

Genetic Control of Development and Immune Defenses

LABORATORY

19

OVERVIEW

The hereditary material within fertilized eggs, or zygotes, of animal organisms holds the key to both structure and function. DNA guides, through time, the structural and functional development of a single cell into an embryo and its morphogenesis into an adult. Even aging is a result of genetic programming.

Biologists are just beginning to understand the genetic control of development. Advanced technologies as well as molecular and recombinant DNA techniques have made it possible to discover more and more about the genomes of organisms and to map the entire genomes of some. Common patterns of gene function are beginning to emerge, suggesting that some day, perhaps, we will be able to explain the mysteries of animal development.

Protection for the organism is also built into the genetic code. A newborn mammal possesses a full set of genetic information for the synthesis of a vast number of different immunoglobulins (antibodies). During development of the B cells of our immune system, however, each cell is modified to produce only a single type of antibody. How could the limited amount of DNA in a single organism produce millions of different types of antibodies? Research in recent years has shown that functional immunoglobulins are assembled from DNA segments that are initially separated and then rearranged during B cell development. Once again DNA exhibits its programming functions—DNA holds the secrets not only for “what” it produces but for “how” and “when” it produces its products.

During this laboratory you will have the opportunity to explore the development of sea urchin, frog, and chick embryos. You will also have an opportunity to investigate antibody activity using the precipitin ring test.

STUDENT PREPARATION

Prepare for this laboratory by reading the text pages indicated by your instructor. Familiarizing yourself in advance with the information and procedures covered in this laboratory will give you a better understanding of the material and improve your efficiency.

PART I DEVELOPMENT

All living things must be able to reproduce and develop. In animals, gametes produced by the process of meiosis unite during fertilization to form a single diploid cell, the zygote. The processes of cell division, cell movement, cellular differentiation, and morphogenesis result in the development of a multicellular embryo that will grow to form an adult.

✓ EXERCISE A Fertilization and Early Development in Sea Urchins

Understanding how DNA regulates cell division, differentiation, and morphogenesis begins with observing the developing embryo. Sea urchins have long been the developmental biologist's favorite organism of study because they are relatively simple to obtain and culture in the laboratory. The cells of the developing sea urchin are also fairly transparent, providing us with a limited ability to "look inside" the embryo.

Early development of the sea urchin is under the genetic control not only of the zygote's DNA but of messenger RNA (mRNA) stored in the egg during its development. These messages include maternal mRNAs synthesized from maternal DNA prior to the meiotic events of oogenesis—mRNAs made from DNA that may not be included in the egg itself. Thus, the story of development begins before fertilization.

The unfertilized sea urchin egg is surrounded by a **vitelline membrane** that lies just above the surface of the cell's plasma membrane (plasmalemma). Within the cytoplasm, yolk granules (sea urchin eggs are microlecithal—they have very little yolk) can be observed. In addition to other cytoplasmic determinants and stored mRNAs, small **cortical granules**, composed of proteins and mucopolysaccharides, lie just beneath the plasmalemma (in the outer rim or cortex of the egg) (Figure 19A-1a). When a single sperm enters the egg plasmalemma, the membrane potential quickly changes as a wave of depolarization spreads from the site of sperm entry. This reaction is often referred to as a "fast block to polyspermy," since no additional sperm can gain entry following the change in membrane potential. Release of Ca^{2+} ions from the egg's endoplasmic reticulum, in response to G protein, causes the cortical granules in the egg's cortex to fuse with the plasmalemma (Figure 19A-1b).

The cortical granules discharge their contents into the space between the plasmalemma and the vitelline membranes. The excess mucopolysaccharide now present in the perivitelline space lowers the water potential of that area, and water flows in. This causes the perivitelline space to increase in diameter, making it appear as if the vitelline membrane is lifting off the surface of the zygote. Addition of proteins to the vitelline membrane hardens (or "tans") it as it is transformed into a **fertilization membrane** (Figure 19A-1c). Formation of the fertilization membrane (often called the "slow block to polyspermy") offers additional protection against multiple sperm entry. Hundreds of sperm can usually be observed still attached to the old vitelline membrane, now the fertilization membrane. These will be removed by the action of enzymes released from the cortical granules.

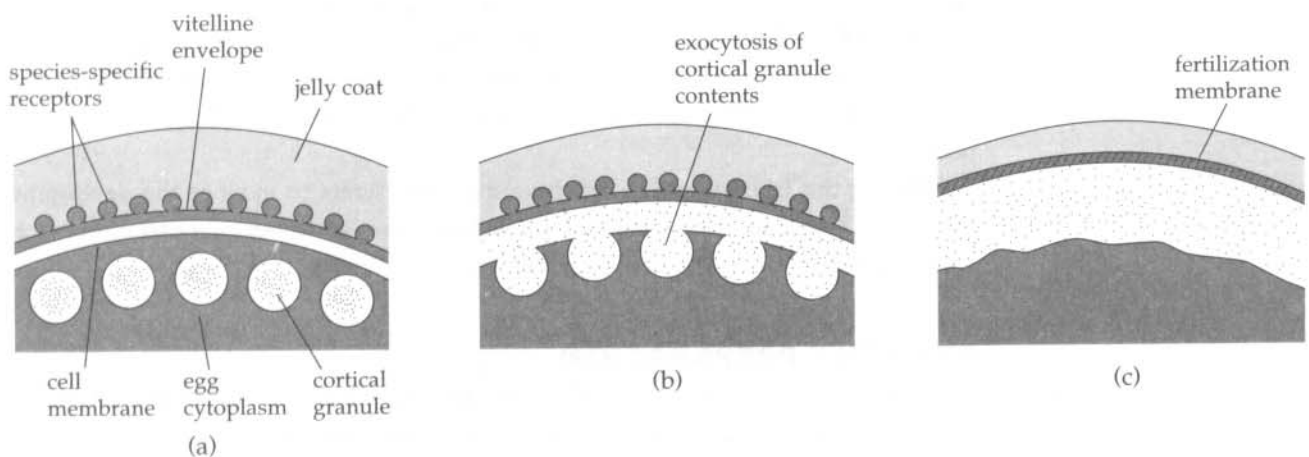


Figure 19A-1 Formation of the fertilization membrane in the sea urchin. (a) The surface layers of an unfertilized egg include a jelly coat; the vitelline membrane or envelope, which bears species-specific receptors for sperm; and the cell membrane (plasmalemma). (b) Contact with a sperm triggers Ca^{2+} release and the fusion of cortical granules with the plasmalemma. (c) Cortical granules release their contents into the perivitelline space, causing the vitelline membrane to rise from the surface to form the fertilization membrane.

Following fertilization, the stored mRNAs are responsible for directing protein synthesis during the early stages of development—cleavage, blastula formation, and gastrulation. In addition to the mRNA and protein already present in the fertilized egg, newly synthesized protein products are responsible for establishing regional differences in the egg cytoplasm. These proteins or **cytoplasmic determinants** are responsible for establishing the cytoarchitecture of the unfertilized egg, reorganizing the cytoplasmic elements in response to fertilization, controlling the direction of the first cleavage divisions, and establishing the axis of the embryo.

In this exercise, you will have the opportunity to observe the earliest stages of sea urchin development—beginning with fertilization. You will also have available later-stage embryos for observation of gastrulation and formation of pluteus larvae.

||||| Objectives |||||

- ☐ Describe the processes of fertilization and cleavage division in the sea urchin.
- ☐ Observe formation of the fertilization membrane as it expands to surround the sea urchin.

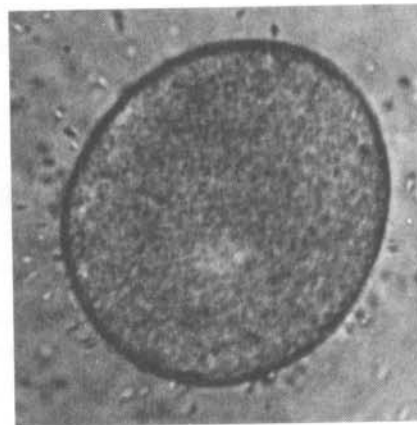
Procedure

1. Inject 0.1 to 1.0 ml of 0.5 M KCl intracoelomically through the soft tissue on the oral surface of the animal. Shake the animal gently after injection to distribute the KCl. (For some urchins, KCl injections will not work, but electrical stimulation is effective. This method requires an adjustable physiological stimulator set to a frequency of 60 Hz. Use 6 to 10 volts AC for smaller urchins and 20 to 30 volts AC for larger urchins. One electrode should be placed directly on the test (body surface) near the gonopore region and the other on a wet cotton ball or tissue on the oral surface.)
2. Gamete shedding should begin minutes after injection. (If you use electrical stimulation, gamete shedding will continue as long as the animal is stimulated, thus you can collect only the amount of material needed.) Once gamete shedding begins, the sex of the urchin can be determined. Semen is gray-white or cream colored. Eggs vary in color, depending on the species, and may be pale yellow, yellow-orange, or reddish.
3. To collect eggs, invert the female over a beaker of seawater and lower the urchin until it is covered by the seawater. Eggs will stream from the gonopores and fall to the bottom of the beaker. If electrical stimulation is used, place the female, after stimulation, right side up (gonopores up) in a beaker of seawater—the urchin should be completely covered with seawater.
4. Using a wide-mouth pipette (a turkey baster is useful), remove eggs that have settled to the bottom of the beaker and resuspend them in clean seawater.
5. Let the eggs settle, decant the seawater, and replace it with clean seawater. (This step removes any traces of coelomic fluid, which might interfere with the fertilization process.) The eggs are now ready to use.
6. It is best to collect sperm in the “dry” condition for maximum concentration. Place the male in a finger bowl of seawater, but make sure that the level of the water is below the gonopores. As the whitish semen is extruded from the gonopores, collect it with a Pasteur pipette. Store the semen in a test tube on ice (it will remain viable for several hours).
7. Return the urchins to a separate aquarium (if the animals continue to shed gametes, they may trigger other animals in the aquarium to shed gametes).
8. Obtain a depression slide and place a sample of egg suspension into the concave depression. Cover with a glass coverslip. Observe the eggs using the 10× and 40× objectives. If you wish to observe the jelly coats, add a drop of 0.1 Janus green before applying the coverslip.
9. Obtain a second depression slide and place another sample of the egg suspension into the concave depression. Cover with a coverslip, but leave a small opening on one side for the

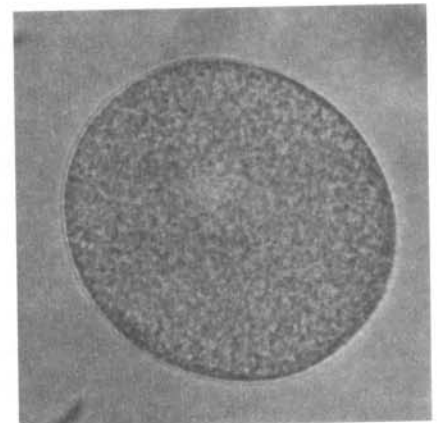
addition of sperm in step 10. Place the slide on the microscope stage and use the 10 \times objective to locate the eggs.

10. Use a Pasteur pipette to place a drop of concentrated sperm suspension on a clean glass slide. Add two drops of seawater. Mix with a toothpick and, using the blunt end of the toothpick, introduce some sperm suspension into the egg suspension on the slide. Observe immediately.
11. Watch for the fertilization membrane to form, then turn off the light on your microscope or remove the slide from the microscope and gently place it in a safe place (away from heat and hot light) for later observation. If the seawater begins to evaporate from your depression slide, add more using a Pasteur pipette.
12. Fusion of pronuclei usually occurs 30 to 45 minutes after gamete fusion. The first cleavage division occurs 60 to 90 minutes after gamete fusion, depending on the species. Check your slide in approximately 45 minutes and then at 10- or 15-minute intervals until you observe the first cleavage division.
13. Your instructor may have additional samples of embryos in other stages of development for you to examine. Prepare wet-mount depression slides of these. See if you can identify four- or eight-cell embryos, early blastulae, hatching blastulae, or gastrula embryos. In the sea urchin, the larval form is called a **pluteus larva**.
14. For a composite photographic description of sea urchin development, refer to Figure 19A-2.

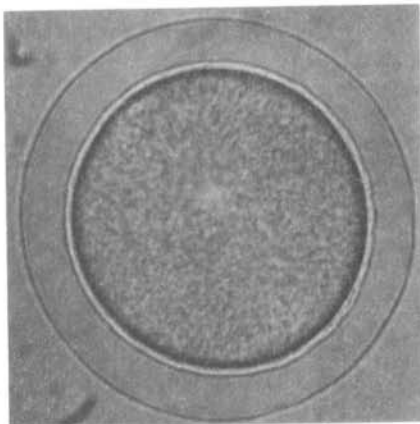
Figure 19A-2 Sea urchin development. (a–h) As the egg divides, the cells become progressively smaller, so that by the blastula stage they are barely distinguishable. (i) Gastrulation begins with the formation of the blastopore, then (j, k) secondary mesenchyme cells break loose to migrate along the inner surface of the blastula. These form long pseudopodia that (l) help to “pull” the forming gut toward the opposite side of the embryo. (m) Spicules form from primary mesenchyme cells within the developing pluteus larva (n).



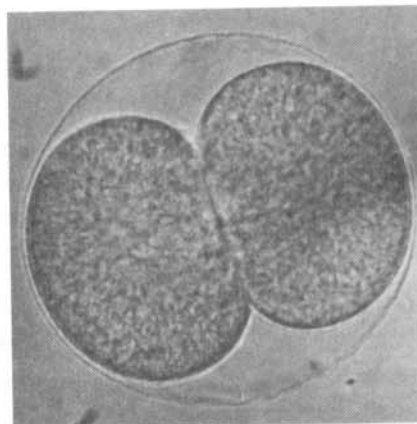
(a) Numerous spermatozoa can be seen surrounding an unfertilized egg.



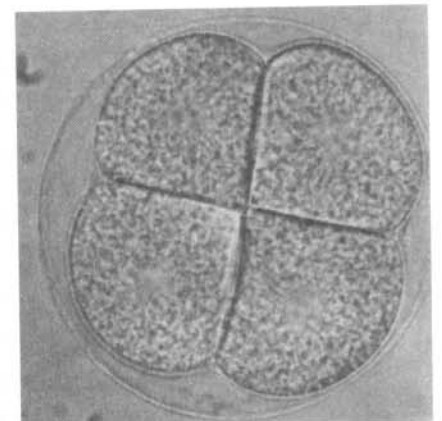
(b) Fertilized egg; the fertilization membrane has just begun to form. The light area slightly above the center is the diploid nucleus.



(c) The fertilization membrane is fully formed. The egg has begun to divide; if you look closely, you can see that there are two nuclei.

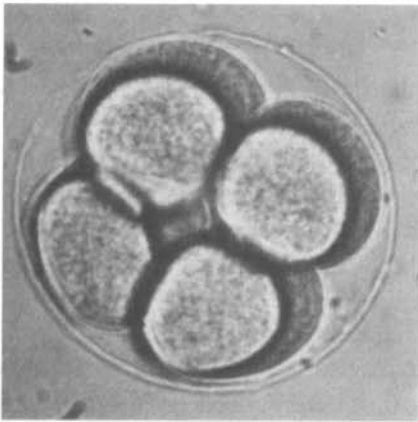


(d) The first division.

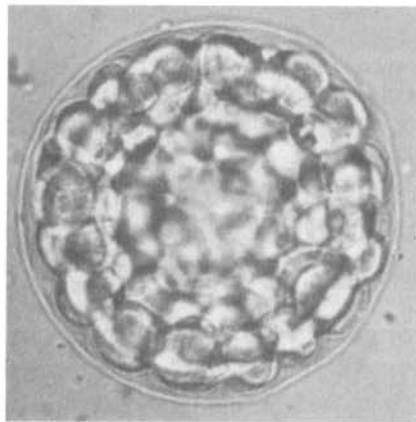


(e) Four-cell stage.

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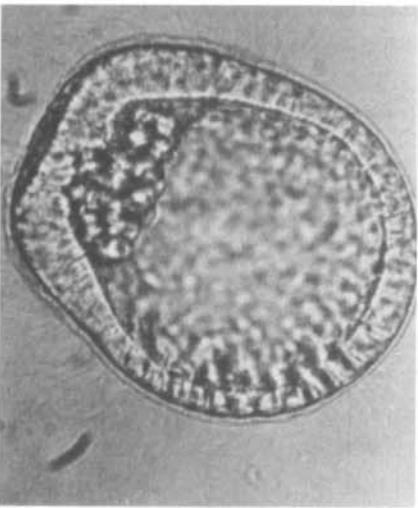
(f) Eight-cell stage.



(g) The blastocoel forms.



(h) The mature blastula.



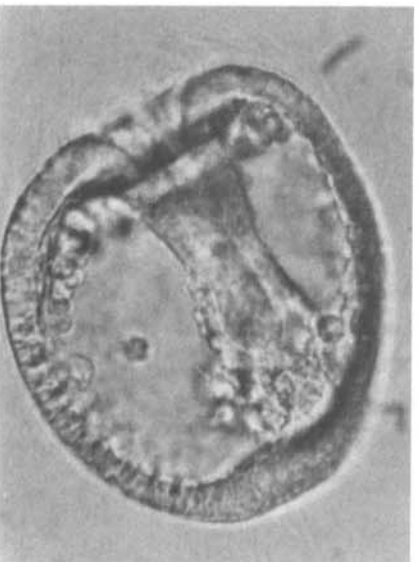
(i) The beginning of gastrulation; the blastopore has begun to form at the upper left, and cells near the blastopore have begun to migrate across the blastocoel.



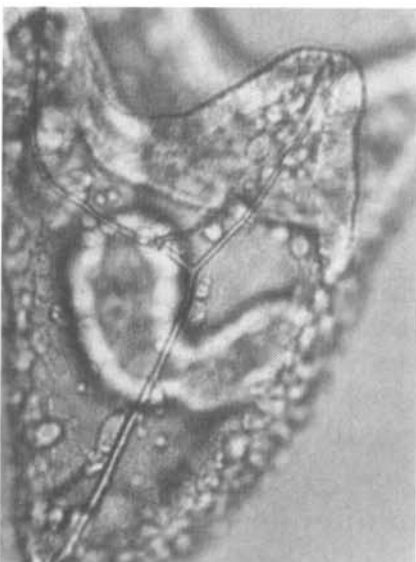
(j) The outer cell layer begins to fold inward at the blastopore, forming the archenteron.



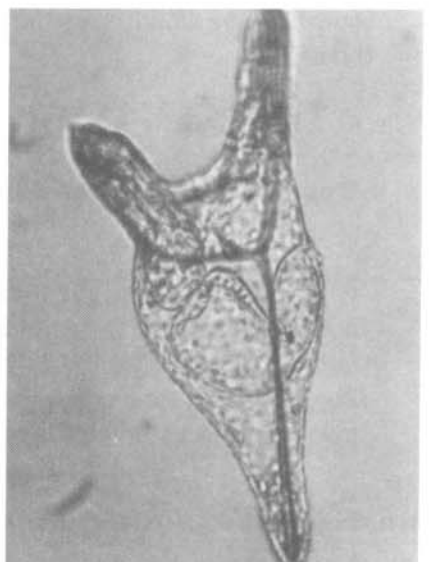
(k) The outer layer of cells continues to move across the blastocoel.



(l) The mature gastrula.

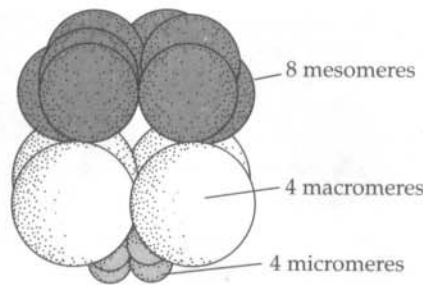


(m) Gastrula cells differentiate and organize to form the pluteus larva.



(n) Within 48 hours after fertilization, the egg has developed into a free-swimming multicellular organism, the pluteus.

Figure 19B-2 Fourth cleavage division in sea urchin. An unequal fourth cleavage division results in 16 blastomeres of three different sizes. A top tier of eight medium-size blastomeres, called mesomeres, will eventually give rise to ectodermal structures. The four large macromeres will develop into endodermal structures associated with the gut. The four smallest blastomeres, micromeres, will produce mesodermal structures.



Procedure

1. Obtain a composite slide of starfish (*Asterias*) development. This slide will have all stages of echinoderm development represented, including unfertilized eggs, fertilized eggs, and cleavage stages (Figure 19B-3). Some of the unfertilized eggs contain a **germinal vesicle**, a large swollen nucleus containing the nucleolus (appears as a black dot). The germinal vesicle breaks down at the end of prophase I and the egg appears as an opaque sphere during the remainder of its maturation.

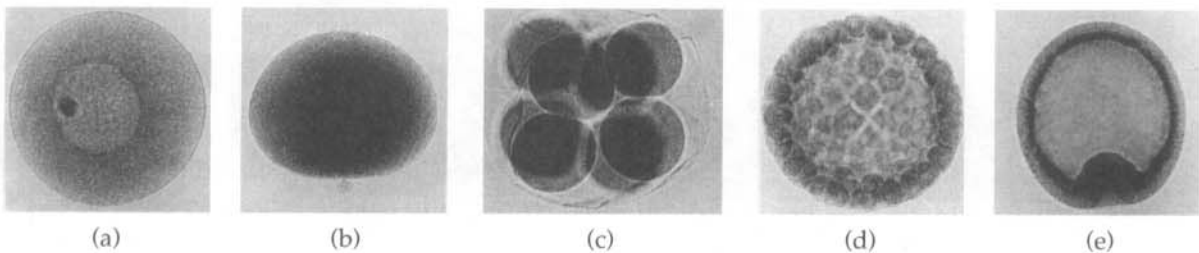


Figure 19B-3 Embryonic development of the starfish: (a) germinal vesicle; (b) zygote with polar body; (c) 8-cell stage; (d) blastula; (e) early gastrula, blastopore apparent.

Locate an unfertilized egg with a germinal vesicle and one without. Label a sheet of paper "Starfish Development" and draw these two examples of an unfertilized egg on the top half of the sheet. Label the nucleus and nucleolus.

- a. Why is the nucleus of the developing zygote so big? _____
2. Find two-cell, four-cell, and eight-cell embryos on your slide of *Asterias* development. Notice that all of the cells in this embryo are the same size. On your sheet of paper, draw what you observe.
 - a. Is the mass of a four-cell or an eight-cell embryo any larger than the mass of the zygote? _____
 - b. What is the effect of cleavage on cell size? _____
 - c. On embryo size? _____

If live sea urchin material is available from Exercise A, observe the first cleavage division and other early cleavage stages. Compare your observations of starfish development with the stages of sea urchin development shown in Figure 19A-2.

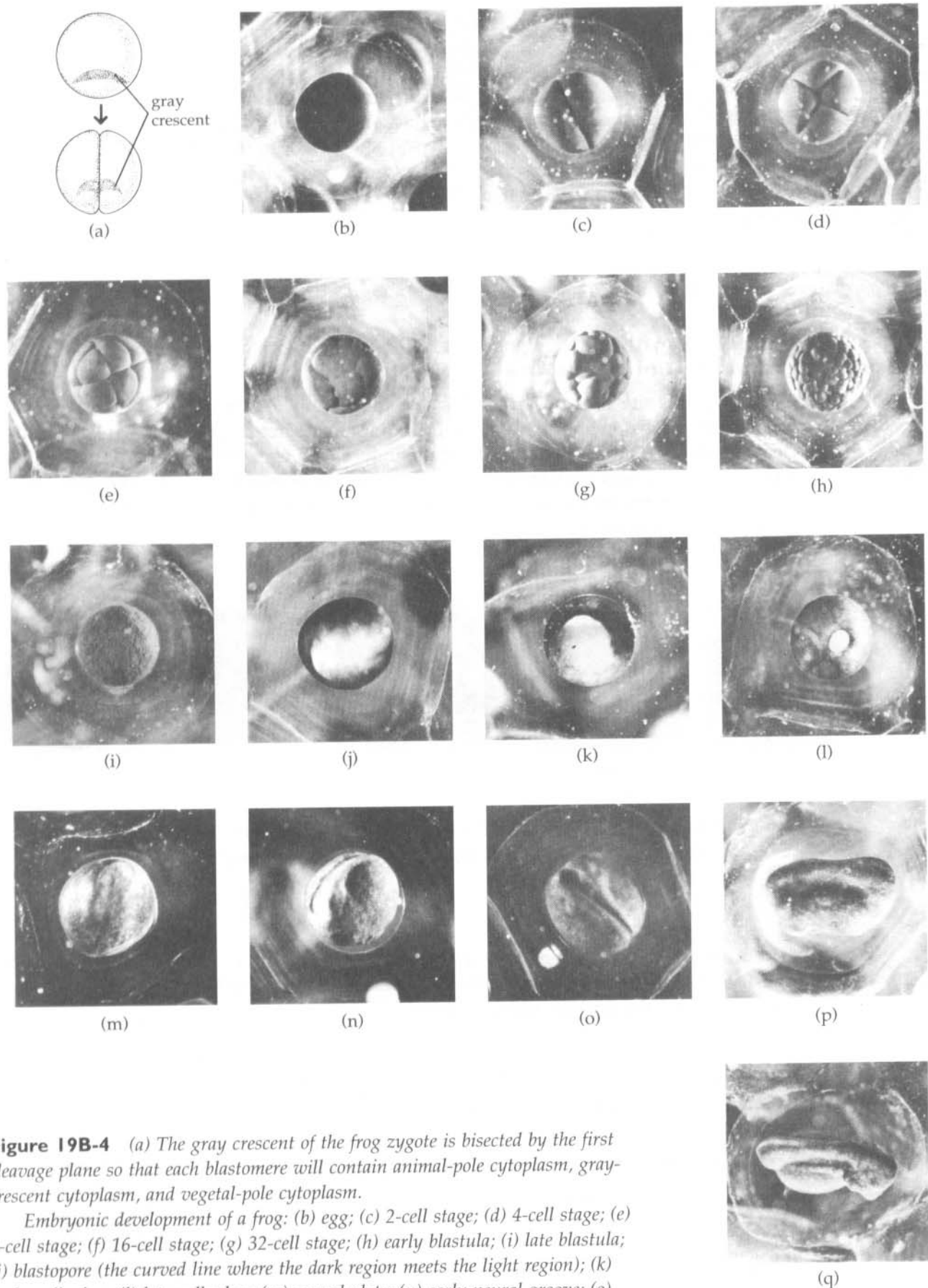


Figure 19B-4 (a) The gray crescent of the frog zygote is bisected by the first cleavage plane so that each blastomere will contain animal-pole cytoplasm, gray-crescent cytoplasm, and vegetal-pole cytoplasm.

Embryonic development of a frog: (b) egg; (c) 2-cell stage; (d) 4-cell stage; (e) 8-cell stage; (f) 16-cell stage; (g) 32-cell stage; (h) early blastula; (i) late blastula; (j) blastopore (the curved line where the dark region meets the light region); (k) early yolk plug; (l) late yolk plug; (m) neural plate; (n) early neural groove; (o) late neural groove; (p) neural tube; (q) tailbud stage.

d. What do you observe? Are the developmental stages similar? _____

3. Examine a fertilized frog egg (preserved specimen) using a dissecting microscope. The frog egg shows how the arrangement of cytoplasm influences the placement of the first cleavage plane. In the frog, fertilization results in a shifting of the pigmented cytoplasm of the animal pole, establishing a grayish, crescent-shaped area (the **gray crescent**) on one side of the zygote (Figure 19B-4a). This area must be cut in half (bisected) by the first cleavage plane, thereby establishing the right and left halves of the future embryo. Use dissecting needles to move the egg around until you see the gray crescent. Do not poke the egg.
4. Now examine an embryo in the two-cell stage. e. Does the cleavage plane cut the gray crescent in half? _____

Interestingly, if you separated these two halves of the embryo, each would develop into a normal tadpole (twins). If, however, you artificially divided the embryo into two halves (by slowly constricting it with a fine hair loop) so that all of the gray crescent material was contained in one blastomere, only the blastomere containing the gray crescent material would develop normally.

5. Examine a sagittal section through an early cleavage stage of the frog zygote. Notice the dark pigment at the surface of the animal pole of the egg. Are the top blastomeres the same size as the lower blastomeres? _____ Label a sheet of paper "Frog Development" and draw the representative section at the top of the sheet.



EXERCISE C Formation of the Blastula

Repeated cleavages will result in formation of a hollow ball of cells called a **blastula**. The cavity inside the blastula is called the **blastocoel**. Even as early as this blastula stage, groups or layers of cells are already destined to become particular organs or organ systems; these layers of cells are known as **presumptive germ layers**. The major germ layers and their derivatives are listed in Table 19C-1.

Table 19C-1 Germ Layers and Their Derivatives

Germ Layer	Derivative
Epidermal ectoderm	Skin
Neural ectoderm	Brain, spinal cord, and neural crest cells
Chordamesoderm	Notochord and spinal disks in some organisms; ganglia
Mesoderm	Skeleton, circulatory system, excretory system, and parts of organs belonging to other systems
Endoderm	Gut and associated outpocketings

In the sea urchin, blastomeres that will give rise to cells of germ layers are already laid out at the 16-cell stage. A **fate map** can be assigned. Mesomeres will give rise to ectodermal structures including the cilia that develop on the blastula's surface. Macromeres will give rise to endodermal structures. Micromeres will be responsible for formation of the body cavity, many internal organs, and the skeletal elements (**spicules**) of the embryo (see Figures 19A-2 and 19B-2).

Objectives

- ☐ Describe the structure of a typical blastula.
- ☐ Relate the structure of the frog blastula to the establishment of the dorso-ventral and antero-posterior axes of the embryo.

Procedure

1. Reexamine the composite slide of starfish development. Locate an early blastula stage. The cells will be large enough to see and will appear as a dark ring of cells surrounding a lighter center. Remember that these are whole mounts and you are looking through a hollow sphere (see Figures 19A-2 and 19B-3). Draw your observations on the sheet of paper labeled "Starfish Development." Label blastomeres and blastocoel.

a. Why does the center appear to be lighter? _____

2. Locate a later blastula stage. The cells of the blastula are now so small that it will be difficult to distinguish individual cells (see Figures 19A-2 and 19B-3). Again, the outer rim appears to be dark. b. Why? _____

At this time, the cells of the blastula are covered by cilia which allow the blastula to spin and move. Draw your observations. Label blastomeres and blastocoel.

3. Examine a sagittal section through a frog blastula. Note the placement of the blastocoel.

c. Why is the blastocoel found closer to the animal pole of the embryo? _____

d. How does the size of the animal-pole blastomeres compare with that of the vegetal-pole blastomeres? _____

Draw the sagittal section on your sheet of paper labeled "Frog Development," and label blastomeres and blastocoel.



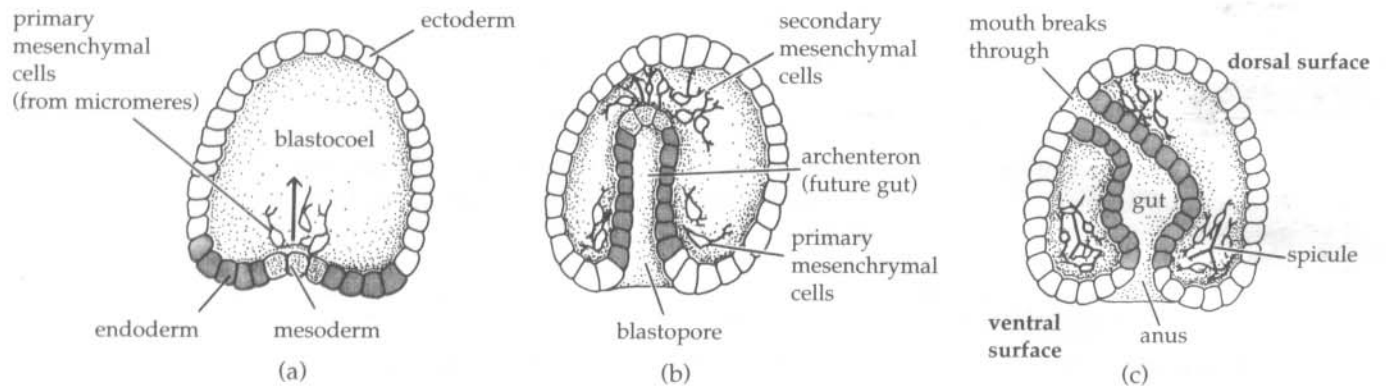
EXERCISE D Gastrulation

As a blastula, most of the cells of the embryo (including those destined to become cells of internal organs and tissues) are on the outside of the hollow ball, and it is obvious that some of the sheets of cells must move, or migrate, to the inside of the blastula. This process of cell movement is called **gastrulation**.

In some organisms, such as the starfish and sea urchin, gastrulation is accomplished simply by buckling or pushing inward, forming a depression or **blastopore**. Endoderm and mesoderm reach the inside of the embryo in this manner. In other organisms, such as the frog or the chick, cells migrate to the interior by way of the blastopore, the portion of the embryo that will eventually contribute to the development of the anus (Figures 19A-2 and 19D-1). Mesoderm, endoderm, and chordamesoderm (notochord material) migrate to the inside. With the change in position of the germ layers, the blastocoel of echinoderms (starfish and sea urchin) and chordates (frog) is eventually obliterated and a new cavity, the **archenteron**, is formed within the gastrula. The archenteron is the primitive gut of the embryo (see Figures 19A-2, 19B-4, and 19D-1).

Note that during sea urchin development, secondary mesenchyme cells help to "pull" the endoderm of the newly forming archenteron (gut) toward the opposite side of the embryo, where it will fuse with the outer layer, or ectoderm, to form the mouth. (Hence the term "deuterostome" or second mouth, the anus forming at the blastopore or first opening.) See Figures 19A-2 and 19D-1.

Gastrulation in the starfish and the sea urchin



Gastrulation in the frog

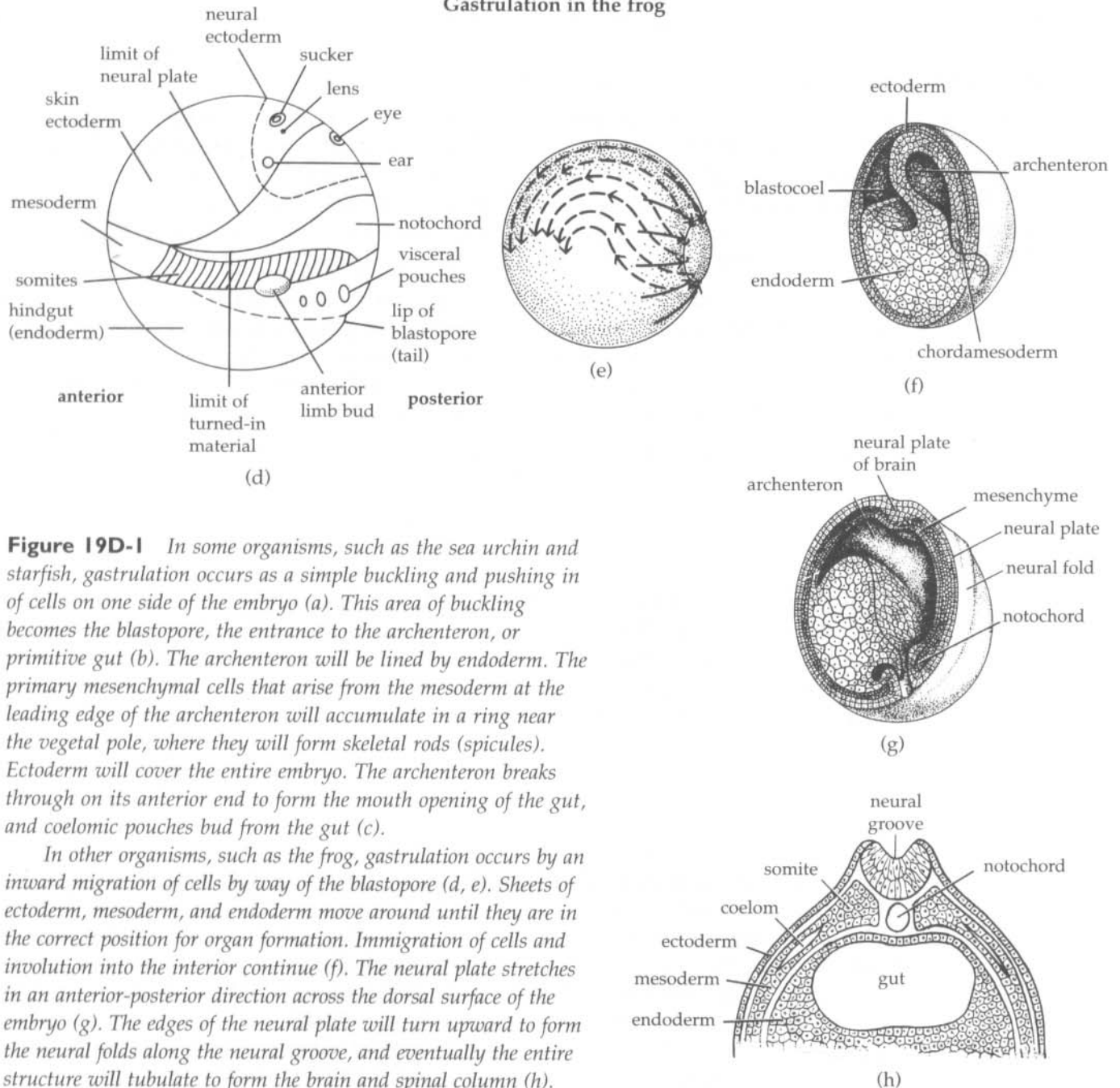


Figure 19D-1 In some organisms, such as the sea urchin and starfish, gastrulation occurs as a simple buckling and pushing in of cells on one side of the embryo (a). This area of buckling becomes the blastopore, the entrance to the archenteron, or primitive gut (b). The archenteron will be lined by endoderm. The primary mesenchymal cells that arise from the mesoderm at the leading edge of the archenteron will accumulate in a ring near the vegetal pole, where they will form skeletal rods (spicules). Ectoderm will cover the entire embryo. The archenteron breaks through on its anterior end to form the mouth opening of the gut, and coelomic pouches bud from the gut (c).

In other organisms, such as the frog, gastrulation occurs by an inward migration of cells by way of the blastopore (d, e). Sheets of ectoderm, mesoderm, and endoderm move around until they are in the correct position for organ formation. Immigration of cells and involution into the interior continue (f). The neural plate stretches in an anterior-posterior direction across the dorsal surface of the embryo (g). The edges of the neural plate will turn upward to form the neural folds along the neural groove, and eventually the entire structure will tubulate to form the brain and spinal column (h).

a. Why do the mesoderm, endoderm, and future notochord need to be on the inside?

b. To what organs or organ systems do these germ layers give rise?

Ectoderm and neural ectoderm remain on the outside of the frog or chick embryo. Eventually, the neural ectoderm will form a tube that is to become the brain and nerve cord, while the epidermal ectoderm spreads to cover this structure and the rest of the embryo.

■■■■ Objectives ■■■■

- ☐ Compare and contrast the structural components of the blastula and gastrula frog embryo.
- ☐ Describe the purpose of gastrulation.
- ☐ Compare the process of gastrulation in the starfish and the frog.

■■■■ Procedure ■■■■

1. Use high power (40×) to examine the composite slide of starfish (*Asterias*) development. Locate both early and late gastrula embryos. You will see the **archenteron** beginning to form as an inpocketing. The front end of the archenteron is expanded and will eventually fuse to the side of the embryo where the mouth will break through. The blastopore (region of inpocketing) becomes the anus. Refer to Figures 19A-2 and 19B-3. Draw the early and late gastrula embryos on the sheet of paper labeled "Starfish Development."
2. Locate a folded and twisted **bipinnaria** larva of *Asterias*. Note that the gut has become differentiated into an **esophagus**, **stomach**, and **intestine**. Two **coelomic pouches** may be seen attached to the gut wall. These are mesodermal and will eventually expand to form the mesodermally lined coelomic cavity. Draw a bipinnaria larva on your sheet of paper. Label the esophagus, stomach, intestine, and coelomic pouches.

c. Recall from your study of diversity that echinoderms are enterocoelous coelomates. Explain how this description reflects the developmental events observed on the starfish composite slide.

3. Compare the structure of the starfish bipinnaria larva to that of the sea urchin pluteus larva (Figure 19A-2). If live specimens of sea urchin pluteus larvae are available from Exercise A, use a depression slide to observe that structure and movement. Observe the skeletal elements (spicules) and internal organs of the digestive system. Do you observe any coelomic pouches forming? In the space below, describe what you observe.

4. Study the preserved frog embryos on demonstration. Using the dissecting microscope, examine the blastopore region at the center of the lower edge of the gray crescent. Cells on the exterior stream toward the blastopore to turn inward (Figures 19D-1e and 19B-4j, k) and then spread out on the inside of the gastrula to take up their proper places. As cells converge on the blastopore, it appears first to form sides and then a ventral lip that completely encircles the yolky cells (the "yolk plug") of the interior (Figure 19B-4l).

Note the changes in the blastopore region in eggs of increasing ages. Identify the yolk plug. Draw these embryo stages on the sheet of paper labeled "Frog Development."

5. Use scanning and low powers (4× and 10×) to observe a sagittal section through a late gastrula or **yolk plug** stage of the frog embryo. Identify the yolk plug and archenteron. Using Figure 19D-1, try to identify the cells of the notochord, ectoderm, and endoderm of the gastrula.

EXERCISE E Neurulation

During this stage of development, a strip of neural ectoderm on the outside of the dorsal surface of the embryo (Figure 19B-4m) turns upward to form a **neural tube** (Figure 19D-1h). The underlying mesoderm and notochord tissue induce formation of the neural tube. The folds of tissue forming the tube are the **neural folds** and the groove between them is the **neural groove**. Eventually the anterior end of the neural tube will expand to form the **brain**; the spinal cord will develop posterior to the brain.

Aggregations of mesoderm (mesodermal **somites**) behind the brain and alongside the spinal cord will form vertebrae (back bones) that protect and enclose the spinal cord. Mesodermal somites also give rise to dorsal skeletal muscles and to the dermis of the skin (Figure 19D-1d). The presence of somites is an indication of the segmented nature of vertebrate embryos. Biologists have recently shown that a series of genes control the development of segmentation in all segmented embryos, whether in fruit flies, mice, or in humans. These genes, called **homeotic genes**, often work in a cascade with other genes that control the basic head-to-tail and anterior-to-posterior architecture of the embryo. Within each homeotic gene, a special sequence or **homeobox** of 180 base pairs codes for a protein that is 60 amino acids long. This is a DNA regulatory protein that can “turn on” other genes involved in segmentation and segment identity (whether wings, antennae, legs, and so forth, are attached). The same homeobox is found in all homeotic genes of segmented organisms—its base sequence has been **conserved** throughout evolution.

The gut will tubulate during this later stage of development, and the ventral unsegmented mesoderm will split to form a mesodermally lined coelom (Figure 19D-1h).

■■■■ Objectives ■■■■

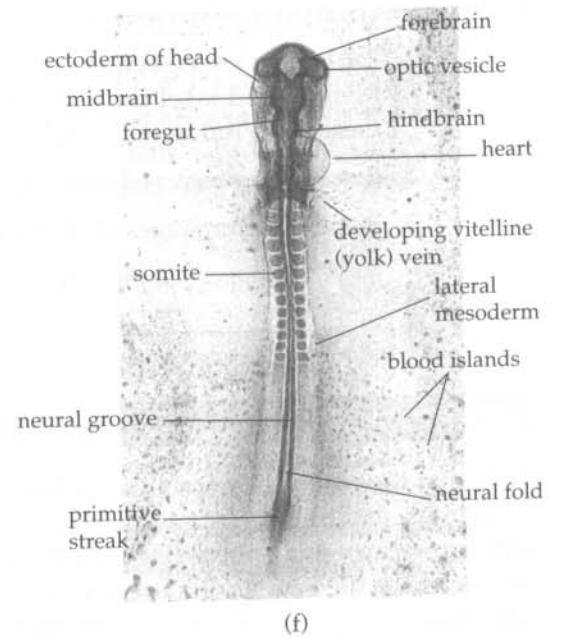
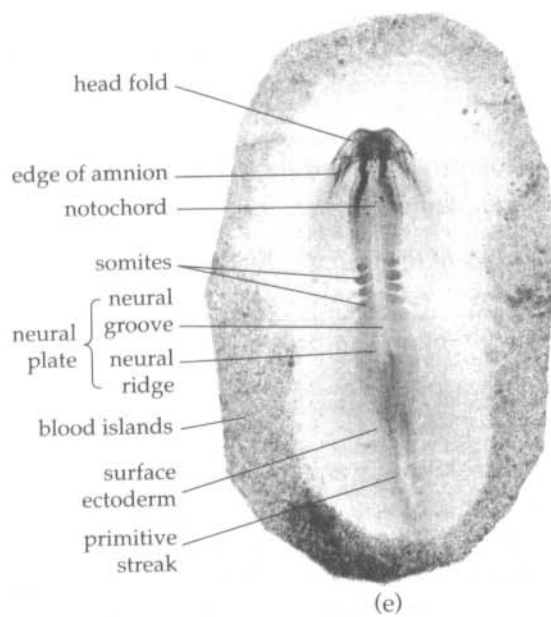
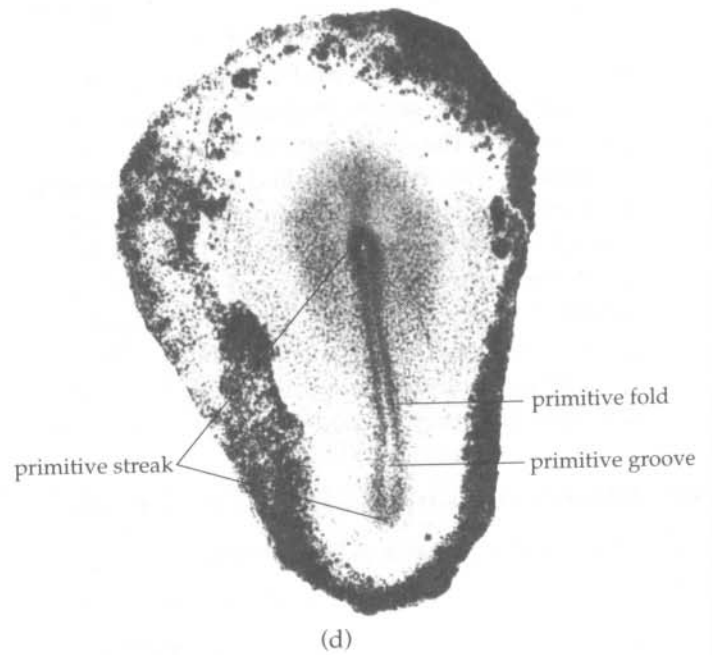
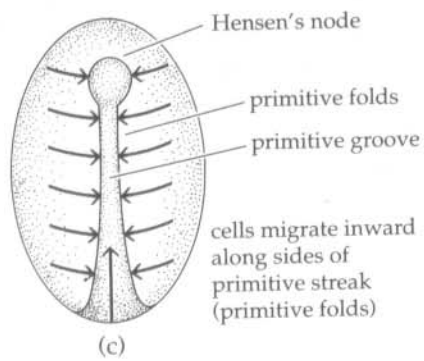
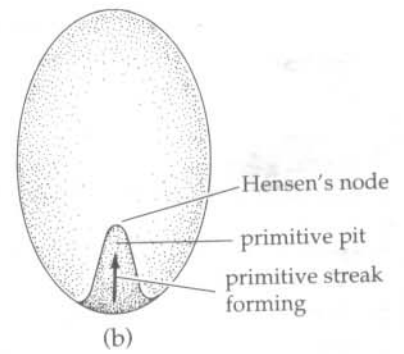
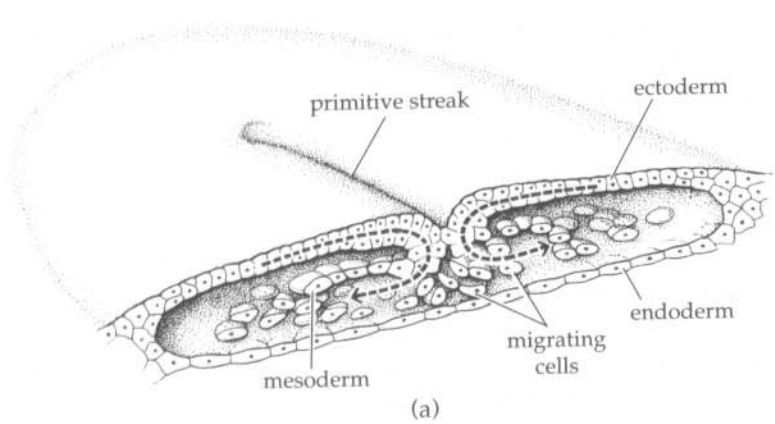
- ☐ Describe the process of neurulation.
- ☐ Describe the function of the chordamesoderm.
- ☐ Relate the development of the brain and the spinal column to the development of the neural tube and neural folds.

■■■■ Procedure ■■■■

1. Examine a cross section of a frog late neurula. Draw this on your “Frog Development” sheet. Identify and label the neural tube, notochord, somite mesoderm, gut cavity, epidermis, and mesoderm lining the coelom.
2. Examine a preserved late-neurula frog embryo. Identify the neural groove and neural folds.
3. Examine later stages of frog embryos (Figure 19B-4).
 - a. What happens to the shape of the embryo after the sides of the neural tube close?

EXERCISE F Development of the Chick

Note that the same body-forming movements occur during gastrulation in the development of chickens as in frogs, except that the end product of the process is three flat layers of cells rather than three concentric rings in a sphere (Figure 19F-1). At the beginning of gastrulation, the disk of cells (blastodisc) on the top of the egg is composed of two layers—the upper layer containing ectoderm, neural ectoderm, notochord, and mesoderm tissue; the lower layer containing endoderm. During gastrulation, notochord and mesoderm cells are moved from the top layer to form a middle layer.



As cells migrate to form the underlying tissue layer, they converge toward the posterior portion of the midline of the blastodisc (Figure 19F-1c). There they sink downward (similar to the way cells sink inward at the blastopore of the frog gastrula). This area, at first triangular in appearance, is called the **primitive streak**. As more cells move into the area, the primitive streak appears to lengthen, forming the **primitive groove** bordered by **primitive folds**, formed by accumulations of cells converging on the area in preparation for invagination. If you made a tube out of the flat layers of the chick after completion of gastrulation, the structure would look surprisingly like a frog gastrula.

☐ Compare gastrulation and neurulation in the chick and the frog.

1. Observe a prepared slide of a 16-hour-old chick and identify the primitive groove and primitive folds.
2. Observe later stages of chick development (Figure 19F-1d, e, f). Note that the neural folds and mesodermal somite blocks can easily be seen very early in the development of a chick (17 to 29 hours). Remember that the chick must tubulate in order for its “flat” layers of tissues to form a circular gut tube. The head is the first area to tubulate, and it appears to be lifted up off the surface of the blastodisc (Figure 19F-1f).

As the chick continues to develop inside the egg, special vascularized membranes develop to serve the respiratory, protective, nutritional, and excretory needs of the embryo.

In the chicken embryo, four membranous sacs are formed by the growth and folding of the sheets of ectoderm, mesoderm, and endoderm at the edges of the blastodisc. These **extraembryonic** membranes are continuous with embryonic tissue, but lie outside (hence “extraembryonic”) the embryo (Figure 19G-1a). The **amnion** is formed around the embryo and fills with fluid to serve a protective function. The vascularized **chorion** lies beneath the shell and serves a respiratory function by picking up oxygen that has diffused through the shell and transporting it to the embryo. The **allantois**, which invaginates from the hindgut, serves as a repository for wastes and eventually fuses with the chorion to aid in respiration. The **yolk sac** invaginates from the gut cavity and becomes highly vascularized by a network of vitelline vessels that pick up nutrients from the yolk and transport them to the embryo (Figure 19G-1b).

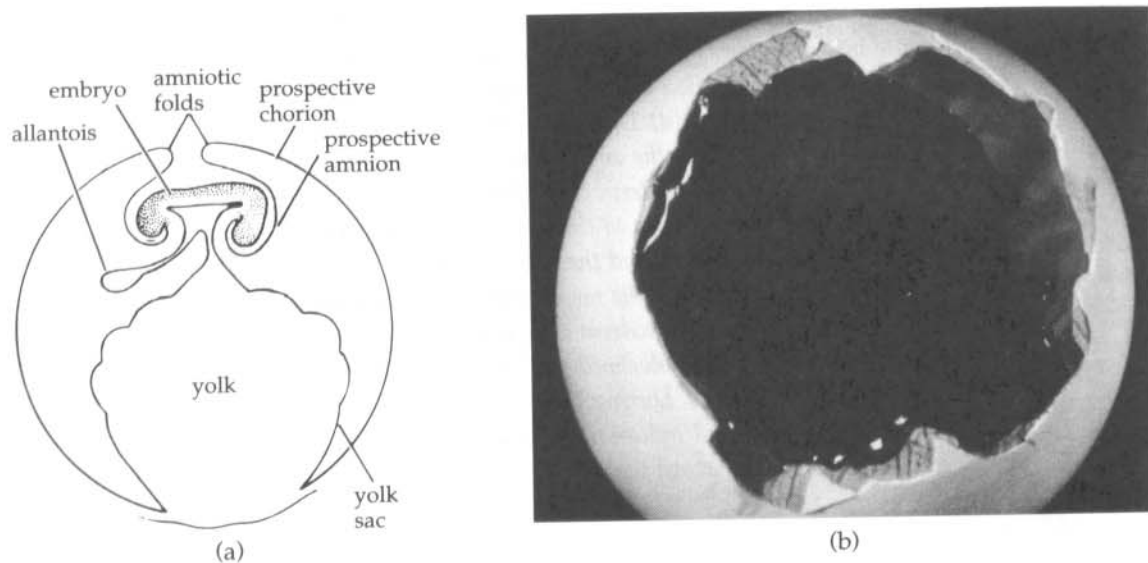


Figure 19G-1 (a) Extraembryonic membranes of the chick. (b) The yolk sac vascularized by a network of vitelline vessels.

■■■■ Objectives ■■■■

- List and give the functions of the four extraembryonic membranes.

■■■■ Procedure ■■■■

1. Work in pairs. Obtain an incubated 96-hour-old or 5- to 6-day-old chicken egg. Place the egg on a towel so that one side faces upward. The embryo will rotate to the top side after 3 to 5 minutes. Meanwhile, fill a small finger bowl with warm (37°C) chick Ringer's solution.
2. Gently crack the egg on the lower surface and allow the egg yolk and embryo to float out of the shell into the Ringer's solution. Do not jerk the two halves of the shell apart or separate them too quickly. It will take some time for the chorion to separate from the shell membranes of the older eggs.
3. Identify the yolk sac with its vessels. How far has the network of vitelline vessels extended. These vessels will eventually cover the entire surface of the yolk.
4. With a blunt instrument, push on the embryo. You will see that it is floating within a sac—the amnion.
5. Look for the allantois. It will appear as a small bubble emerging from the posterior end of the embryo.
6. With a pair of forceps, lift up the outermost tissue layer lying above the embryo. This is the chorion. How far does it appear to extend?

PART II IMMUNE RESPONSES

Consider that a mammalian organism can make more than 10 million different types of antibodies. However, there is not enough DNA in the organism's entire genome to code for all of these molecules. How can we account for the variety of antibodies, or **immunoglobulins**, that protect our bodies?

The answer lies in the observation that the DNA of eukaryotic chromosomes can rearrange itself, making new coding combinations from old sequences. Each antibody molecule is made up of two **heavy** (long) **chains** and two **light** (short) **chains**. A portion of each chain, the **variable region** (containing approximately 100 amino acids), is responsible for antibody diversity and function (Figure 19II-1).

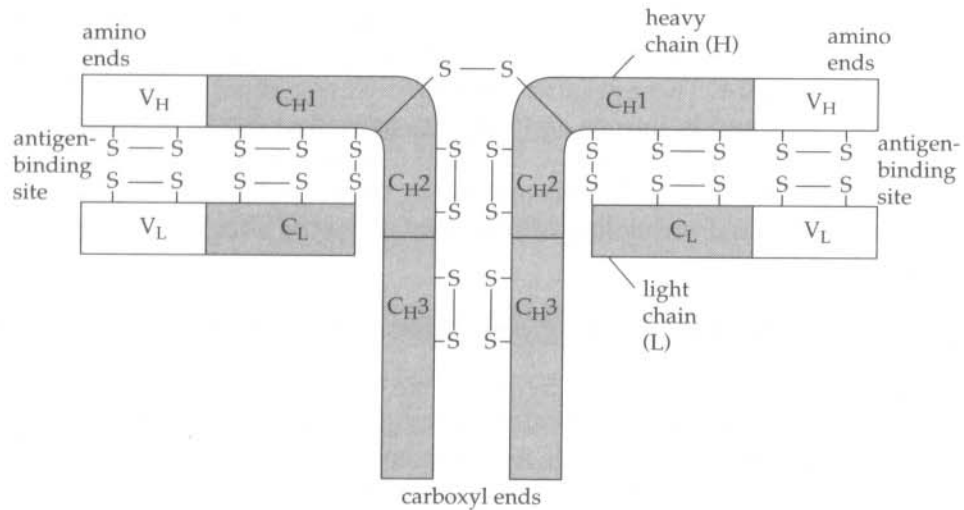


Figure 19II-1 The immunoglobulin molecule. An antibody (immunoglobulin) molecule has two light (L) and two heavy (H) polypeptide chains, each of which has variable (V) and constant (C) regions. The heavy and light chains are connected to each other by disulfide bonds.

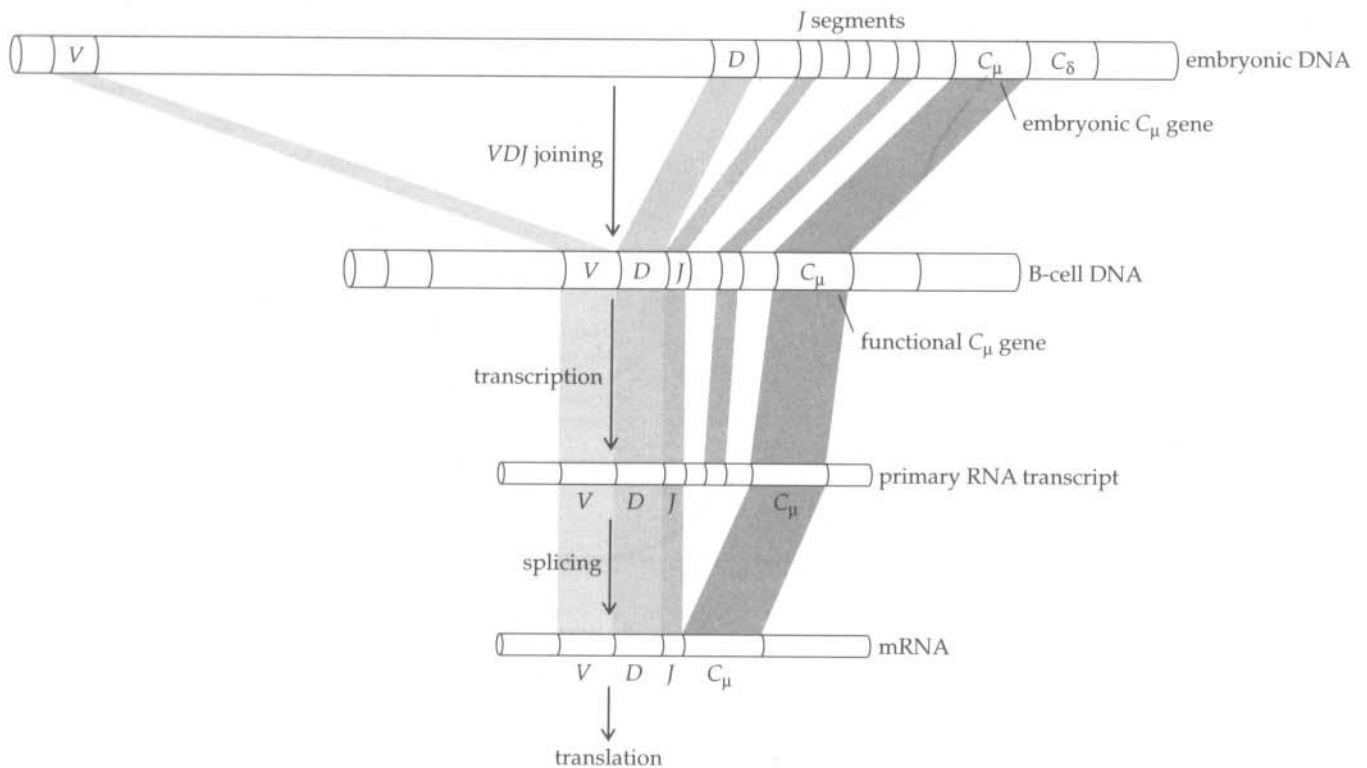


Figure 19II-2 Rearrangement of the DNA segments of the genes for the heavy chains of immunoglobulins. The V, D, J, and C segments are joined to form the gene. After transcription, the primary RNA transcript is spliced to remove the transcripts of introns and extra J segments. The mature mRNA is then ready for translation.

During production of the variable portions of heavy or light immunoglobulin chains, various combinations of DNA segments are put together by “selecting” and splicing together *V* (variable), *D* (diversity), and *J* (joining) segments of DNA. These are then combined with selected *C* (constant) regions for immunoglobulins I, M, A, D, and E. Introns are removed, as well as extra *J* segments, during splicing. The final product is a single and unique chain of *V*, *D*, *J*, and *C* segments (Figure 19II-2).

Understanding this genetic control of diversity among immunoglobulins has led us to a better understanding of their biochemical and physiological functions.



EXERCISE H Demonstrating the Immune Response by the Precipitin Ring Test*

Immunology is the study of the mechanisms by which the body protects itself against disease-causing microbes. Any foreign substance (whether of microbial origin, including viruses, or from another species of animal) will trigger the immune response if that substance is sufficiently large (a molecular weight of at least 10,000), is sufficiently complex (such as a protein composed of at least 20 different amino acids), and is biodegradable—that is, can be broken down by naturally occurring biochemical interactions. Some large synthetic molecules, such as many plastics, are nonbiodegradable—tend not to interact with or be broken down by substances within the body—and therefore do not normally trigger an immune response. Foreign substances that cause an immune response are called **antigens**.

When antigens enter the body (for example, when you are injected with a flu vaccine), they set off a complex series of reactions which are demonstrable in a variety of ways. One of the easiest ways to demonstrate the immune response is to do a blood **serum titer analysis**. A titer is a measure of the amount of antibodies in the blood serum that can react with a specific antigen. (Serum is the liquid part of the blood remaining after cells and fibrin have been removed. Antibodies circulate in the blood serum.) If a series of measurements is taken over a period of several weeks after the antigen is injected, the time course for the development of antibodies looks like the graph in Figure 19H-1. The initial injection triggers the primary immune response; the effect of a “booster shot” is shown by the secondary immune

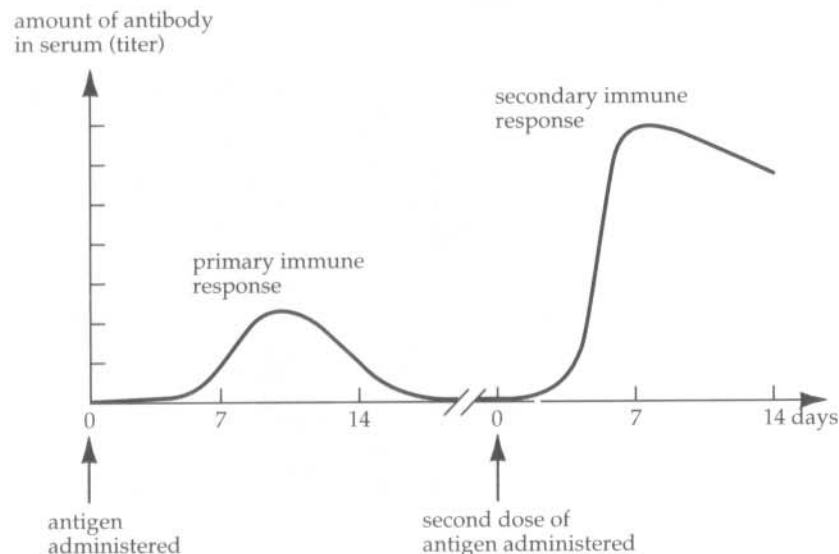


Figure 19H-1 Changes in serum antibody levels in response to two injections of an antigen administered at different times.

*This exercise was developed by Dr. Fred Stutzenberger and Jzuen-Rong Tzeng, Clemson University, Clemson, South Carolina.

solution, introduce a similar amount of buffer into tube 2 and of normal serum into tubes 3 and 4, as indicated in Table 19H-1.

2. Gently overlay each volume of solution with an equal volume of the buffer or antigen indicated in Table 19H-1. *Be careful not to mix these two layers of solution.* This can be avoided by slightly slanting the tube and carefully introducing the buffer or antigen solution along the wall of the tube near the surface of the bottom solution, then gradually pulling up the Pasteur pipette. Place the rack of tubes in front of the light source and observe after 10 to 15 minutes at room temperature.

Table 19H-1 Ring Test for Detection of an Antigen (BSA) or an Antibody (anti-BSA)

	Tube Number*				
	1	2	3	4	5
Top layer	B	Ag	B	Ag	Ag
Bottom layer	Ab	B	N	N	Ab
Reaction					

*B = buffer, Ag = antigen, Ab = antiserum, N = normal serum.

3. A positive reaction is indicated by formation of a milky white ring at the interface. Record reactions in Table 19H-1.

d. Explain why you had a positive reaction or no reaction in:

Tube 1 _____

Tube 2 _____

Tube 3 _____

Tube 4 _____

Tube 5 _____

e. How might the ring test be used to check whether an individual demonstrates immunity to a particular antigen, perhaps to a disease such as measles or chicken pox?

f. How is this reaction similar to the type of reaction that would occur if a person with type B blood received a transfusion of type A blood by mistake? _____

Laboratory Review Questions and Problems

1. Fill in the following table to summarize the major events of early development.

	Formation of the Blastula	Early Gastrula	Late Gastrula	Neurula
Processes occurring				
Type of structure formed				
Characteristics of structure				
Significance of stage				

2. Compare and contrast major developmental events in the sea urchin, frog, and chick by completing the following table.

	Sea Urchin	Frog	Chick
Type of egg			
Type/pattern of cleavage			
Distinguishing characteristics of blastula			
How gastrulation occurs			
Events of neurulation			
Distinguishing characteristics of later development			

3. What are cytoplasmic determinants? How do they affect early development, including cleavage of the zygote?
4. How can maternal DNA affect the development of an embryo when the maternal genes are not included in the egg produced as a result of meiosis?
5. What are homeotic genes? How do they control the development of segmented organisms?
6. How is the heavy chain of an immunoglobulin molecule of the M type constructed?
7. How can you explain the tremendous variability among immunoglobulins if DNA does not have enough genes to code for each molecule individually?
8. What are antigens and antibodies? What types of cells make antibodies?
9. Why would it be important to be able to determine whether a person had antibodies to a specific antigen? Can you think of a medical crisis in which this knowledge would be helpful?
10. Describe the function of each of the following extraembryonic membranes in the chicken embryo: amnion, chorion, allantois, yolk sac.
11. What is the function of the vitelline blood vessels in the chicken embryo?
12. In development of the human embryo, the trophoblast is like the chorion, surrounding the embryo in its allantois. The trophoblast becomes the placenta during development of the fetus. How does the function of the human placenta compare to the chorion of the chicken embryo?
13. During human development, a rudimentary allantois is formed but is never used. Why would it not be used? How are the functions that the allantois performs in the chicken embryo carried out during human fetal development?