25 STUDY OF PREPARED SLIDES OF THE DEVELOPMENTAL STAGES OF FROG

25.1 INTRODUCTION

Animal development begins usually with the fertilization of an egg by a sperm. The nuclei of the egg and sperm fuse, and the male and female parent's genes determine the characteristics of the offspring. Compared to other biological processes, embryonic development is relatively slow. New cells, tissues and organs make their appearance in the embryo over a period of hours, days or weeks.

In this laboratory exercise, we shall study the development of frog with the help of prepared slides and stained sections, from the unfertilized egg to the tadpole stage. However, before we do so, in order to make the exercise more meaningful, we will begin with a brief description of the major events in the development of frog. You may also read units 13, 14 and 15 of Block 3 of LSE-06 course.

Description of Frog Embryology

The frog is an semiaquatic animal and deposits its eggs in water. The egg is of mesolecithal type having a moderate quantity of yolk which is concentrated in the vegetal (lower) hemisphere. Fertilization occurs minutes after ovulation and is essential for further development to proceed. The developmental stages from ovulation until tadpole stage occurs in water. The ovum has polarity even before fertilization and is enclosed in three jelly layers. The egg itself has two distinct regions (1) The animal hemisphere—the darkly pigmented region, which occupies more that half of the egg and contains the nucleus and (2) the vegetal hemisphere the light coloured, yolk filled heavier region (Fig. 25.1).

Fertilized egg (zygote) — The entry of the sperm into the egg, results in the formation of zygote. The sperm can enter only at the animal pole and when it does so, usually a grey crescent forms on the side opposite the sperm entry (Fig. 25.2). On the entry of sperm other changes also occur (Refer to unit 13 of LSE-06 course). The vitelline membrane, bound tightly around the egg lifts away, allowing the zygote to rotate so that animal pole becomes uppermost if it was not so before.

Early cleavage and morula formation — Development is rapid after fertilization so that within a few hours at normal temperature the zygote cleaves or divides into many cells (blastomeres). Cleavage is holoblastic (entire egg cleaves) and unequal; that is the blastomeres are not of equal size. Those at the animal pole being smaller in size are called micromeres and those at the vegetal pole are called macromeres. Early cleavage results in a solid ball of blastomeres called morula. The total cluster of blastomers is still no larger than the original fertilized cell.

Blastula — After about the fourth or fifth cleavage, a cavity called blastocoel develops from the morula giving rise to a blastula. This fluid filled cavity is more towards the animal pole and can be only viewed in a section of the embryo. The division of the blastomeres is no longer synchronous (Fig. 25.3). The blastomeres towards the animal pole divide more rapidly than the yolk laden ones present at the vegetal pole. Thus externally the blastomeres of the animal pole cover most of the blastula.

Gastrula — Gastrula appears as a dark pigmented ball of cells with a circular yolk plug of larger vegetal cells. A gastrula is formed when the single layered blastula is transformed into a three layered structure with an (i) outer ectoderm. (2) intermediate mesoderm and (3) an internal endoderm. Gastrulation involves division and movement of cells and these activities follow a definite pattern depending on the amount of yolk (Fig. 25.4a).

Formation of gastrula begins by the pushing in or invagination of the cells at the animal pole at a position somewhat below the equator of the blastula, at the boundary of the animal and vegetal hemispheres in the area of the grey crescent. The invagination forms a crescent shaped lip and is called blastopore. The position of the blastopore marks the

future posterior end of the embryo. The future anterior end of the embryo will develop at the animal pole. The cells of the animal pole continue to divide and move around the embryo (epiboly) and then turn inward from the blastopore (invagination or involution). A new cavity the archenteron is formed which expands at the expense of the blastocoel and is connected to the blastopore. The archenteron later develops into the gastrocoel. After the completion of all the events mentioned so far, it is possible to identify the three primary layers of the gastrula in a vertical section of the embryo (Fig. 25.4b).

Neurula — Near the end of gastrulation, there is a thickening of the ectoderm along a longitudinal axis on the dorsal surface to form a noticeable flattened strip called the neural plate. The edges of this neural plate fold upward and toward each other, finally to form a closed tube called the neural tube in the midline (Fig. 25.5). The neural tube of ectodermal cells running from the anterior to the posterior end of the embryo detaches and sinks below the ectoderm (which was previously lying above it). The neural tube thus gets covered by the epidermis and in time differentiates into brain and spinal cord.

Meanwhile the notochord also develops from a row of cells (of mesodermal origin) in the mid-dorsal wall of the archenteron as a cylindrical rod and comes to lie beneath the neural tube and above the archenteron. During this time, mesodermal tissue ventrolateral to the notochord and distinct from the notochord also develops laterally. The mesoderm splits into splanchnic and somatic layers within the body cavity. The coelom lies between these two layers.

The morphogenetic movements of gastrulation and neurulation bring various groups of cells into position so that the ground plan is laid for their subsequent interaction and differentiation into the final tissues of the adult in later developmental stages. The head begins to form at the anterior end. The mouth breaks through the anterior end of the archenteron and gill slits develop in the lateral walls of the archenteron. A tail bud forms at the posterior end which develops into a tail.

The neurula thus gets transformed into a young larva or tadpole with four distinct characteristics of all chordates; a tube-within a tube body plan, dorsal nerve cord, notochord and gill slits (Fig. 25.6).

Free swimming larva — The larva or young tadpole soon becomes capable of independent existence and emerges from the jelly mass or gelatinous covering to become a free swimming organism. At this stage the herbivorous larva no longer depends entirely upon its store of yolk for nutrients. Furthermore a fold of skin called 'operculum' grows backwards and covers the internal gills.

After growing for a few months, the larva metamorphoses into an adult. This process involves marked external and internal changes (Fig. 25.7, 25.8, 25.9). The mouth, teeth and tongue change as the herbiviorous larva becomes a carnivorous frog. Circulatory modifications accompany the change from gill to lung circulation. The musculature adapts from that for swimming in a fish-like manner to locomotion using the limbs.

Objectives

After completing this laboratory exercise you should be able to:

- identify and describe the representative stages in frog development from fertilized egg upto tadpole stage as observed in microscopic preparations.
- draw and label the early and late embryonic as well as the larval stages in the prepared slides.

25.2 MATERIALS REQUIRED

prepared microscope slides of frog development.

unfertilized egg

blastula entire and its vertical section

gastrula entire and its vertical section

neurula entire and its vertical section

whole mount of tapole larva of frog

T.S. through ear of tadpole

T.S. through eyes of tadpole

T.S. through the head and gills of tadpole

microscope.

25.3 PROCEDURE

Before you start studying the slides, carefully go through the description of frog embryology given in the introduction and the various figures provided throughout the laboratory exercise. This will help you to become familiar with the various developmental processes occurring in frog and to relate them with the embryological slide material you will be studying. First by examining whole mounts of embryonic stages become familiar with the general and overall changes taking place in frog development. Make drawings of these stages in your record notebook by observing them under the microscope. This study should be followed by the study of the sections of the developmental stages and you should be able relate them to the study whole mounts.

Your counselor will arrange the slides of various stages of frog development. Examine the slides carefully first under the low power and if necessary under the high power of the compound microscope and compare what you have observed with the description and figures provided in the lab exercise. After viewing one slide you may move to next until you complete viewing the entire series.

In you notebook sketch, label and write the description of all the stages of the development which you have observed.

The stages which you will be examining and for which descriptions are provided are listed below:

1. Unfertilized egg
2. Fertilized egg
3. Blastula entire and V.S. of blastula
4. Gastrula entire and V.S. of late gastrula
5. Intact neurula and V.S. of neurula
6. Tadpole larva of frog
7. T.S. of tadpole through eye region
8. T.S. of tadpole through auditory vesicles (ears)
9. T.S. of tadpole through head and gills.

A Unfertilized Egg of Frog

1. The egg is moderately telolecithal or mesolecithal
2. The egg or ovum is surrounded and covered by three distinct layers (a) the innermost vitelline membrane which is secreted by the egg itself (b) chorion, the intermediate layer secreted by follicular cells of the ovary and (c) the gelatinous, tertiary egg membrane or albumen consisting of 3-4 gelatinous rings which is secreted by the wall of the oviduct.
3. More than one half of the surface of ovum appears blackish due to the presence of the melanin pigment while the rest appears almost whitish due to the presence of yolk (Fig. 25.1).
4. The pigmented region will form the future animal hemisphere, while the non-pigmented region will form the future vegetal hemisphere.
5. The clear cytoplasm and haploid nucleus of the egg are located in the animal pole.
6. The vegetal hemisphere contains the bulk of the yolk, which provides the necessary nutrients for embryonic development.

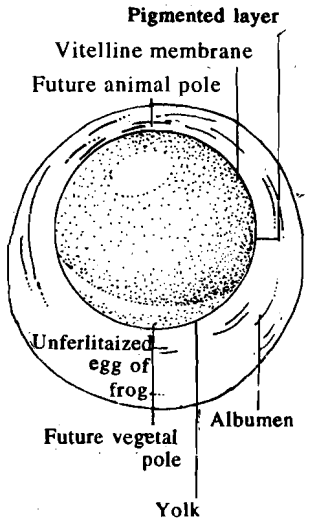

Fig. 25.1 : Unfertilized egg.

SAQ 1

Can you explain the necessity for the three jelly coats in the ovum?

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B Fertilized Egg

1. The centre of the animal and vegetal hemispheres are called animal and vegetal pole respectively (Fig. 25.2).
2. The animal hemisphere contains the diploid nucleus formed by the union of sperm and egg nucleus.
3. The vitelline membrane is now called fertilization membrane. Soon after fertilization the egg secretes a protein material which absorbs water and swells up, causing the vitelline membrane to lift up from the egg.
4. A grey crescent is visible opposite the point of entry of the sperm at the margin between the animal and vegetal pole, largely in the animal pole. It is more lightly pigmented than the animal hemisphere. The grey crescent later forms the posterior and dorsal side of the embryo.

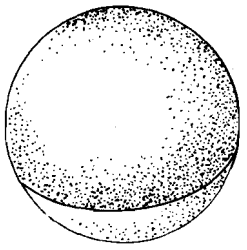
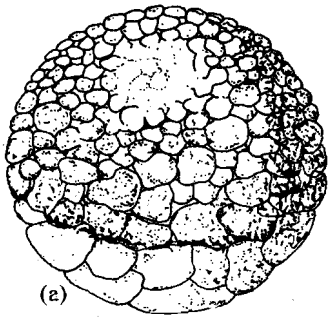


Fig. 25.2 : Fertilized egg.

SAQ 2

With the help of labelled diagrams show the differences between the unfertilized and fertilized frog eggs.



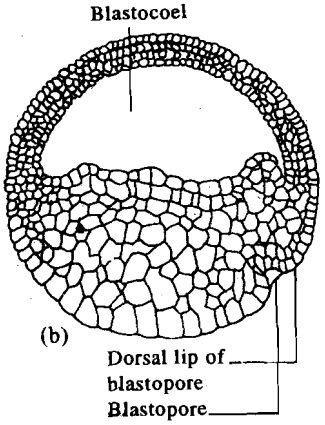


Fig. 25.3 : Blastula a) entire, b) V.S.

C Blastula entire and V.S.

1. The blastula is a ball of cells with a central cavity the **blastocoel** (Fig. 25.3).
2. The section of blastula shows that it contains an excentric blastocoel cavity surrounded by unequal sized blastomeres.
3. The smaller pigmented blastomeres are located in the animal hemisphere and are called micromeres, while the larger yolk filled blastomeres occupying the vegetal hemisphere are called megameres.
4. The micromeres at the animal hemisphere form the thin, multilayered roof over the blastocoel, while the megameres of the vegetal hemisphere form the floor of the blastocoel.

SAQ 3

Are all cells in the vegetal pole slightly larger, much larger or many times larger than those at the animal pole?

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SAQ 4

Draw a labelled diagram of the entire blastula and its vertical section.

D Gastrula entire and V.S.

The blastula gets transformed into a gastrula, which appears externally as a ball of dark pigmented cells, with a circular, light coloured yolky plug.

1. The size of the gastrula is still as small as the zygote (Fig. 25.4a).
2. Gastrula is formed by rearrangement of cells of the blastula. Gastrulation reorganises the embryo completely and results in three germ layers (1) external ectoderm (2) The middle mesoderm and (3) innermost endoderm. The various organs of the animal are derived from these three layers. The various layers of the gastrula are well differentiated and are clearly visible in vertical section (Fig. 25.4b).
3. The ectoderm will give rise to epidermis, cutaneous glands, nervous system, eye parts and linking of mouth cavity and cloaca.
4. Endoderm will form the lining of alimentary canal, liver, pancreas, lung urinary bladder and primordial germ cells.
5. The mesoderm appears as a small area towards the posterior and will give rise to the musculature, connective tissue, vascular system, genital organs, excretory organs, skeleton and notochord.
6. Other structures seen in the section are dorsal lip of blastopore, yolk plug, ventral lip of the blastopore.
7. The notochord or chorda cells and neural plates are well differentiated and lie at the dorsal side.
8. The cavity of the gastrula called archenteron lies towards the dorsolateral side of the anterior part of the embryo, and the archeneteron will develop into gut in the future.

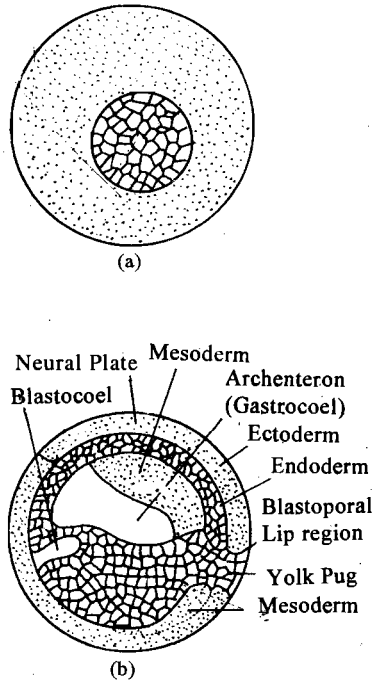

Fig. 25.4 : Gastrula (a) entire (b) V.S.

E Neurula

1. The embryo is called neurula as the two neural plates of the gastrula join to form a neural tube along the longitudinal axis (Fig. 25.5).
2. Externally the neural tube in the embryo appears as a thickened ridge along the longitudinal axis.
3. In vertical section of the neurula, the neural tube which is of ectodermal origin lies above the notochord.
4. The notochord, a rod-like structure arises from the mesoderm in the middorsal region and lies below the neural tube and above the archenteron. The notochord runs along the longitudinal axis of the embryo.
5. On either side of the neural tube and notochord the extensive mesodermal tissues are seen.
6. The mesodermal tissue which flank the neural tube form the somite mesoderm.
7. The lateral mesoderm a continuation of the somite mesoderm appears split into (1) The splanchnic mesoderm which lies nearest to the gut endoderm and (2) the somatic mesoderm which lies nearest to the endoderm.
8. A new fluid filled cavity the coelom is present between the splanchnic and somatic mesoderm.

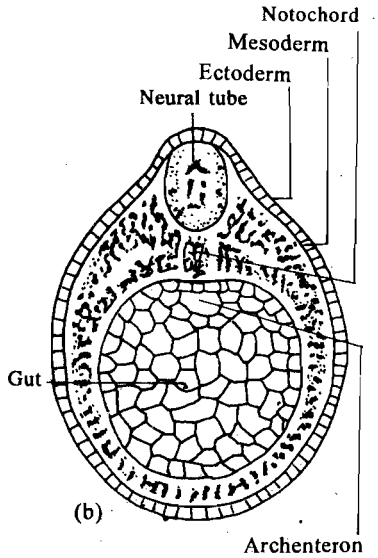

Fig. 25.5 : V.S. of Neurula

F Tadpole Larva of Frog

1. The egg hatches into a free living herbivorous larva (Fig. 25.6).
2. The larva measures about 5-7 mm in length.
3. The body of the larva is differentiated into body proper and tail with fin.
4. The larva contains mouth, sucker, external gills, rudiments of eye, olfactory pits, gut, anus and myotomes (muscles).
5. Mouth bears horny jaws or teeth.
6. Three pairs of external feathery gills act as functional respiratory organs.
7. Tail is long and bears the tail fin on its ventral and dorsal surface.

8. The herbivorous larva feeds on vegetation and has a coiled intestine. The larva metamorphoses into an adult.

G T.S. of Tadpole through Eye Region

1. Eyes develop very early and are of ectodermal origin. They are the most conspicuous and protuberant structures of the tadpole.
2. Eyes develop from the optic vesicles which originate from a pair of diverticula given off from the prosencephalon of the brain on each side.

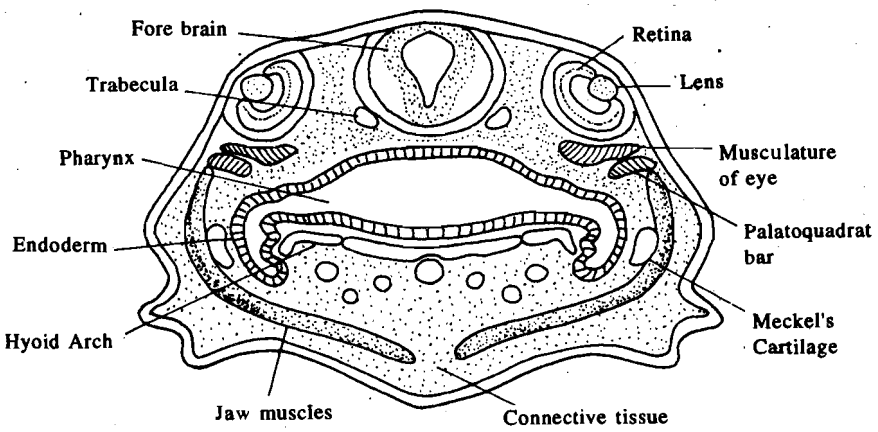

Fig. 25.7 : T.S of tadpole through eye region.

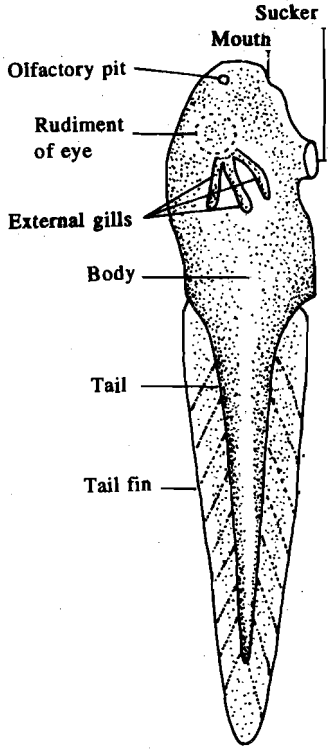

Fig. 25.6 : Tadpole of frog

3. Transverse section of the tadpole through the eye region (Fig. 25.7) shows the forebrain in the centre on the doral surface.
4. The eyes are seen on the lateral sides of the brain and are composed of lens, sensory layer of retina and eye muscles.
5. The pharynx lies in the centre of the transverse section.
6. Hyoid arch lies just below the phanynx in the section.
7. The palatoquardate bars are placed dorsal to the phaynx on the lateral sides.
8. Meckel's cartilage are present on the lateral sides of the pharynx.
9. Other structures seen in the section are jaw muscles, trabaculae, epidermis and connective tissue.

H T.S. of Tadpole through the Auditory Vesicles (ears)

1. Ears develop as a pair of auditory pits from the epiblast on the sides of the hind brain.
2. The auditory vesicles develop at an early tadpole stage and are found on the dorso lateral side of the section, below the hind brain (Fig. 25.8).

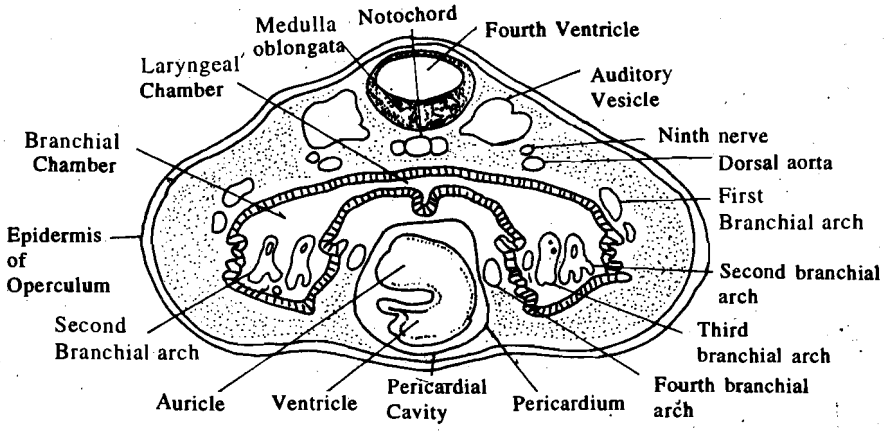

Fig. 25.8 : T.S. of tadpole through auditory vesicles.

3. The auditory pits become vesicle like and enclose the middle ear. The semicircular canals develop as outgrowths from the wall of the auditory vesicles.
4. The medulla oblongata of the hind brain is located in the middorsal region of the section between the two auditory capsules and encloses the fourth ventricle.

5. The notochord lies below the medulla oblongata.
6. The branchial chamber having the laryngeal chamber lies below the notochord and auditory vesicles.
7. The dorsal aorta and the first, second, third and fourth branchial arches are located on the lateral side of the section.
8. The pericardial cavity enclosing the developing auricle and ventricle is located on the ventral side of the section, below the laryngeal chamber.

I T.S. of tadpole through Head and Gills

Transverse section of the tadpole (Fig. 25.9) through head and gills shows the following details.

1. The medulla oblongata enclosing the fourth ventricle lies in the middorsal position.
2. The membranous labyrinth are present on both the lateral sides of the medulla oblongata.
3. The notochord lies just below the medulla oblongata.
4. The pharynx lies in the centre of the section.
5. Larynx is seen in continuation with pharynx.
6. The heart enclosed in the pericardium is seen just below the larynx.
7. The branchial arches and internal gills are present on the lateral sides of the pericardium.
8. The lateral dorsal aorta is located above the pharynx.
9. Lymphatic spaces lie on both the lateral sides.

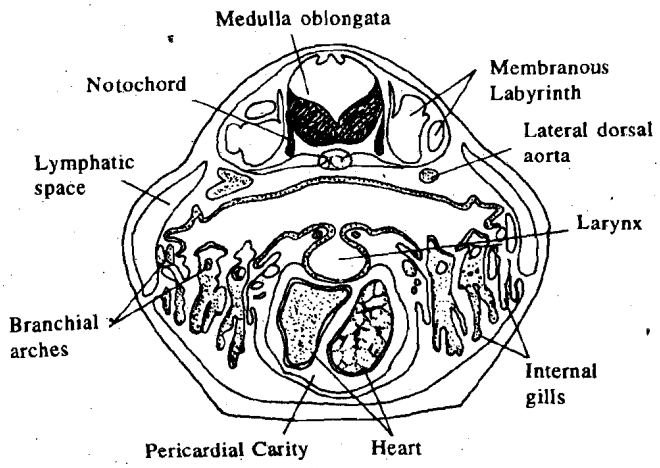

Fig. 25.9 : T.S. of Tadpole through the head and gills.

SAQ 5

1. Fill in the blanks:

a) A hollow ball stage in early embryonic development, preceeding gastrula stage is called a _____ .

b) The frog egg is called mesolecithal as it has _____ amount of yolk.

c) A solid ball of cells in embryonic development is called _____ .

d) The cavity which forms during embryonic development and gives rise to the gut in the adult is called _____ .

e) The coelom is a fluid filled cavity that separates the _____ mesoderm from the _____ mesoderm.

f) Cleavage is _____ but slow in the _____ hemisphere due to the presence of yolk in frog development.

g) _____ is the opening in the gastrulla through which the ectodermal cells invaginate.

h) _____ is infolding of cells through the blastopore leading to differentiation of endoderm and mesoderm.

2. Ultimately what is the fate of the blastocoel?

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3. From which germ layer does the nervous system develop?

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4. Our study of the early developmental stages of frog has shown the formation of three germ layers from which the major organs develop. In the table below indicate the germ layers and their derivatives as discussed in this lab exercise.

	Germ layer	Organs developed
1.		
2.		
3		

5. How does the size of blastula compare with one cell stage? These are about 1000 cells by late blastula stage in frog development. How many division would this represent. What assumption is required for such a calculation? Are all the cells similar in the blastula?

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6. Draw clear labelled diagram of all the stages you have observed.