chapter 9  Early Development of the Chick  *Gallus domesticus*

The common domestic chick, *Gallus domesticus*, is traditionally studied in embryology classes. Its development is an international language common to all students of embryology, and this alone is reason enough to learn about it. There are other reasons as well. Living chick embryos are easily obtained. Since chickens breed in any season, chick embryos often are no further away than the nearest farm. Above all, chick embryos can teach you a great deal about vertebrate development in general and will help you understand comparative vertebrate anatomy and vertebrate evolution. You will see the vertebrate body plan being laid down, learn how the organs form, and witness how they relate developmentally to one another. You will see the structures form that are diagnostic characters of the phylum *Chordata* and subphylum *Vertebrata*. And you can extrapolate from chick to human development and suddenly know a tremendous amount about what must go right for a normal baby to be born and what can go wrong that causes birth defects. Learn chick developmental anatomy well, and I promise it will serve you well.

Throughout the chapter, you will find a number of questions (easy ones). Remember to record answers to these questions in your laboratory notebook.

**The Chick Egg**

The female chicken lays an **amniote egg**, the same type of egg laid by other birds, reptiles, and **monotreme** mammals. An evolutionary invention of the early reptiles, it is one of the major changes that freed vertebrates from the water, allowing them to lay eggs on land. The design is an engineering masterpiece. An outer shell protects against physical damage and dehydration while still allowing for gas exchange. Packed within is enough food and water to survive the long journey to hatching—and a long journey it is. In the chick, it takes 21 days. Stranded out on land, the embryo cannot rely on an aquatic feeding larval stage, as do, for example, most amphibian embryos. The provisions packed within the shell must be enough to nourish all of development through to the hatching of the juvenile. In a medium-size chick egg (about 60 gm), these provisions include more protein (7.2 gm) than there is in a frankfurter; as much fat (6 gm) as is in 2 teaspoons of butter but with about seven times the cholesterol (300 mg); and about 8.5 teaspoons of water (40 gm). Despite the bad reputation of its cholesterol, the egg truly is an excellent food source.

In order to permanently alter the way you look at an egg in the kitchen, you will be dissecting one in the laboratory. Obtain an egg that was collected from the wilds of a local grocery store. Examine the shell and see that one end is blunt. This marks the region of an air space that you will see upon opening the egg. Prior to opening your egg, put it briefly into hot water (water that has been brought to a boil,
but is no longer boiling), or watch someone else do this with their egg. What happens? What does this tell you?

Now open your egg very carefully into a finger bowl without breaking the yolk. Do this by giving the egg a sharp crack against the edge of the bowl, putting your two thumbs in the crack, and pulling the two halves apart, with the egg close to the bottom of the finger bowl. If you are lucky, you have kept the chick ovum (the yolk) intact. No wonder flipping a fried egg without bursting the yolk is an art—that yolk is one cell. It’s huge! Only a fragile cell membrane overlain by a vitelline envelope protects it from the slice of your spatula. Compare the yolk to a more typically sized cell—a piece of dandruff, for example, which represents a single epidermal cell. The difference is remarkable! Most of the bulk in the chick ovum is not cytoplasm, but spheres of yolk lipid suspended in a sea of yolk protein. The yolk is so disproportionately abundant compared to the cytoplasm that the cytoplasm is displaced to the animal pole and sits as a puddle on top of the yolk. The large quantity of yolk classifies the egg as **macrolecithal** (*macro*, large; *lecithal*, yolk).

Examine the eggshell. Ninety-eight percent of it is calcite, which is calcium carbonate. Calcium is the major component of bone as well (in the form of calcium phosphate, or apatite). The calcium that goes into the shell comes both from the hen’s diet and from her bones. Weigh the shell. Realize that a truly remarkable hen can lay an egg a day. Approximately how much calcium will a one-a-day hen lose in a week? In a year, a hen can put into her eggshells over 25 times her own skeletal weight in calcium. Obviously, this must be replaced. The standard feed given to laying hens is 3 to 4% calcium, and one hen receives about 100 gm of feed a day. Based on the weight of the shell, is this sufficient to replace the calcium lost in a superhen laying every day?

Examine a piece of shell under a dissecting scope, turning it so that you can see it in cross section. Notice that it has three layers (Figure 9.1): two calcified layers, the inner mammillary layer and the outer spongy (or crystalline) layer; and outermost the thin shiny cuticular membrane. In the mineralized layers, calcium carbonate is laid down as crystals perpendicular to the surface of the egg—this gives the shell tremendous strength. The outer cuticular membrane is made of glycoprotein. Prior to laying, it is wet and slippery, which helps with oviposition, but soon after laying it dries out and serves to protect against invasion by microorganisms.

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**Figure 9.1**
Schematic diagram of a chick egg.
Notice the shell has numerous pores. Use food coloring to dye the shell. When the dye dries, a greater amount will have collected in the dips of the pores, and these will appear as dark spots. Notice that the dark spots are irregularly sized and spaced, and that they are more abundant in the area over the air space than anywhere else. Can air pass through these pores? How can you tell? Think back to what happened when you put the egg into hot water. If air could not pass through the shell, what would happen to the embryo? (Write your answers in your laboratory notebook.) Wherever air can pass, water vapor will follow. Balancing the need for gas exchange against the risks of dehydration while retaining strength is always a major task in shell design.

On the inside of the shell you should see two shell membranes, an inner and outer one, separated from one another at the region of the air space (air cell) at the blunt end of the egg (Figure 9.1). Peel the two membranes apart from one another. Which of the two membranes is thicker? Look at a piece of each membrane under the dissecting scope, then stain them with 0.1% toluidine blue and mount each on a slide with a coverslip using water as mounting medium. You will see that the membranes are a dense, crisscrossing mat of fibers. These elastin-like fibers retard water loss, give mechanical support, and provide a defense against microbes. Where else do you find a high proportion of elastin? (In ligaments: attaching bone to bone, they must be able to s-t-r-e-t-c-h; in the cartilage of the external ear: pull on your ear—does it droop, or bounce back into shape?) What does this tell you about the mechanical properties of the shell membranes? Experiment to see how much stretch the membranes have.

Look at the air space at the blunt end of the egg. This space forms between the two shell membranes after the egg is laid. As the egg cools from the internal temperature of the hen (about 41°C) to room temperature, the internal contents contract more than the shell, creating the air space. Try cooling a warm egg by putting it into the refrigerator, and see if the air space becomes even larger. Do you think the air space serves a function? Could it help to absorb shock when the egg is jarred? When the chick begins to hatch, it first breaks through into the air space using its beak and fills its lungs with air. The confined space soon becomes fouled with carbon dioxide, which triggers the chick to start breaking through its shell (a process referred to as pipping, a word most of us have heard without knowing its meaning. (In the folk song “I Gave My Love a Cherry,” one verse goes, “A chicken when it’s pipping, it has no bone.” Does this make any sense?)

Now look at the egg albumen. Notice that it has varying viscosities. Around the yolk is a narrow band of thin albumen, surrounded by a layer of thick albumen, and external to this another layer of thin albumen (Figure 9.1). The albumen is 88% water and is a major source of water for the developing embryo. The rest is primarily glycoproteins, the most abundant of which is ovalbumen. Some of the proteins in the albumen serve antibacterial functions: lysozyme, for example, is an enzyme that disrupts bacterial cell walls; ovotransferrin binds iron and avidin binds the vitamin biotin, making these nutrients—which are necessary for bacterial growth—unavailable to the bacteria. Two other proteins, ovomucin and cystatin, are thought to have antiviral activity. In addition, the albumen has a pH that prevents bacterial growth. What pH is this? Measure the pH using pH paper. (Did your parents, when making lemon meringue pie, ever tell you not to lick the meringue bowl because of the raw egg whites? What they were worried about was the avidin, which can cause a vitamin deficiency called egg white disease. Explain. Why are cooked egg whites all right to eat?)

Notice that part of the albumen is very dense and is coiled into two cordlike structures attached tightly to the yolk membrane. These are the chalazae (pronounced ka-lay-zee) (Figure 9.1). They suspend the yolk in the middle of the albumen and allow it to rotate. The yolk will rotate, always orienting to gravity so that its animal pole (and the embryo) face upward. Why do you think this is important? (By definition the animal pole of an egg is the pole where the polar bodies form. In the chick, this is also where the blastodisc is. The vegetal pole is opposite the animal pole.) Remove the
Chalazae using scissors and fine forceps and place them side by side on a slide, keeping track of which end was attached to the yolk. Do the fibers twist the same way? Add a drop of 0.1% toluidine blue stain to help distinguish the fibers. You probably noticed that one chalaza twists clockwise and the other counterclockwise. This twisting occurs during egg formation as the yolk is slowly rotated during its descent through the oviduct. This twists the fibers of the chalazae and draws them taut, pulling the yolk to the center of the egg. You can visualize this using a cork, two rubber bands, and thumbtacks. Attach the rubber bands with thumbtacks to opposite ends of the cork. Holding the free ends of the rubber bands, slowly rotate the cork as the yolk would rotate coming down the oviduct. Notice that the rubber bands twist in opposite directions, just as the chalazae do. Notice also that as the slack in the rubber bands is taken up, the cork is suspended between the two ends. Unlike rubber bands, however, the chalazae do not tend to unwind. Using microneedles, try to tease apart the fibers of the chalazae and notice how resistant they are to untwisting.

Examine the yolk. Where does the yolk sit in relation to the albumen? Does it float, or is it submerged? Remember that bowl full of eggs before you made your last omelet. Did the yolks float or sink in that sea of whites? What does this tell you about the relative densities of yolk and albumen? About the importance of the chalazae? Notice the blastodisc, the puddle of cytoplasm that should be oriented upward. It is here that the egg nucleus is found. With forceps and fine scissors, remove the blastodisc by puncturing the vitelline envelope and cell membrane with the forceps, and holding on to both with the forceps while cutting around the blastodisc with the scissors. Carefully lift the blastodisc to a slide. A disc of white yolk will be attached to the underside of the cytoplasm. Examine the blastodisc under the dissecting and compound microscopes. Remember that the nucleus is partway through a meiotic division, so there is no nuclear membrane, and the chromosomes are aligned on a metaphase plate; they are very difficult to detect.

If you see a spot of blood in the yolk, this can be either an indication of a developing embryo (it would have to be in the location of the blastodisc—look at it carefully) or simply the incorporation of a blood clot from the ovary at the time of ovulation.

The yellow color of the yolk is due to carotenoid pigments—the same pigments seen in carrots and other yellow vegetables. (Your body uses carotenoids to make vitamin A, which has a long list of functions. When transformed into retinal, for example, it becomes part of an eye pigment, rhodopsin, necessary for vision; when transformed into retinol, it is needed for skin maintenance. Egg yolk has about one-ninth as much carotenoid as carrots.) The carotinoid pigments are absorbed from the hen's food and deposited rapidly in the yolk. Consequently, greater amounts are deposited during the day when the hen is eating than at night when the hen is sleeping. This results in the yolk having concentric layers of dark and light yolk (Figure 9.1), and provides us with a daily record of the yolk deposits. You can see this by removing an unbroken yolk with a spoon to some boiling water and boiling it for 10 minutes. Then, using a razor blade or sharp scalpel, carefully bisect the yolk through the middle, starting at the animal pole where the blastodisc is. Count the rings. Each sequence of a light and dark ring equals one day. How many days did it take the hen to form your yolk? Remember when and where the yolk was being packed into the ovum (in the ovary prior to ovulation).

The Female Chick Reproductive Tract

The female chick has only one fully developed ovary and oviduct (usually the left), while the other remains vestigial. Why do you think this is so? If your laboratory has a female chick reproductive tract on display, examine this, using Figure 9.2 to study each region discussed below.
Look at the **ovary**. Those large lumps are oocytes developing to maturity. These are ovulated as **secondary oocytes**, when the egg is in metaphase of its second meiotic division. **Meiosis** is not completed until after fertilization. Fertilization takes place high in the oviduct, in the region called the **infundibulum**. Why would it be difficult for fertilization to occur farther down in the oviduct? After **ovulation**, the egg remains fertilizable for only about 40 minutes. This does not mean, however, that mating must have occurred near the time of ovulation. Sperm can be stored in the female tract and remain viable there for as long as 35 days, though viability is reduced after the third week. Look back at the slides of sperm from an earlier laboratory session to recall the shape of chicken sperm. At the time of fertilization, many sperm enter the egg, but only one sperm **pronucleus** fuses with the egg pronucleus. This unusual phenomenon is called **physiological polyspermy**.

Notice that the mouth of the **oviduct** is not attached to the ovary. It is not a given that every egg that is ovulated successfully enters the oviduct. In fact, as many as 5% of eggs ovulated normally miss the opening and fall into the body cavity, where they are usually absorbed. (A hen that does this a high percentage of the time is called an “internal layer.”) Once in the oviduct, the ovum packed with yolk is moved along by muscular contractions of the oviduct. After passing through the **infundibulum**, albumen begins to be secreted around the yolk. Most of the **albumen** is secreted by cells in the region of the oviduct called the **magnum**. The egg passes from the magnum through the **isthmus**, where the chalazae and the inner and outer **shell membranes** are added. In the **uterus**, also called the **shell gland**, water and salts traverse the shell membranes and are added to the albumen, **plumping** out the shell membranes. The shell is then deposited, and the egg travels on through the **vagina** and into the **cloaca**.
Here the egg is turned completely around. Having traveled with its small end first, its orientation is now reversed, and the egg is laid with the large blunt end first. The entire process from ovulation to laying takes about 24 hours, the major portion of which (about 19 hours) is spent in the shell gland. If the egg was fertilized, development of course has been taking place all this time, and the embryo is already undergoing gastrulation by the time it is laid.

**Cleavage**

Cleavage stages cannot be observed in the living egg under normal conditions, since they occur while the egg is still in the oviduct. You will be studying cleavage, therefore, by examining models and diagrams of the events (Figures 9.3 and 9.4). Remember the function of cleavage. The fertilized egg already contains within its cytoplasm all of the germ-layer components. Cleavage divides this single cell into a lot of smaller building blocks that then can be rearranged and molded into the multicellular organism. Use the models and diagrams to identify all of the structures printed in boldface in the next two paragraphs.

The first thing you should notice is that the entire egg does not cleave; only the puddle of cytoplasm sitting on top of the yolk cleaves. Dense yolk creates a formidable impediment to a cleavage furrow. You will see this later in the mesolecithal egg of the amphibian, in which the moderate amount of yolk concentrated in the vegetal half slows down the cleavage furrows. Here in the chick egg, however, the huge amount of yolk stops the cleavage furrows altogether. Cleavage initially, therefore, is necessarily partial, or meroblastic. Cleavage furrows, which cut down from the animal pole toward the yolk, stop when they hit the yolk. The cleavage pattern in the chick is also often called discoidal, since it is restricted to the circular disc of cytoplasm, the blastodisc.

The cells produced during cleavage are called blastomeres, and together they make up what is called the blastoderm. In the center of the blastoderm, the cells become separated from the underlying yolk by a space called the subgerminal cavity. The space makes the central area of blastoderm look lighter and more translucent than the surrounding area. This central region is called the area pellucida (a pellucid stream is transparent; a pellucid explanation is clear and easily understood). The surrounding area of blastoderm, still connected to the underlying yolk, looks darker and opaque; it is called the area opaca.

**Figure 9.3**

Early cleavage stages in the chick. The blastoderm is shown removed from the yolk and viewed from above, starting with the 2-cell stage and showing progressively more advanced stages.
By the time the egg is laid, the embryo is undergoing gastrulation. The cell movements during this stage have been traced by scientists working with great patience and steady hands. The normal procedure is to label tiny regions of the blastodisc with vital dyes and other markers, to watch the paths of movement these markers make during gastrulation, and to determine where they eventually end up, thereby determining the final fate of the cells. In this way, maps have been constructed showing the location of each germ layer prior to gastrulation and its route of travel during gastrulation. These maps, called fate maps, are a gift to any experimenter, providing a blueprint for a vast array of experiments. As you study them, think of experiments you would do using the fate maps as a ground plan. Briefly outline one of your experiments in your laboratory notebook.

To begin your study of gastrulation, first remember what gastrulation achieves. It separates the germ layers so that they are in appropriate positions for the organ formation that will follow. Since endoderm forms the epithelium of the gut and gut derivatives, it must become a centrally located tube. The ectoderm, which forms the epidermis and nervous system, must cover the outside. And in between must come all the packing material provided by the mesoderm—muscle, skeleton, circulatory system, kidney, and dermis. Now look at the fate map of the chick blastoderm (Figure 9.5). Notice that it is laid out flat as a pancake, a configuration forced upon it by the huge inert yolk. This is a problem. Embryos such as those of sea urchins and amphibians don’t face this problem. Because they form a hollow sphere as blastulae, they are able to gastrulate by simply moving the mesoderm and endoderm inside the sphere and then arranging these germ layers appropriately within the sphere. But in the chick, what is a sphere in these other organisms is laid out flat, and the germ layers are forced to separate in a flat plane forming a three-layered sandwich with endoderm on the bottom, ectoderm on top, and mesoderm in between. The flat germ layers later get tucked and folded to form the gut tube and other structures. Meanwhile, the entire sandwich is growing outward over the surface of the yolk and eventually surrounds the yolk.

It is only the central region of the gastrula sandwich that will form the body of the embryo. The outer regions will form extraembryonic membranes: the amnion, chorion, allantois, and yolk sac. (The amnion and chorion serve to protect the embryo; the allantois stores nitrogenous waste and where it is attached to the chorion provides a vast respiratory surface that also resorbs calcium from the eggshell for the growing embryo; the yolk sac absorbs yolk and transports it back to the embryo in its vitelline blood vessels. You will see these in later chapters.)
Look at the blastoderm fate map again, and imagine how you would orchestrate the gastrulation movements to create a three-tiered sandwich. Now look at Figures 9.6 and 9.7 and at the models of chick gastrulation to see how close your plan of gastrulation came to what actually happens. The first step in chick gastrulation is the splitting off of the major bulk of endoderm from the underside of the blastoderm to form a layer of endoderm below the rest of the cells. This splitting is called delamination. What molecular mechanisms must be involved in delamination? Obviously, the endoderm must lose its stickiness to the rest of the blastoderm; it must change its affinity by changing its cell surface adhesion molecules. If you were able to conduct experiments, how might you test for this?

Delamination produces an upper epiblast, consisting of the presumptive ectoderm, mesoderm, and some endoderm; and a lower hypoblast, consisting of the extraembryonic endoderm. The extraembryonic endoderm will participate in forming extraembryonic membranes. The presumptive endoderm remaining in the epiblast, the embryonic endoderm, will form the endodermal derivatives of the embryo itself, but it must first join the hypoblast before doing so.
The embryonic endodermal cells move to the hypoblast by turning inward along a line of ingress and then moving downward to the hypoblast. The line along which cells are moving inward from the epiblast, or ingressing, is called the primitive streak. The prospective mesoderm cells are the next to enter the primitive streak and leave the epiblast. However, they do not move down to join the hypoblast but instead make a U-turn and migrate outward between the epiblast and hypoblast to form a separate layer between the two.

Compare this type of gastrulation to that of a sea urchin or amphibian. What in the sea urchin or amphibian would be analogous to the primitive streak? Yes, the blastopore. Remember the dorsal lip of the blastopore in an amphibian gastrula? It is where notochordal mesoderm cells involute to form the notochord. There is an analogous structure in the chick embryo at the top of the primitive streak called Hensen’s node (or primitive node). It is here that the notochordal cells converge, ingress, and migrate forward, rather than laterally, to form a streak of notochordal cells down the midline.

Look at various stages of gastrulation, noticing that gastrulation begins in anterior regions first and progresses to more and more posterior regions. Anterior regions may have finished gastrulating and gone on to organ formation while posterior regions are still gastrulating. This anterior-to-posterior wave of progression results in the primitive streak becoming confined to ever-more posterior regions. This is often referred to as the regression of the primitive streak.

24-Hour Chick Whole Mount

Molding a body out of the three-tiered sandwich of a chick gastrula is a task that takes a lot of folding and pinching. The beginnings of this process are well illustrated in a 24-hour chick embryo (by tradition, this means an embryo incubated for 24 hours—total time of development has been more than 24 hours, since development began prior to egg laying) (Figure 9.8). You will be looking at whole mounts in which the cellular region has been removed from the yolk and mounted on a slide. Because of the transparency of the embryo at this stage, all levels can be seen by focusing up and down through the embryo. Use both your dissecting and compound microscopes, experimenting with different settings for
contrast and lighting. Be able to identify any of the structures printed in boldface below, and write in your laboratory notebook answers to any questions posed. Realize that you yourself looked very much like this embryo at one time (about three weeks into your development).

By 24 hours of incubation, the anterior half of the embryo is undergoing neurulation while the posterior half is still gastrulating. Notice that the area opaca has greatly expanded. In fact, it now occupies too large of an area to be mounted on your slide, so much of it already has been trimmed away. Even so, you still should be able to identify the two subdivisions of the area opaca: the outer area vitellina (vitellina means yolk), where cells contain many yolk granules; and the inner area vasculosa (referring to vascularization), where blood islands are forming. Blood islands are masses of blood-forming cells that will later anastomose to form a capillary network that will bring yolk nutrients back to the embryo proper. Look at the area pellucida, the clear area around the embryo; it looks almost like a footprint. Just in front of the embryo’s head is a region of the area pellucida that is particularly clear. This is called the proamnion (an unfortunate name whose meaning, “before the amnion,” has nothing to do with the structure—suggest a better name and we’ll vote on it). The reason the proamnion is so translucent is that it consists only of ectoderm and endoderm. The spreading mesoderm has not yet reached this area.

Focus now on the embryonal area. The folding and pinching has already begun. At the anterior end you will see a head fold. This is where an anterior fold has undercut the developing head and raised it

![Figure 9.8](image-url)
above the level of the blastoderm. Look at the models on display to see this more clearly. Notice how the head fold has caused the endoderm to fold into a closed tube to form the foregut. Posteriorly, where the endoderm is still flat, is the open midgut, and the transition between the closed foregut and open midgut is the anterior intestinal portal (portal means door). This is best visualized by looking at models. Now look carefully on your whole mount for the darker outline of the foregut and the crescent-shaped line of the anterior intestinal portal. Look at models to see the mesoderm lying directly underneath the foregut and lateral to it. This mesoderm will be forming the heart later on and is therefore called the cardiac mesoderm. On your whole mount, it should look somewhat darker than the surrounding region.

One of the most notable features of the 24-hour embryo is the invaginating neural ectoderm. Previously it was an open neural plate, but by 24 hours, the anterior region has folded upward to form a closed neural tube. A major part of this folding is caused simply by a change in shape of the neural plate cells from cuboidal to truncated-pyrimidal. Convince yourself of this by making a sheet of cells (neural plate) out of small cubes of clay. Now change the shape of each cube by pinching its apical end (a contraction due to an apical belt of actin and myosin microfilaments in the cells of the neural plate) and elongating it along its apical-basal axis (caused by an elongation of microtubules in this axis). Now fit the clay cells back together, and you will see that your sheet of cells has folded upward.

Look at your whole mount again. Notice that neurulation is taking place earlier anteriorly than posteriorly. Identify regions of closed neural tube (if any), open neural plate, and in between regions where neural folds have formed but have not closed. Notice that at the anterior tip of the embryo, the neural plate does not close completely but leaves an open channel called the anterior neuropore. This will be closed by 36 hours. (Sometimes the neural folds in the spinal region fail to close during development, causing an extremely severe birth defect called spina bifida with myeloschisis. Occurring in humans as well as chicks, vertebrae do not form properly in the area, and the spinal cord is left an unprotected, flattened mass of neural tissue.)

One thing you cannot see in the whole mount, which you will see later in serial sections, are the neural crest cells lying on top of the neural tube. As soon as the tube closes, they start migrating away from the neural tube, sometimes to distant regions. These are fascinating cells and will be discussed more completely in the next chapter. For now, just keep in mind that they are unique to the vertebrates and form a variety of structures including cartilage and membrane bones of the head, pigment cells of the skin, and spinal ganglia.

Look at the open midgut region, and notice that the mesoderm has segmented into blocks of somites on either side of the neural tube. Count and record the number of somites your embryo has. As the embryo develops, additional somites will form from the more posterior unsegmented somite mesoderm. Recall what the somites will be forming: the axial skeleton (vertebrae and ribs), muscles of the axial skeleton and limbs, and dermis. Between the two rows of somites, in the midline underneath the neural ectoderm, you should see a streak of condensed tissue. This is the notochord. How far does it extend anteriorly and posteriorly on your embryo?

At the posterior end of the embryo, notice the region of the primitive streak and Hensen’s node, showing that ingression is still occurring in this region. Can you think of any reason why an embryo would spend more time making its anterior end than its posterior end?

Now that you are thoroughly familiar with your embryo, you can stage it more accurately than simply calling it a 24-hour embryo. You can use the staging series developed by Hamburger and Hamilton; a truncated version of this series is given in the next chapter. This is the most widely used staging series for the chick. Record the stage of your chick. By convention, when using this staging series, you would precede the stage number with the initials HH.
Accompanying Materials

See Vade Mecum: “Chick Early.” This chapter of the CD shows step by step how to open the egg and identify its parts. It shows early development in a series of movies developed from models and histological whole mounts.


Selected Bibliography


Deeming, D. C. and M. W. J. Ferguson (eds.). 1991. Egg Incubation: Its Effects On Embryonic Development in Birds and Reptiles. Cambridge University Press, Cambridge. This is a collection of papers from an international meeting. The comparisons between bird and reptile are fascinating. For example, did you know that although bird eggs require turning during incubation, turning a reptilian egg usually kills the embryo?


Hamburger, V. and H. L. Hamilton. 1951. A series of normal stages in the development of the chick embryo. J. Morphol. 88: 49–92. This is the original printing of the Hamburger-Hamilton chick staging series. It contains an exquisite set of photographs and drawings along with the written description of each stage.


Romanoff, A. L. and A. J. Romanoff. 1949. The Avian Egg. John Wiley and Sons, New York. This is a classic. Though some of the biochemical information is now outdated, the wealth of anatomical details is well worth the task of carrying such a heavy book around.


Spratt, N. T., Jr. 1946. Formation of the primitive streak in the explanted chick blastoderm marked with carbon particles. J. Exp. Zool. 103: 259–304. This is a classic paper showing the original ways in which fate maps were determined.


**Suppliers**

Prepared microscope slides of chick embryos can be obtained from suppliers such as:

**Turtox/Cambasco, Macmillan Science Co., Inc.**
8200 South Hoyne Ave.
Chicago, IL 60620
1-800-621-8980

**Connecticut Valley Biological Supply Co., Inc.**
P.O. Box 326
82 Valley Road
Southampton, MA 01073
1-800-628-7748

**Ward’s Natural Science Establishment, Inc.**
5100 West Henrietta Road
P.O. Box 92912
Rochester, NY 14692-2660
1-800-962-2660
[www.wardsci.com](http://www.wardsci.com)

Models of chick development are available from Turtox, listed above.

**Glossary**

**Actin microfilaments:** The thin microfilaments found in the cytoplasm of a cell. Made up of G-actin, the globular protein subunits. The filamentous form of actin is called F-actin. F-actin forms an extensive cytoskeletal network in the cortical cytoplasm. Myosin (the thicker of the two types of microfilaments) can move along F-actin, creating a contractile force.

**Air cell:** In the chick egg, the air space at the blunt end of the egg between the inner and outer shell membranes. It provides a cushion for the developing embryo as well as a the air for the chick’s first breath during hatching.

**Albumen:** Egg white. The albumen of a chick egg is 88% water and is a major source of water for the developing embryo. The rest is primarily glycoproteins.

**Allantois:** The extraembryonic membrane found in amniotes that forms off the hindgut of the embryo. It stores nitrogenous waste and in the chick will eventually fuse with the chorion to form the chorioallantoic membrane. It is vascularized and is formed from the splanchnopleure, a layer made up of endoderm and splanchnic lateral plate mesoderm.

**Amnion:** The extraembryonic membrane found in amniotes that surrounds the embryo. It contains amniotic fluid, which protects the embryo by providing an isotonic fluid and buffering the embryo
against being jarred. It forms from the somatopleure, a layer made up of epidermal ectoderm and somatic lateral plate mesoderm.

**Amniotes**: Vertebrates that have an amnion during development: reptiles, birds, and mammals.

**Amniote egg**: The type of egg typical of the amniotes, and an egg that can survive on land. It consists of a hard outer shell that protects the embryo from physical damage and dehydration, and a large yolk along with a watery albumen that provide the nutrients and water necessary until hatching. The embryo develops four extraembryonic membranes, the chorion, amnion, yolk sac, and allantoic membranes.

**Anamniote**: Vertebrates that do not have an amnion during development: the fishes and amphibians.

**Animal pole**: The point on the surface of the egg where polar bodies are given off. Eggs usually orient to gravity with their animal pole oriented uppermost.

**Anterior intestinal portal**: In the chick embryo, the opening between the closed foregut and the open midgut. The opening continues to shift posteriorly as development proceeds.

**Anterior neuropore**: In the chick embryo, the opening at the anterior end of the brain. It later closes.

**Area opaca**: In the chick embryo, the outer extraembryonic region that is still connected to the underlying yolk.

**Area pellucida**: In a chick embryo, the inner extraembryonic region that is separated from the yolk by a subgerminal cavity. This area looks lighter and more translucent than the surrounding area because of the space below it.

**Area vasculosa**: The inner region of the area opaca where blood islands are forming.

**Area vitellina**: The outer region of the area opaca where cells contain many yolk granules. (Vitellina refers to yolk.)

**Avidin**: An enzyme that inhibits bacterial growth by binding the vitamin biotin, making it unavailable to the bacteria. It can be found in the albumen of a chick egg.

**Biotin**: One of the B vitamins, it is water-soluble and sulfur-containing. It is important for a number of enzyme functions and for healthy nails, hair, digestive enzymes and sugar metabolism. It is destroyed by avidin, found in raw egg white.

**Blastoderm**: The layer of cells formed in the embryo during cleavage. In *Drosophila* this layer is formed in the periphery of the yolky egg. In the chick, it is formed as a disc-shaped area at the animal pole of the yolky egg.

**Blastodisc**: In a chick egg, the puddle of non-yolky cytoplasm that sits atop the vast quantity of yolk. The nucleus of the egg sits in the blastodisc, and it is here that development begins.

**Blastomeres**: The cells that are created through cleavage during early embryonic development.

**Blastopore**: In a gastrulating embryo, the point at which cells are moving inward to form the archenteron. In deuterostomes, the blastopore becomes the anus, in protostomes, the mouth forms at or near the blastopore opening.

**Blastula stage**: The stage in embryonic development following cleavage and preceding gastrulation. The embryo may form a hollow blastula (coeloblastula) or a solid blastula (stereoblastula). The cavity formed in a coeloblastula is called the blastocoel.

**Blood islands**: In the chick embryo, the beginnings of blood vessel formation out in the area vasculosa. These are masses of blood-forming cells that will later anastomose to form a capillary network that will bring yolk nutrients back to the embryo proper. They form from splanchnic lateral plate mesoderm.

**Calcite**: Calcium carbonate (CaCO₃). One of the commonest minerals and is found in a variety of crystalline forms. It is the form of calcium found in the egg shell of a bird.

**CAM graft**: A chorioallantoic membrane graft.
Cardiac mesoderm: The mesoderm that will form the heart.

Carotenoid pigments: The yellow-orange pigments found in leafy-green, yellow-to-red vegetables, from which the body makes vitamin A. The yellow color of yolk is from carotenoid pigments, derived from the bird’s food.

Chalazae: In a chick egg, the portion of albumen that is very dense and coiled into two cordlike structures that attach tightly to the yolk membrane. The chalazae suspend the yolk in the middle of the egg and allow it to rotate.

Chordata: The phylum that includes the vertebrates and two invertebrate groups, the urochordata and the cephalochordata. Diagnostic features of the phylum are the presence of a notochord (hence its name), a dorsal hollow nerve cord, and the presence of pharyngeal pouches during development.

Chorioallantoic membrane: The fused chorion and allantoic membranes. In the chick, this serves a major function in respiration. It becomes very large, and presses against the inner aspect of the shell, allowing for efficient gas exchange.

Chorioallantoic membrane graft: A graft placed on the chorioallantoic membrane of an embryonic chick. The highly vascularized membrane makes an excellent and inexpensive incubator for growing embryonic tissues.

Chorion: The extraembryonic membrane found in amniotes that lies outside of the amnion. It protects the embryo, and, in the chick will, eventually fuse with the allantoic membrane. It forms from the somatopleure, a layer made up of epidermal ectoderm and somatic lateral plate mesoderm.

Cleavage: The stage in embryonic development, normally following fertilization, in which the egg, or portions of the egg, divide by mitosis into a number of cells. Cleavage results in the formation of the blastula.

Cloaca: Literally, means sewer. In a chordate, the terminal region of the endodermal hindgut. In general, it receives the ducts and products from the gonads, and the digestive and excretory organs.

Cuticular membrane: The outermost shiny membrane of a chick egg shell. It is made of glycoprotein that, prior to laying, is wet and slippery, thereby helping with egg laying. Soon after laying, it dries out and serves to protect against invasion by microorganisms.

Cystatin: A protein that is thought to have antiviral activity. It can be found in the albumen of a chick egg.

Delamination: A type of gastrulation movement in which a multilayered sheet of cells splits into two sheets. In the chick embryo, at the beginning of gastrulation, delamination separates the blastoderm into two layers, an upper epiblast and a lower hypoblast.

Deuterostome: Among the bilaterian organisms, the phyla in which during embryonic development the blastopore becomes the anus. These include the echinoderms, hemichordates, and the chordates (urochordates, cephalochordata, and vertebrates).

Discoidal cleavage: The pattern of cleavage found in certain macrolecithal eggs such as the chick egg, where cleavage is restricted to the blastodisc. Initial cleavages are meroblastic; the cleavage furrows cut down from the animal pole towards the yolk and stop when they reach the yolk.

Ectoderm: The germ layer in an embryo that gives rise to the epidermis and nervous system. In many cases, as in vertebrates, an anterior and posterior invagination of ectoderm gives rise to the stomodeum and proctodeum, respectively.

Endoderm: The germ layer in an embryo that gives rise to the epithelium (lining) of the gut and gut derivatives.
Early Development of the Chick

Epiblast: In the chick embryo, following delamination which separates the blastoderm into two layers, the epiblast is the upper layer of cells that contains the presumptive ectoderm, mesoderm and some endoderm. It is separated from the lower hypoblast.

Extraembryonic membranes: The membranes that form in association with the embryo proper. In the amniote, four extraembryonic membranes form, the amnion, chorion, yolk sac, and allantois.

Fate maps: Maps of the developing embryo that have been constructed to show the location of each germ layer prior to gastrulation and its route of travel during gastrulation.

Foregut: In the chick embryo, the region of anterior gut that has been closed into a tube.

Gallus domesticus: The species name for the common domestic chicken.

Gastrulation: The stage in embryonic development that follows cleavage. During gastrulation, cells rearrange themselves, with the endoderm and mesoderm cells moving inward, and the ectoderm cells spreading around the outside, with some ectoderm invaginating to form such structures as the stomodeum and proctodeum.

Germ layers: The ectoderm, mesoderm, and endoderm of a developing embryo.

Headfold: In the chick, the undercutting fold of the blastoderm in the area pellucida of the head region which separates the head from the extraembryonic regions, lifting the head up off of the remaining blastoderm.

Hensen’s node (or primitive node): In the gastrulating chick embryo, the top of the primitive streak where the notochordal cells are ingressing and migrating forward.

Hypoblast: In the chick embryo, following delamination which separates the blastoderm into two layers, the hypoblast is the lower layer of cells that contains the extraembryonic endoderm.

Infundibulum: In the adult female chicken, the region of the oviduct where fertilization takes place. It is high up near the beginning of the oviduct.

Ingression: During gastrulation, the type of cell movement that involves cells moving inward as individual cells. In the chick embryo, mesoderm and embryonic endoderm move through the primitive streak by ingestion. In the sea urchin embryo, the primary mesenchyme moves inward by ingestion.

Involution: A type of gastrulation movement in which a sheet of cells folds inward and spreads over an inner surface.

Isthmus: In the oviduct of the female chicken, it is the region where the chalazae and the inner and outer shell membranes are added to the egg as it descends.

Lysozyme: An enzyme that disrupts bacterial cell walls, thereby killing the bacteria. Lysozyme can be found, for example, in the albumen of a chick egg.

Macrolecithal egg: An egg with a large quantity of yolk, as in the chick, fish, and insect eggs.

Magnum: In the oviduct of the female chicken, it is the region that secretes most of the albumen around the ovum as it travels down the oviduct.

Mammillary layer: The inner calcified layer of the chick egg shell, lying next to the outer spongy layer. The crystals of calcium carbonate lie perpendicular to the surface of the egg, giving the shell tremendous strength.

Meiosis: The type of cell division that halves the number of chromosomes in cells, taking them from a diploid (2N) to a haploid number (1N) of chromosomes. This occurs only in germ cells.

Mesoderm: The germ layer in an embryo that gives rise to the muscular and circulatory systems and most of the skeletal and urogenital systems.

Mesolecithal egg: An egg with a moderate amount of yolk, as in the amphibian egg.

Microfilaments: The thin filaments, actin and myosin, within the cytoplasm of a cell.
**Microlecithal egg:** An egg with a small amount of yolk, as in the sea urchin egg and those of many other invertebrates that have small, feeding larval stages.

**Microneedle:** A needle that is very thin, suitable for microdissection under a dissecting microscope. It can be made inexpensively from inset pins, a wooden dowel, and superglue.

**Microtubules:** Thin tubules found in the cytoplasm of a cell. Microtubules are made up of tubulin subunits. Microtubules function both as a cytoskeletal support and for transport within the cell. Motor-molecules, associated with the microtubules, are able to ferry such things as organelles along the microtubules.

**Midgut:** The region of gut between the foregut and hindgut.

**Monotreme:** A primitive type of mammal that lays eggs. Includes the duck-billed platypus and the spiny ant-eater.

**Myosin microfilaments:** Microfilaments found in the cytoplasm of cells. Myosin microfilaments are the thicker of the two types of microfilaments; actin microfilaments are the thinner. Myosin can move along actin microfilaments in a ratchet-type mechanism that creates a contractile force within the cell.

**Neural crest cells:** Cells found only in vertebrate embryos that start out residing on the crest of the neural tube and subsequently migrate to become a host of structures: pigment cells (except for the pigmented retina), membrane bones in the face and skull, dentine-secreting cells of the teeth, the adrenal medulla, and sympathetic ganglia of the autonomic nervous system are just some of the structures formed.

**Neural ectoderm:** The region of ectoderm that forms the neural tube.

**Neural plate:** The neural ectoderm prior to its invagination to form the neural tube.

**Neural tube:** The tube formed by the neural ectoderm. The precursor to the brain and spinal cord.

**Neurulation:** The morphogenetic movements of the neural ectoderm that form a neural tube out of the neural plate.

**Notochord:** The axial supporting structure in a chordate embryo formed from notochordal mesoderm. Notochordal mesoderm has been called the “primary organizer” because of its inducing functions that initiate an axial organization of the embryo. The notochord is a diagnostic characteristic of the chordates.

**Ovalbumen:** The most abundant glycoprotein found in the albumen of a chick egg.

**Ovary:** The female gonad in which germ cells form ova.

**Oviduct:** The duct in a female that carries eggs from the ovary.

**Oviposition:** The laying of an egg.

**Ovomucin:** A protein that is thought to have antiviral activity. It can be found in the albumen of a chick egg.

**Ovotransferrin:** An enzyme that inhibits bacterial growth by binding iron, making it unavailable to the bacteria. Ovotransferrin can be found in the albumen of a chick egg.

**Ovulation:** The release of an egg (ovum) from the ovary.

**Physiological polyspermy:** An unusual event that occurs in some organisms such as the chick, it is the entry of multiple sperm into an egg at fertilization, but with the result that only one sperm pronucleus fuses with the egg pronucleus.

**Pipping:** At hatching, the process of a baby bird breaking through its shell.

**Primitive node:** Hensen’s node.

**Primitive streak:** In the chick gastrula, the line along which embryonic endoderm and mesoderm cells ingress. Analogous to a blastopore.
**Proamnion:** In the chick embryo, the region of area pellucida in front of the embryo’s head that is particularly clear, consisting only of ectoderm and endoderm. The spreading mesoderm has not yet reached this area.

**Pronucleus:** A haploid maternal (from the egg) or paternal (from the sperm) nucleus within a fertilized egg prior to the fusion of these nuclei into a single diploid nucleus.

**Protostome:** Among the bilaterian organisms, the phyla in which the mouth, during embryonic development, forms at or near the blastopore opening. These include phyla such as the arthropods, nematodes, annelids, molluscs, and platyhelminthes.

**Retinal:** A form of vitamin A from which the pigment rhodopsin is made.

**Rhodopsin:** A pigment in the eye that is necessary for vision. Retinal, derived from vitamin A, is used to make rhodopsin.

**Secondary oocyte:** A developing ovum in the secondary gonocyte stage. A secondary oocyte is haploid. Each of its chromosomes consists of two sister chromatids. The first meiotic division divides the primary oocyte asymmetrically, producing one secondary oocyte and one tiny polar body. At the end of this stage, the secondary oocyte enters the second meiotic division.

**Serial sections:** Histological sections of a specimen that are taken sequentially, and kept in order. Usually every section is mounted in correct order for microscopic observations.

**Shell gland:** In the female chick, the region of the oviduct where water and salts are added to the albumen and the shell is deposited. Also called the uterus.

**Shell membranes:** Membranes on the inner aspect of the calcareous portion of an egg shell. In the chick egg, there are two shell membranes. These protect against desiccation and invasion by microorganisms.

**Shell pores:** Pores within an egg shell that allow for gas exchange.

**Somites:** Blocks of mesoderm that become subdivided into a sclerotome, myotome, and dermatome. Sclerotomes form vertebrae and ribs, myotomes form skeletal muscles for the back and appendages, and dermatomes give rise to dermis.

**Spina bifida:** A congenital defect in which the neural plate doesn’t fully close when making the neural tube, and parts of the neural tube remain open.

**Spongy layer:** Also called crystalline layer. The outer calcified layer of the chick egg shell, lying next to the inner mammillary layer. The crystals of calcium carbonate lie perpendicular to the surface of the egg, giving the shell tremendous strength.

**Staging series:** An embryonic timing series that identifies stages of development. A complete staging series is valuable, and once a good one for an organism has been published, this is usually used for many years. The Hamburger-Hamilton staging series for the chick, for example, has been used since its publication in 1951.

**Unsegmented somite mesoderm:** The region of somitic mesoderm that has not yet formed blocks of somites.

**Uterus:** The lower end of the oviduct that is modified for a specific function. In a female placental mammal, it is where the embryo implants. In the female chick, it is where water and salts are added to the albumen and the shell is deposited. In the chick, also called the shell gland.

**Vagina:** The posteriormost region of the duct system leading from the ovaries to the outside.

**Vegetal pole:** The pole that is opposite the animal pole. Yolk is more concentrated at the vegetal pole. Eggs usually orient to gravity such that their vegetal pole is oriented downward.

**Vertebrata:** The class of chordates that include the fish, amphibians, reptiles, birds and mammals. Diagnostic features of the vertebrates are a vertebral column (hence its name), neural crest cells during development, and the presence of bone.
Vitelline: Of the yolk or having to do with the yolk.
Vitelline envelope: An extracellular coat surrounding an ovum.
Whole mounts: A histological preparation of a specimen in which the whole specimen is mounted. The specimen is usually mounted on a microscope slide, and can be mounted either in a water-mountant such as glycerin jelly or a non-water mountant such as the plastic mounting media used routinely for stained paraffin sections.
Yolk: A term commonly used to indicate the ovum of an amniote egg. The yolk is a single cell and contains a large amount of yolk protein in the form of yolk platelets. In a bird egg, the yolk is denser than the albumen and would sink in the albumen if it weren’t suspended by the chalazae.
Yolk sac: The extraembryonic membrane that surrounds the yolk. In the amniote, it is vascularized so that it can bring yolk nutrients from the yolk back to the embryo proper. It forms from the splanchnopleure, a layer made up of endoderm and splanchnic lateral plate mesoderm.