenly thickened recurved lines above the margin of anterior intestinal portal . Better view can be observed in ventral side.
I. Margin of Anterior Intestinal Portal – a thin dark recurved line (concave) outer to the splanchnic mesoderm. It marks the extent of growth of the gut.
J. Margin of Foregut – thin line on each side of the head region. It appears like a translucent veil beneath the head fold and continuous with the tip of the margin of splanchnic mesoderm.
K. Somites – paired blocks of cells between the neural folds and neural plate.
L. Unsegmented mesoderm – vertical mass of undifferentiated cells below the last formed somites.
M. Notochord – elongated tube flanked by the neural folds. Note that it is larger at the posterior end than the anterior. This is more visible on the ventral side.
N. Hensen's Node – dark mass of cells below the notochord..
O. Primitive Streak – short thin line below the Hensen's node.

Label the parts of a 24-hour chick embryo (Whole mount).



Exercise 10.2 - 24-Hour Chick Embryo (Serial Transverse Sections)

I. Level of the Anterior Neuropore

A. Anterior Neuropore - a large open cavity formed by the unfused neural folds at the most anterior region of the embryo. ***At what hour will it close?***

B. Foregut – a narrow elliptical cavity surrounded by a thick dense layer of cells below the neural folds.
C. Oral Plate – median portion of the ectoderm that is in contact with the floor of the foregut.
D. Subcephalic Pocket – a deep recess beneath the head of the embryo.
E. Mesoderm – a thin layer of cells between the ectoderm and the endoderm.
F. Endoderm – dense layer of diffused cells below the mesoderm.

II. Level of Amnio-Cardiac Vesicle

A. Neural Tube – rounded structure in which the neural folds unite enclosing a central cavity, the neurocoel. This is the region of the future midbrain.
B. Head Mesenchyme – lighter-staining cells scattered around the neural tube and other structures. The nuclei are the only ones visible. Note that there is a space around these cells.

C. Notochord – a round mass of cells below the neural tube.
D. Amnio-Cardiac Vesicle – paired space ventrolateral to the foregut.
E. Splanchnopleure – double layers of cells below the subcephalic pocket.

III. Level of Anterior Intestinal Portal

A. Anterior Intestinal Portal – a large median space lined with endoderm. It leads to the pharyngeal pouches on both sides.
B. Pharyngeal Pouches – fingerlike spaces dorsolateral to the anterior intestinal portal.These are formed by the evagination of the pharyngeal wall.
C. Head Fold – downward curve of the head ectoderm separating the head of the embryo from the yolk.

IV. Level of Midgut

A. Midgut– A wide chamber continuous with the foregut. It is a floorless gut, appearing as a large space diverging on the upper sides of the embryo.
B. Somatic Mesoderm – layer of cells parallel to the ectoderm. This fused with the splanchnic mesoderm to enclose the future pericardial coelom.
C. Cardiac primordia – thickened splanchnic mesoderm ventrolateral to the anterior intestinal portal. ***What is the immediate fate of this?***

V. Level of Somite

A. Neural Groove – large V-shaped cavity bounded by thick neural folds.
B. Somites – paired triangular structures lateral to the notochord.
C. Intermediate Mesoderm – a thin and short strip of cells connected to the somites. They are also called nephrotome. ***Why?***

D. Lateral Plate Mesoderm – paired layers of cells connected to the nephrotome. They split into two layers: somatic and splanchnic mesoderm.
E. Coelom – a narrow cavity inside the lateral plate mesoderm.

VI. Level of Primitive Pit

A. Primitive Pit – a shallow V-shaped cavity in the median region bounded by the primitive folds.
B. Area Opaca Vasculosa – region surrounding the embryo where yolk and blood islands are found.
C. Blood Islands – large cord of cells lateral to the mesenchyme. These appear as coarse, dense granules scattered around the area pellucida.
D. Area Opaca Vitellina – clear region lateral to the area opaca vasculosa. It is free of mesoderm and blood islands.

Label the parts of a 24-hour chick embryo (Serial Transverse Section).

Level of the anterior neuropore

Level of the amnio-cardiac vesicle

Level of the anterior intestinal portal

Level of the somite

Level of the primitive pit

Questions:

1. Why would somites be useful in identifying the stage of the chick embryo? Is this reliable? Justify your answer.

2. How do you distinguish if the level is at the region of the neural tube or at the primitive streak?

3. What factors account for the appearance of head fold? What are the consequences of such event?

Exercise 11.1 -- 33-Hour Chick Embryo (Whole Mount)

During the intervening period between 24 to 33 hours of incubation, the chick embryo has undergone extensive changes through rapid growth and differentiation of certain parts. The most conspicuous transformation is the differentiation of the neural tube into three brain vesicles, elevation of the ectoderm into a crescentic fold (amniotic head fold), the ventricular loop, and the increase in the number of somites.

At this point, the chick embryo exhibits a straight cranio-caudal body axis and lies with its ventral side on the yolk. Yet, it will soon reorient itself. The linear embryo is in the process of early organogenesis, in particular, the rudiments of the CNS and the circulatory system. Neurulation occurs on the anterior region while primitive streak is regressing on the posterior region.

I. Ectodermal Derivatives

A. Neural Tube – fused neural folds which have differentiated into three primary brain vesicles:

a.1. Prosencephalon - anteriormost part with paired lateral evaginations called the *Optic vesicles* with a cavity called the *prosocoel.* Ventral depression on its floor is called the *infundibulum*. ***What is the fate of the infundibulum?***

The *anterior neuropore* is the open or unfused neural tube which may or may not be seen depending upon the actual stage of the embryo.

a.2. Mesencephalon – middle part with very thick walls and shaped like a vase. Its cavity is called the mesocoel.

a.3. Rhombencephalon – lowermost part of the brain (hindbrain), prominent

and separated by a slight constriction along its length. They are called neuromeres. ***How many neuromeres are there?***

B. Notochord – a thin or faint line viewed at the middle of the neural tube. May be vaguely seen when viewed dorsally.
C. Primitive Streak – most posteriorly located below and between the neural folds. Seen as a mass of cells or dark area.
D. Spinal Cord – paired straight "lines" below the myelencephalon. It is found below and above the heart. It extends midway to the most posterior segment.
E. Sinus Rhomboidalis – swollen end of the spinal cord which appeared to enclose the notochord and primitive streak of the embryo.
F. Auditory Vesicle – a small, C-shaped structure seen on the left side only. May be seen lying between the atrium and the ventricle, adjacent to the last neuromere of the myelencephalon.

II. Endodermal Derivatives:

A. Foregut – faintly visible as a clear homogenous oval organ beneath the neural tube. It extends from the mesencephalon to the rhombencephalon where the contour of the paired vitelline veins are clearly visible.
B. Anterior Intestinal Portal – the opening of the foregut into the yolk. Located just above the first somite.

III. Mesodermal Derivatives

A. Somites – differentiated into three parts:

a.1. Epimere or segmented mesoderm. Blocks of mesoderm lateral to the spinal cord.
a.2. Mesomere – middle portion of the somite between epimere and hypomere.
a.3. Hypomere – most lateral portion of the somite.

B. Heart – usually concealed by the rhombencephalon in dorsal view. Shaped like a reverse C protruding on the right side of the embryo.

b.1.Ventricle – the anterior bulging part of he heart continuous with the short and narrow ***Bulbus Cordis or Conus Arteriosus.***
b.2. Atrium – posterior part of the heart continuous with sinus venosus and receives the right and left omphalomesenteric veins.
b.3.Omphalomesenteric Veins – stout vessels directly connected to sinus venosus and posteriorly to the vitelline veins.

b.4. Aortic arches – paired vessels looped around the foregut and connecting the dorsal aorta and the ventral aorta.

b.5. Vitelline Veins and Vitelline Arteries – paired blood vessels which convey yolk from the yolk sac to the embryo. It is connected to the omphalomesenteric veins.

b.6. Vitelline Plexus – a network of fine vessels located in the area vasculosa vitellina.

b.7. Sinus Terminalis – a dense circular vessel on the peripheral margin of area vasculosa. These vessels become connected with the vitelline vessels

Label the parts of a whole mounted 33-hr chick embryo as described.

Exercise 11.2 -- 33-Hour Chick Embryo (Serial Transverse Sections)

I. Level of Prosencephalon – most anterior and dilated region of the brain of the brain. Prosencephalon is also called the forebrain.

A. Optic Vesicles – lateral outgrowths of the walls of the forebrain that resemble the ears of a rabbit. The extreme lateral wall is in contact with the ectoderm. ***What is the consequence of this?***

B. Optocoel – central large cavity of the forebrain that is continuous with the other cavities of the brain vesicles.
C. Infundibulum – median depression on the floor of the prosencephalon.
D. Ectoderm – dense outer layer of cells surrounding the head. This part is separated from the developing germ layers below it as a consequence of folding and rapid elongation.
E. Mesenchyme –small diffused mass of cells lying between the ectoderm and forebrain.
F. The three germ layers – seen as three parallel layers with a long median cavity. Note that the three germ layers seemed to merge as a thin line at the middle where the forebrain is located.

f.1. Ectoderm – layer immediately next to the forebrain.
f.2. Mesoderm – differentiated into somatic and splanchnic with the open coelom on each side. The somatic mesoderm is closely apposed to the ectoderm while the splanchnic mesoderm is closely apposed to the endoderm.
f.3. Endoderm – a thin layer next to the splanchnic mesoderm.

G. Blood Islands – irregular round or oval structures attached to the endoderm.
H. Head Fold – downward curved part of the prosencephalon.
I. Lateral Body Fold – marginal bend of the embryo continuous with the head fold. It is also called the *lateral limiting sulcus*.

II. Level of Mesencephalon

A. Mesencephalon – small, round organ with a central cavity called the mesocoel.
B. Notochord – dense central structure below the mesencephalon.
C. Dorsal Aortae – paired vessels lined with a thin layer lateral to the notochord and mesenchyme. Connected to the ventral aorta on the dorsal region.
D. Mesenchyme – loose cells seen as granular structures that fill the spaces between ectoderm and other parts of the embryo.
E. Foregut – small chamber seen as inverted triangle below the dorsal aortae.
F. Oral Plate – a thickened layer of cells composed of apposed ventral endoderm and ectoderm.
G. Ventral Aortic Roots – paired vessels seen as oval structures ventrolateral to the

foregut. This arise from the ventral aorta and continuous with the conus arteriosus.

H. Amniotic Fold – the upward curve of the somatopleure lateral to the headfold.
I. Somatopleure– apposed layers of ectoderm and outer mesoderm arising from the splitting of the lateral mesoderm
J. Splanchnopleure– apposed layers of endod
K. Extraembryonic Coelom – large cavity lined with splanchnic mesoderm distal to the embryo.

III. Level of the Heart

A. Rhombencephalon – small round structure at the anterior region of the head.
B. Heart – tubular organ which appears as large round organ in the body cavity. Note that it has double layers: epimyocardium and the inner endocardium. It is suspended into the cavity via *dorsal mesocardium.*
C. Pericardial Coelom – a wide cavity which arose from the confluence of the left and right amniocardiac vesicles of the paired cardiac primordia.
D. Otic Placode – thickened ectoderm surrounding the anterior region of the rhombencephalon.
E. Thyroid – thickened dark region medioventral to the foregut.

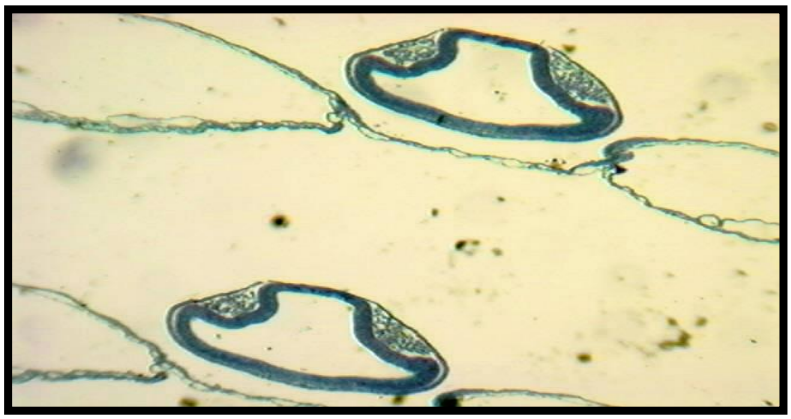
IV. Level of Midgut

A. Spinal Cord – oval structure at the center of the embryo.
B. Neural Crest – a small cluster of cells perched dorsal to the spinal cord.
C. Midgut – appeared as inverted triangle within the coelom. It opens directly into the yolk, hence a "floorless gut".
D. Anterior Intestinal Portal – narrow opening of the midgut to the yolk.
E. Omphalomesenteric Veins – paired vessels seen as oval structures ventrolateral to the midgut. These are later seen as the vitelline plexus composed of fine network of vessels in the yolk sac.

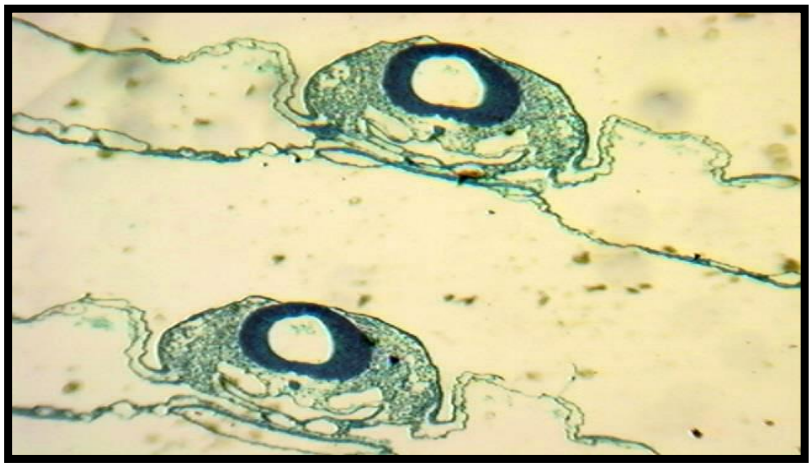
V. Level of Somite

A. Neural Tube – small oval structure with slit-like cavity.
B. Somites – paired triangular structures with round central cavity lateral to the neural tube. The cavity is called *myocoel*.
C. Nephrotome – thin and short strip of cells attached to the somites.
D. Lateral Plate Mesoderm – double layer of cells extending from the nephrotome and outwardly.
E. Coelom – a narrow space between the somatic and splanchnic mesoderm.
F. Dorsal Aortae – paired vessels seen as round structures with a central cavity.

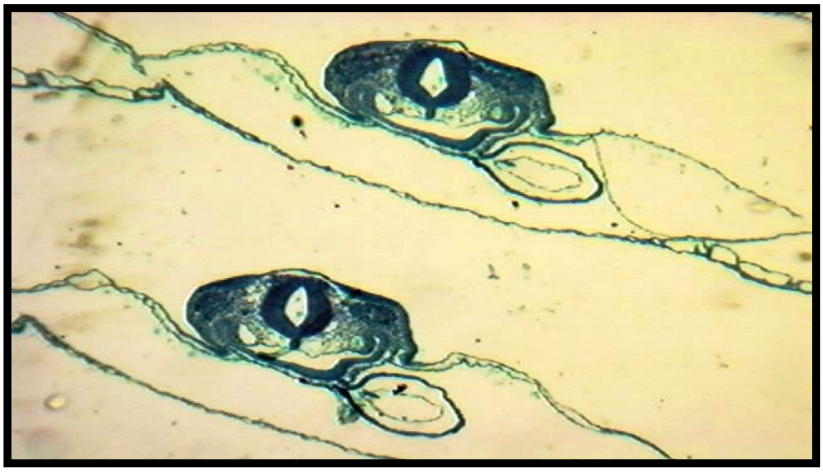
Label the parts of 33-hour chick embryo (Serial Transverse Section)



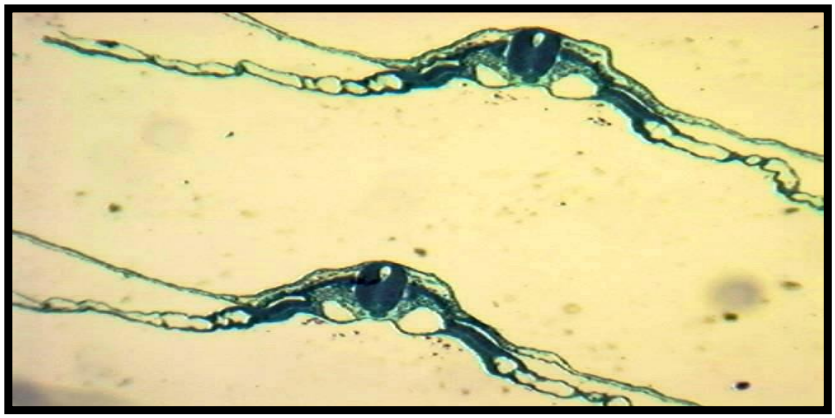
Level of the prosencephalon



Level of the mesencephalon



Level of the heart



Level of the midgut

Questions:

1. What are the most remarkable morphological changes in a 33-hour chick embryo?

2. What processes/factors caused the heart to bend or fold on the right side?

3. Give the adult fates of the following structures:

   a. Brain vesicles
   b. Vitelline veins
   c. Vitelline arteries
   d. Notochord