Observation of chick embryos and shell-less culture

The development of chick embryos has been studied since Aristotle. It is one of the most intensely studied organisms. One reason for this is that there are great similarities between avian and mammalian embryology. Another advantage is that the embryo is relatively accessible and can be operated on or treated with teratogens (substances that perturb development) while still *in ovo* (in the egg). Finally, fertilized chicken eggs are available year round and can be "held" at 14°C for several weeks before being set, producing embryos upon demand.

A. Observation of plastic embedded specimens

Examine plastic embedded specimens of "13- or 16-hour", "24-hour", "33-hour", "48-hour" and "72-hour" chick embryos. These terms refer to classic studies of chick development. In reality, chick embryos will develop at different rates for a number of reasons, including incubation temperature. In modern times, chicks always take longer to reach these stages. Two more accurate ways of describing the relative developmental age of a chick embryo are (1) the number of somites and (2) comparison to a staging series, such as the one devised by Hamburger and Hamilton (H&H). The somites are the blocks of tissue on either side of the neural tube. Try to determine the number of somites and the H&H stage number of your embryos, and to identify the structures indicated in the diagrams. Pay particular attention to the developing circulatory system. Try to determine which germ layer forms each structure. Highlights include:

13- to 16-hour. Identify the area opaca and area pellucida. Locate the primitive streak. Hensen's node is located at the anterior end of the streak.

24-hour. Locate the neural folds and Hensen's node. Identify the anterior end of the embryo.

33-hour. The embryo is lying along the center of the blastodisc, dorsal side up. The heart is to the right, along the side of the hindbrain. It is a simple, looped tube; connected to the vitelline vein and the ventral aorta. What is the function of the vitelline vein? Identify the forebrain, midbrain and hindbrain. The optic vesicles are outpockets on either side of the forebrain. The neural plate runs along the dorsal midline and is not yet closed. You may also be able to see the notochord running along the midline.

48-hour. The head has bent forward and the body has begun to twist. The optic cup (eye) and the otic vesicle (ear) have formed. The neural tube has closed, except in the region of the hindbrain. A series of aortic arches run between the pouches in the pharynx and converge into the dorsal aorta. The heart has begun to differentiate into an atrium and a ventricle.

72-hour. The flexure of the embryo is now pronounced and the embryo is lying on its left side. The brain has further subdivided and the olfactory pit (nose) is present over the tip of the forebrain. The lens of the eye should be visible. Lateral swellings representing the limb buds and a curved tail are also present.

B. Observation of living embryos (3-day and 4-day)

1. Obtain an egg and clean scissors, forceps, a plastic spoon. First prepare a small petri dish with Howard's Ringers solution. Gently rotate egg, clean with 70% ethanol and set down for a few minutes to allow the embryo to float to the top. Open up the blunt end of the shell and carefully peel back the shell membranes. Observe the embryo under the dissecting scope. Measure the heart rate and observe any other movement. Grab the outer ring of the blastodisc near embryo with fine forceps in your non-cutting hand. Try not to pinch any blood vessels. Quickly cut around embryo.

Note: an alternate method of embryo isolation is to break the egg open into a finger bowl of Howard's. This is similar to cracking an egg for culinary purposes, but the yolks of warm eggs are more fragile than those of refrigerated ones. First, place the egg in a horizontal position to allow the embryo to float to one side. Next, keeping the same orientation, crack the egg gently against the side of a finger bowl filled with Howard's. Submerge the egg and gently pull the ends apart to crack the egg open. Isolate the embryos as above.

Embryo in shell

Crack egg into finger bowl

2. Keeping hold of the embryo with the forceps, pick up a spoon with your cutting hand and slide it under the embryo. Continue to hold onto embryo, lift with the spoon and transfer to the petri dish filled with Howard's and examine with the dissecting microscope. Have your lab partner stand by with scissors in case the embryo wasn't completely detached. If the dish is cloudy with yolk, transfer embryo to a fresh dish of Howard's. Carefully trim away membranes the surround embryo.

3. Examine embryos. Pay particular attention to the heart and circulation, and to the developing neural tube. Measure the heart rate. Which side was towards the yolk?

4. For each embryo, determine the H&H stage. How do these embryos compare to the stained specimens?

5. Clean your instruments well with warm water, distilled water and 70% ethanol. Dry before returning to case. Discard the shells in the trash and the yolk/albumin remains in the sink.

C. Shell-less cultures of chick embryos

1. Squirt section of PVC pipe with 70% ethanol and allow to dry in laminar flow hood. Label with your name and the date.

2. Create a cradle with generic plastic wrap; be careful not to touch the middle of what will be the inside of the cradle. Secure with a rubber band. Cover with the lid of a sterile petri plate.

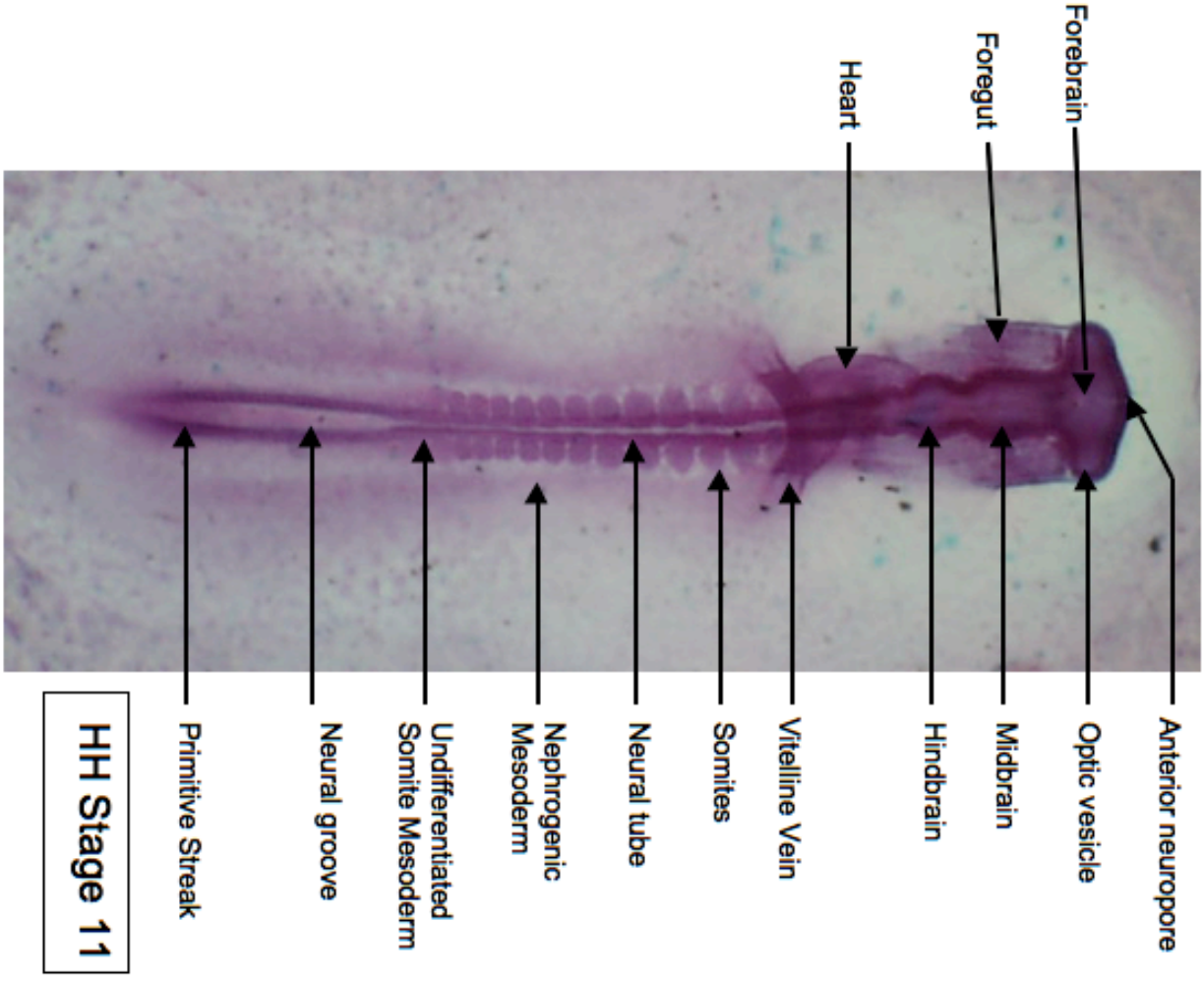
3. Wipe 3-day egg with 70% ethanol. Allow it to sit on its side in the 37° C incubator for 5-10 minutes to allow the embryo to rotate to the top side.

4. Remove lid from cradle, leave in hood. Firmly crack lower side on edge of finger bowl. Resolutely open into cradle. Cover with lid and return to 37°C incubator. The embryo will float to the surface. Obviously the embryo will develop best if the yolk is not broken. Warm, incubated eggs break more easily than refrigerated ones, unfortunately, but embryos will usually survive for a while if there are only small tears in the yolk.

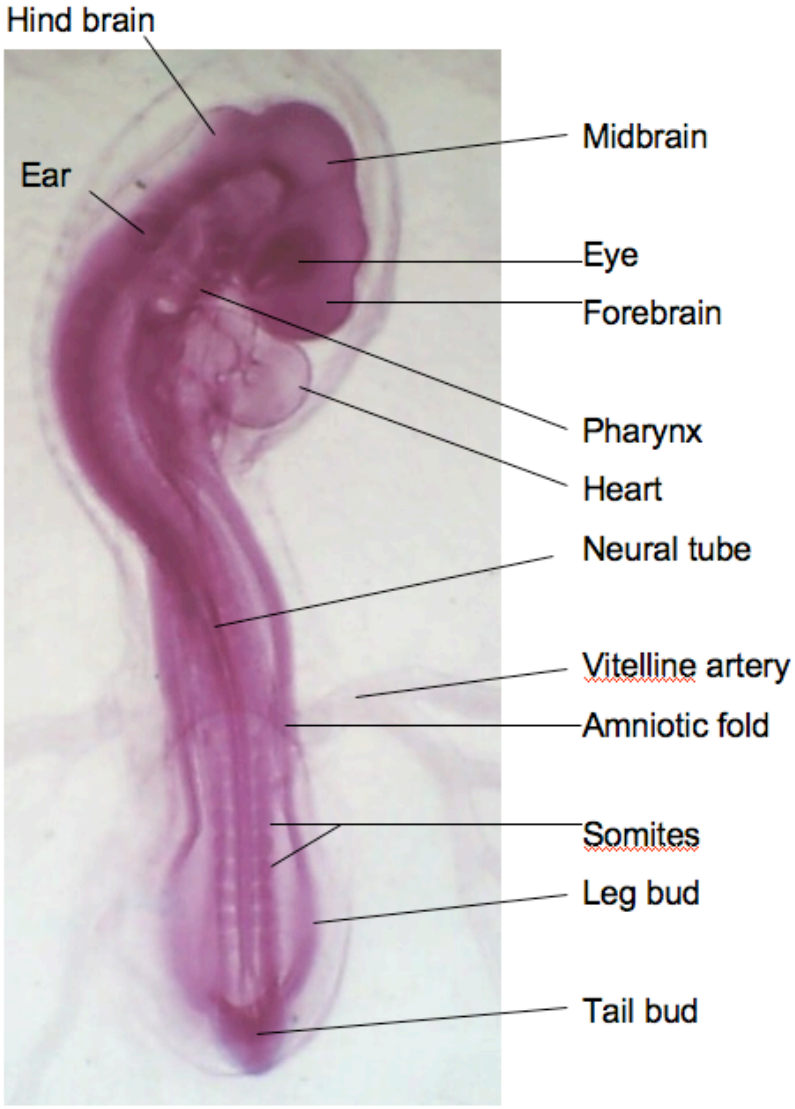
5. Observe periodically to watch for organ development and extraembryonic membrane formation. Do not keep outside the incubator for more than 5 minutes. Remove dead embryos promptly to freezer. We will examine the survivors next week and compare them to control embryos incubated in the shell.

6. If the yolk is too damaged to permit survival, isolate the three-day embryo. Locate the peripheral ring of blood vessels. Hold onto the membrane near the blood ring and cut around it with sharp scissors. Position the spoon near the embryo with your cutting hand and lead the embryo onto it. Do not let go with forceps. Quickly transfer to small petri dish filled with Howard's. Position under dissecting scope. Pipet out yolk and straighten embryo gently with forceps. Add additional solution if necessary or transfer to fresh dish.

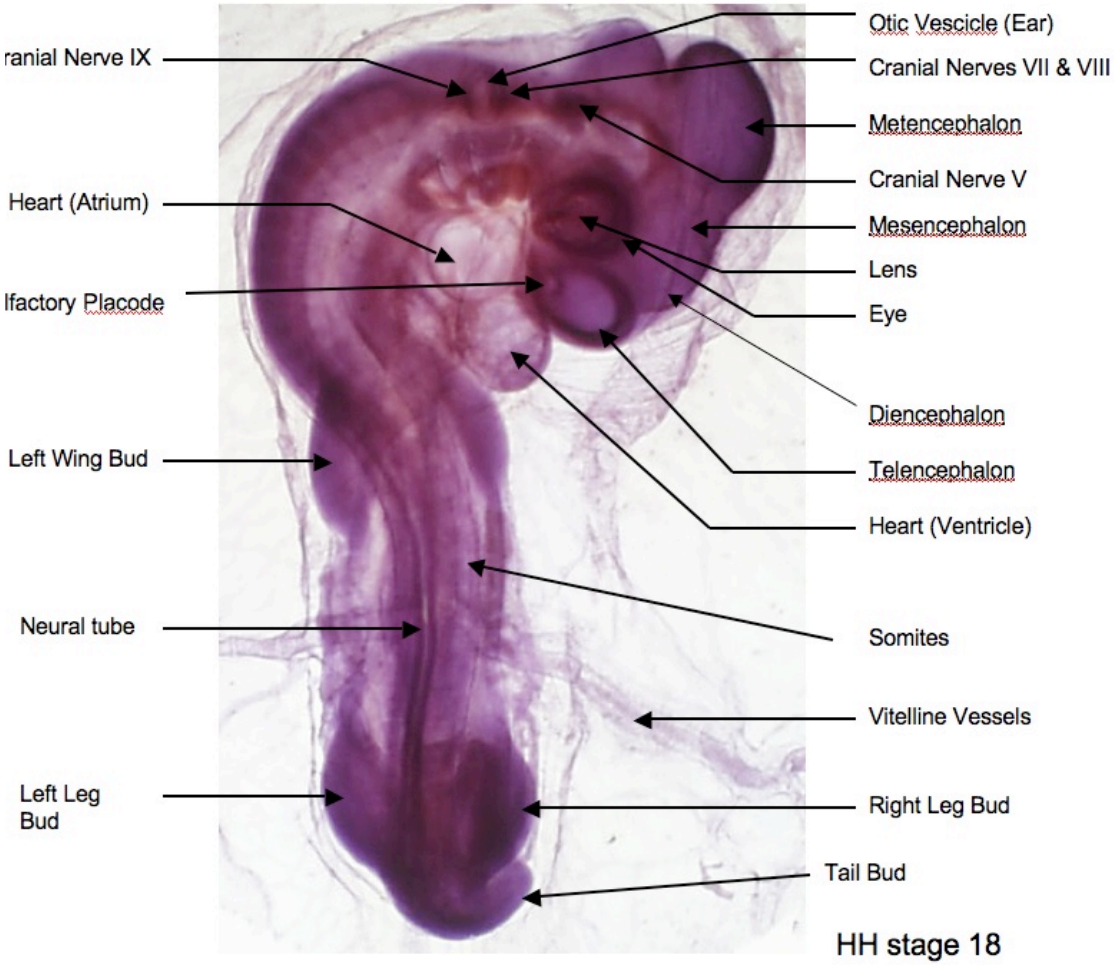
3-day embryo in shell-less culture



HH Stage 11



Hamburger & Hamilton Stage 16



HH stage 18

References

http://www.millersville.edu/~jcebrathomas/cebra_thomas/DB_lab/Chick/Chick_Observe.html

http://www.millersville.edu/~jcebrathomas/cebra_thomas/DB_lab/Chick/Shell-less.html

Hamburger, V. and H. Hamilton. 1951. A series of normal stages in the development of the chick embryo. *J Morphol*. 88: 49-92.

Tuan, R.S., and M.H. Lynch (1983). Effect of experimentally induced calcium deficiency on the developmental expression of collagen types in chick embryonic skeleton. Dev. Biol. 100:374-386.

Prep List

Dissecting microscope
60 mm plastic petri dishes
transfer pipets
kimwipes
bench paper
finger bowls (optional)
70% ethanol in squirt bottle
dissecting tools
- plastic spoon or Moria spoon
- 2 Dumont #5 fine forceps
- 1 Dumont #6 bent forceps
- 1 pair iris scissors (curved best)

Howard Ringer's solution
3-day and 4-day eggs incubated at 37°
PVC pipe sections (approx. 100 mm diameter)
100 mm petri dishes, sterile (glass preferable)
Generic plastic wrap
large rubber bands
Copies of Hamburger & Hamilton staging series

Howard Ringer's solution

14.4 g	NaCl
0.34g	$CaCl_2$ (anhyd.) or 0.46g $CaCl_2.2H_2O$
0.74g	KCl

dH_2O to 2 liter