LAB

Development: Building an Animal From the Ground Up

FROM THE *Irvine Valley College Life Science Labs Series*

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The sequence of events that converts a collection of organic and inorganic molecules into a living organism is without doubt one of the most amazing phenomena known. Consider the material that comes out of a chicken egg when you crack it into a pan to cook it – yellow yolk and clear egg white. It is mostly a collection of carbohydrates, lipids, proteins, and nucleic acids. Guided by the sequential expression of thousands of genes, this seemingly homogenated material will become a chicken in 27 days.

This exercise focuses on understanding the earliest stages of embryological development in starfish (A.K.A. sea stars) and chickens. Even at these very early stages, the basic design of the animal begins to take shape at this time.

Activity 1: A First Observation

Activity 2: Starfish Development

Activity 3: Development of the Chick

Activity 1: A First Observation

Your instructor will begin the lab by showing a film depicting the stages in the development of an animal embryo, beginning with fertilization. The film has been significantly accelerated so that hours become seconds.

➲A. What kinds of organisms are being depicted in the film?

➲B. Name three major events during the development of the embryos in the film.

Sexual reproduction and development involves:

- the production of the sex cells or gametes;
- fertilization of the egg by fusion of the male and female gametes;
- the production of a large number of cells from the single fertilized egg via mitosis;
- the organization and movement of these cells into specialized tissues; and
- the layering of these tissues to form organs

The major events in development can be summarized as follows:

- The fusion of ovum and sperm is a process called fertilization. The sperm cell, equipped with a special enzyme-filled vesicle called an *acrosome*, comes in contact with the jelly coat of the ovum. On contact, the acrosome releases its enzymes (*acrosomal reaction*), which make a hole through the jelly coat. The membranes of sperm cell and ovum fuse, and the sperm nucleus is released into the ovum's cytoplasm. The fusion of the two haploid nuclei follows (called k*aryogamy*).
- To prevent additional sperm cells from entering, the ovum initiates a *cortical reaction*, in which an impenetrable fertilization membrane forms, and *fast block*, which involves a change in the polarity of the egg surface (i.e. electrical charge).
- The fertilized egg first undergoes a series of rapid divisions by the process of simple mitosis. Essentially, the fertilized egg divides into two cells (*primary cleavage*), then both of these cells divide in concert producing four cells (*secondary cleavage*), then each of the four cells divides, producing eight cells, etc. The result of these successive divisions is a solid ball of cells known as a *morula*.
- Cells within the morula continue to divide. Next, a cavity begins to form in the center of the morula. This cavity, known as the *blastocoel*, continues to grow, turning the solid morula into balloon of cells, one cell layer thick, called a *blastula* (see figure 1). The exact shape of this mass varies from animal to animal.

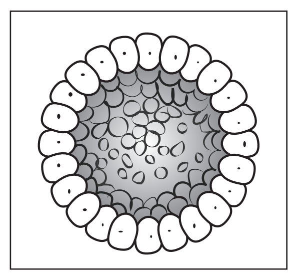

Figure 1.

- The blastula next experiences a period of not only cell division but cell migration. Cells in one region of the blastula's surface begin to migrate inward forming a pocket (see figure 2). This pocket, called an *archenteron* continues to grow until it greatly compresses the blastocoel. This process, known as *gastrulation*, results in the formation of the *gastrula*. The archenteron will eventually form the gut.

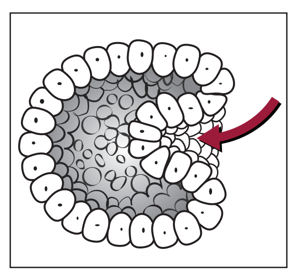

Figure 2.

- The gastrula is comprised of 2 cell layers. These cell layers are referred to as the primary germ layers of the embryo. All tissue and organs will develop from these layers. The outer cell layer is called the *ectoderm*. The ectoderm will develop primarily into the skin and the nervous system of the organism. The inner layer, called the *endoderm* will primarily become the organs of digestion. A third layer, called the *mesoderm* will immediate form between the first two germ layers. Mesoderm will develop into muscle and the skeleton (if the organism has a skeleton!), as well as a variety of other organs, such as the kidneys and gonads.
- Once the primary tissue layers have been formed, the cells in each can replicate and arrange themselves into the shape of adult organs, these are called *organ rudiments*. For instance, cells of the ectoderm form the beginnings of the nervous system in the process of *neurulation*. The formation of organs is called *organogenesis*. As organs and body parts take form, the individual cells of each must "learn" to perform their respective functions in a process termed *cell differentiation*.

Development is well studied in most major groups of animals including humans. The sequence of events described above is essentially the same for major groups of complex multicellular animals. There is, however, considerable variation among various animal groups at each step as you will discover in this laboratory exercise.

The first part of this exercise focuses on the development patterns in *Asterias* (starfish or sea stars). The early development sequence in *Asterias* is very similar to that in humans and is much easier to visualize and understand.

The second part of the lab exercise emphasizes the study of human development using chicken eggs. This is possible because development through the embryonic stage in chicks and humans is almost identical; so much so, that it is difficult to distinguish a chicken embryo from a 56-day old human embryo at a similar stage of development.

Activity 2: Starfish Development

This part of the exercise focuses on developmental events that convert an unfertilized egg into a starfish (*Asterias*), with special emphasis on the events of fertilization, cleavage and gastrulation (see figure 3).

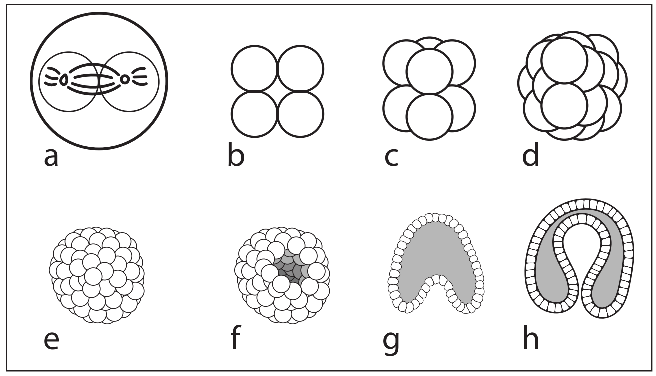

Figure 3. Starfish development: a-d (early cleavage); e (morula); f (blastula); g-h (gastrula formation)

Asteria embryos are especially suitable for study for two reasons. *Asteria* eggs are of the *isolecithal* type, which means that the yolk material is evenly distributed throughout the egg. During cleavage, such eggs divide completely at each division producing cells of about equal size, and therefore cell layers are more visible.

In addition, the absence of shells or opaque membranes enhances observation. As you make your examinations, realize that the focus is on the most readily observable events. Development also involves very complex biochemical changes and cell movements that are **not** visible. Developmental stages of cleavage and gastrulation are represented below.

Compare this type of division to the incomplete division in chicken eggs. These eggs are easily visible in the light microscope using a 10X objective lens.

Materials Needed:

- Microscope
- Set of *Asterias* development slides

Procedure:

Unfertilized egg cell:

1. Obtain slide of unfertilized starfish from center table and view it using a 10X objective lens. Draw a representation of the egg in **Figure ➲C**.

Fertilization:

Sperm are very small and difficult to see using the light microscope, so you will not observe them here. As the sperm recognize the egg, one sperm attaches to the surface of the egg and enters the egg cytoplasm. In response to sperm penetration, the egg immediately produces a protective coating of molecules over its entire surface. This coating is called the fertilization membrane. It prevents penetration by other sperm cells.

2. Obtain a slide of a fertilized starfish egg. View it at 10X and then draw a presentation in **Figure ➲D** and label the fertilization membrane.

Early Cleavage:

The process of cleavage in the starfish is a simple affair. The original egg divides into two equal size cells by mitotic division producing a two-cell stage (*primary cleavage*). Both of these cells divide producing four cells, each of which divides again producing eight cells, and so on. These divisions produce the 4-cell, 8-cell, 16-cell, etc., stages of cleavage.

3. Obtain a slide of early cleavage and identify and draw the 2-cell, 4-cell, and 8-cell stage in **Figure ➲E**.

Late Cleavage: Morula and Blastula:

These rapid divisions of early cleavage produce a solid ball of cells called the *morula*. A cavity, the *blastocoel*, forms within the center of the morula. The result is a *blastula* composed of a single layer of cells surrounding a hollow, fluid-filled center.

Obtain a slide of late cleavage. Observe the developmental stages presented on this slide. Identify the morula and blastula and draw them in **Figures ➲F** and **➲G**, respectively.

Gastrulation:

The cells of the blastula are undifferentiated. They are genetically, physically, and biochemically alike. The next stage of development involves the organization of these cells into the three distinct groups: ectoderm, mesoderm, and endoderm. As this organization occurs, cells become differentiated such that they are restricted in terms of their roles in the future adult. In addition, as these layers form, the primitive gut cavity of the animal also forms. This series of events composes the process gastrulation in starfish.

Gastrulation begins when a few select cells of the blastula begin to divide rapidly and migrate into the center of the blastula. To visualize this process, imagine a **hollow** ball of soft clay. If you gently pushed in one side of the ball to form a cup and pushed the edges of the cup lip close together so there was only a small opening, you would have a cup with two layers of clay and a small opening. The cells of the blastula grow inward in a similar fashion, producing a cup with an outer layer of cells (the ectoderm) that is lined with a layer of cells (the endoderm). This has a fairly narrow mouth (the *blastopore*) and a hollow interior (the *archenteron*, or primitive gut). Some of the cells of the endoderm then grow into the space between the ectoderm and endoderm to form the mesoderm.

4. Obtain a slide of a starfish gastrula. View it at 4X and 1OX and then draw it in **Figure ➲H**. Label the ectoderm, endoderm, blastopore, and archenteron of the gastrula. There are several additional slides of later development. Examine these later larval stages but you do not need to draw them.
5. Obtain a slide of the larval and the immature starfish for observation only you do not need to draw them.

Activity 3: Development in the Chick:

We will next focus on the chick embryo as a type of embryonic development different from starfish. We can actually study and understand stages of human development using chicken eggs because development through the early embryonic stage in chicks and humans is almost identical. Let's describe human development first. The life cycle of a human begins at conception (fertilization of the egg) and proceeds through intrauterine development; birth growth and development to adulthood; and preparation for conception of the next generation, repeating the cycle. The events that characterize the life cycle are set in motion at the time of conception. At this instant in time a single haploid cell from the female fuses with a single haploid cell from the male to produce the fertilized egg or zygote. As in sea urchins, this fertilized egg is but a single cell with no clear features. It must undergo drastic change in order to produce the millions of cells that form the very complex adult body. See figure 4.

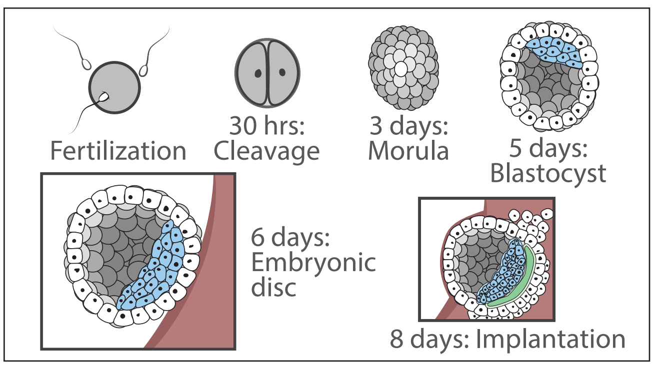

Figure 4. Human.

Cleavage and the Germinal Period:

Immediately after fertilization the egg begins to divide, forming the mass of cells needed to begin construction of the embryo. In humans, the rapid divisions following fertilization produce a morula and then a hollow ball of cells similar to the blastula of the starfish. This hollow ball has an outer layer of cells called the *trophoblast* which forms membranes such as the placenta needed by the developing embryo to obtain nutrients from its mother in utero, hence the name: tropho = to feed; blast = to become. On the inside of the hollow ball is a mass of cells (the inner cell mass) which separates from the trophoblast and forms a two-layered disc in the trophoblast interior. This disc is essentially a flattened blastula called a *blastodisc* and is the beginning of the embryo. Development to this stage takes 14 days and includes the implantation of the trophoblast into the uterine wall; these 14 days is called the *germinal period*. You will not observe these human developmental stages in this laboratory directly. Instead, you will focus on the chick as an example of early embryonic development.

Development of the chick embryo, which will be used for observation and comparison during this lab exercise, also produces a *blastodisc* (figure 5). But because developing chicks do not produce extra embryonic membranes such as the placenta, the events leading to blastodisc production are different. The blastodisc consists of a bilaminar layer of cells comprised of embryonic endoderm and ectoderm. The blastodisc divides rapidly and moves over the yolk. This structure will not be observed in this lab exercise. Further descriptions of development in this exercise refer to humans, although you will be observing the various phases of development and the specific features of each phase in chick embryos.

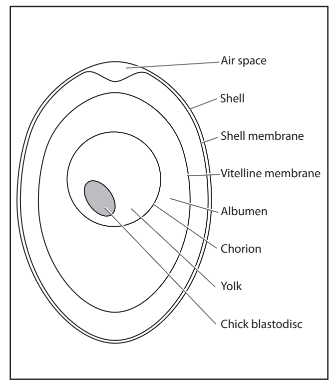

Figure 5.

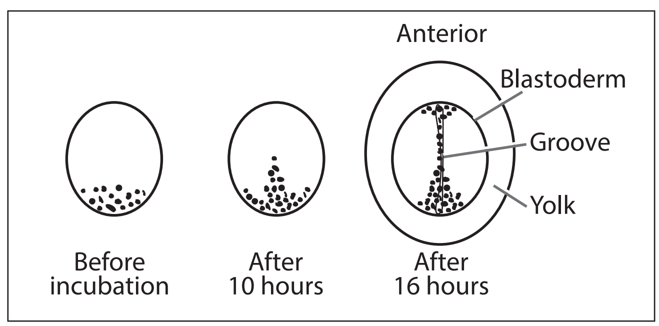

Figure 6.

Gastrulation:

Gastrulation is similar in both the chick and human embryo. A long groove begins to form on the upper surface of the blastodisc. This is called the *primitive streak* (see figure 6). Cells then migrate from the upper surface of the disc toward the groove. They move into the groove and then into the space between the upper and lower layers (see figure 7).

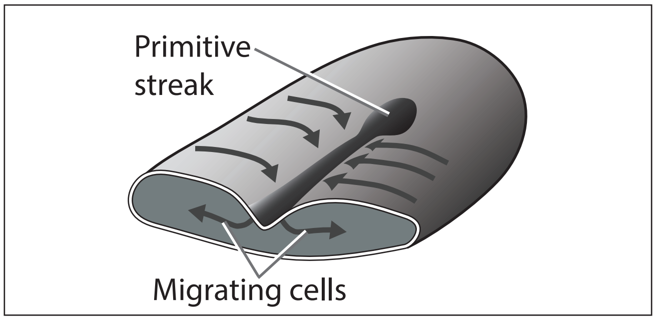

Figure 7.

During cell migration, the interaction between the migrating cells and the upper and lower cell layers results in the formation of three groups of cells. The upper layer becomes the ectoderm, the lower layer of cells forms the endoderm, and the cells in between form the mesoderm.

You will not observe human gastrulation in this exercise. The chick embryo develops essentially the same way, so observation of this organism will suffice. The mesoderm, originating from the primitive streak, migrates between the two layers.

Materials Needed:

- Microscope
- Series of 3 chick embryo slides

Procedure:

Obtain a slide of a 16-hour-old chick embryo. View this slide using a 4X objective. Locate the primitive streak. Draw a representation of the blastodisc and label the primitive streak in **Figure ➲1**.

Neurulation and Somite Formation:

Organogenesis (i.e, the formation of the organs) begins just prior to completion of the primitive streak and thus overlaps primitive streak formation somewhat. The first event in organogenesis is the formation of the neural tube, the precursor to the nervous system (see figure 8).

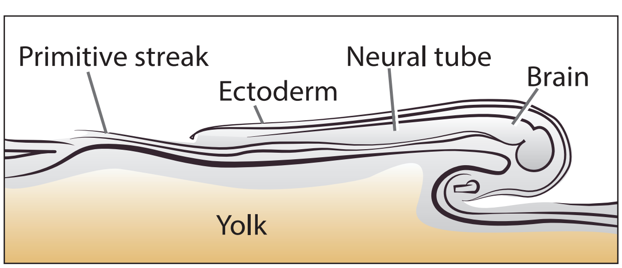

Figure 8. Sagittal section

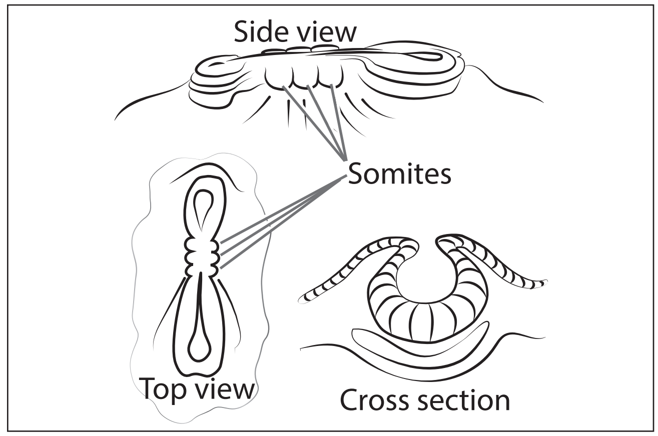

Figure 9. Neural groove formation

We will refer only to the visible phases in this description. As the primitive streak nears completion the neural groove begins to form in the same location. Cells from the ectoderm migrate toward the neural groove and form a crest of cells on each side. These are the neural crests. As the crests build on each side of the groove they begin to lap over the groove and come in contact with each other. To imagine this event, picture two waves approaching the groove from opposite directions, breaking just as they reach the neural groove (see figure 9).

When the upper edges of the waves make contact, they create a hollow space underneath them. The first contact occurs about the middle of the embryo and continues toward both ends, eventually creating a hollow tube that extends from the anterior to the posterior end of the embryo on its upper or dorsal surface. This structure becomes the brain and spinal cord of the adult.

At the site where the neural crests first make contact, mesoderm gathers into small groups along either side of the nerve cord just underneath the ectoderm. These are paired structures called *somites*. As the neural tube 'zips up' from the center, paired somites form along its entire length. It is the paired groups of mesoderm which give rise to the segmentation seen in humans, birds, and other segmented groups. Its human segmentation is expressed clearly in the vertebral column and ribs. The somites will grow, differentiate and form the segmented muscle blocks of the vertebrate body (refer to the 'washboard stomach' of athletic humans).

Later Development in the Chick Embryo:

The primitive streak and primitive pit will become the anus of the chick embryo; the embryo thus develops anterior to the streak. As the ectoderm, endoderm, and mesoderm spread anteriorly and laterally over the yolk, they rise up and form a *headfold* in the head region. The headfold contains an endoderm-lined cavity that represents the beginning of the *tubular gut*. Note how relatively small the primitive streak now appears compared to the rest of the embryo.

1. Obtain a slide of a 20- to 22-hour-old chick embryo from the center table. Observe this slide first by simply holding it up to the light to get a general view of the embryo. Try to identify the neural groove and/or neural tube, and the somites that have formed along both sides of the tube. View the slide using the 4X objective lens. In **Figure ➲J** draw an outline of the embryo and add the neural groove, somites and other structures which you see.
2. Obtain a slide of a 27- to 29-hour-old chick embryo from the center table. View this slide using the 4X objective. Using a different color pencil than the one used to draw the neural tube and somites, add any organs you see to the drawing in **Figure ➲K**.

➲L. What does the ectoderm, mesoderm, and endoderm become in the adult chick and human?

➲M. What role do stems cells play in embryological development?

➲N. What happens to the abundance of stem cells as you get older? Why?

➲O. It is only possible to split the embryo to make clones up to, but not beyond, the 32-cell stage of cell division? Why?

Making cloned mice for laboratory research has been done for many years. The process involves fertilizing a mouse ovum in a petri dish and letting it develop for several cell divisions. Next, the ball of cells (a morula) is carefully broken up into individual embryonic cells. These cells are implanted into the receptive womb of surrogate female mice. These embryos, all clones of each other, will develop into healthy mice.

➲P: Why is the use of clonal mice in lab experiments so desirable?

➲Q: How does the cloning of mice in this way differ from the more controversial cloning techniques used in the creation of the famous sheep named Dolly?

BOTH ENDS OF EMBRYOLOGICAL DEVELOPMENT

Within the Animal Kingdom, there are two great groupings defined by the fate of the blastopore. For the vast majority of species, the blastopore will eventually form the mouth in the adult organism. These species, which include the arthropods, molluscs, and the annelid worms, are called *Protostomes*. In the other group, called the *Deuterostomes*, the blastopore does not become the mouth, but the anus in the adult organism. The mouth forms at a secondary opening in the primitive gut. Echinoderms (e.g., sea urchins and star.

WORKSHEET
Development: Building an Animal from the Ground Up

Name:	
Date:	
Instructor:	
Lab Time:	

➲A

➲B

➲C	➲D

➲E	➲F

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