Observation and culture of early chick embryos (2-days and younger)

1. Use sterile technique. Prewarm agar/albumin culture dish to 37°C. Set up several small petri dishes with Howard's Ringers. Clean your dissecting equipment and wipe with 70% ethanol.

2. Clean a 2-day egg and allow the embryo to float to one side. Crack egg into large sterile petri dish. Use cut-off sterile disposable pipet to remove some of the albumin, so that top half of yolk is uncovered. Save some sterile albumen for culture dishes.

3. The embryo may not be visible to the naked eye. The blastodisc is located above a small ring of white yolk. Drop a filter paper disc around your embryo. Hold on to the filter paper with fine forceps and cut around the ring with your sharp scissors. Transfer the filter paper ring with embryo to a small petri dish with Howard's Ringer's solution (see Figures 1 & 2). Stage embryo. Gently wash away yolk.

4. Examine the embryos. Identify the heart and the developing neural tube. Do you see evidence of blood circulation? The reddish spots on the 2-day blastodisc are the blood islands, the sites of hematopoesis. The embryo is covered with a clear protein layer known as the vitelline membrane. This may start to peel away from the embryo. Photograph embryo.

5. Transfer a small quantity of albumen to agar/albumin dish. Gently lift the embryo by the filter paper ring and transfer to dish, filter paper-side down (ventral side of embryo is up).

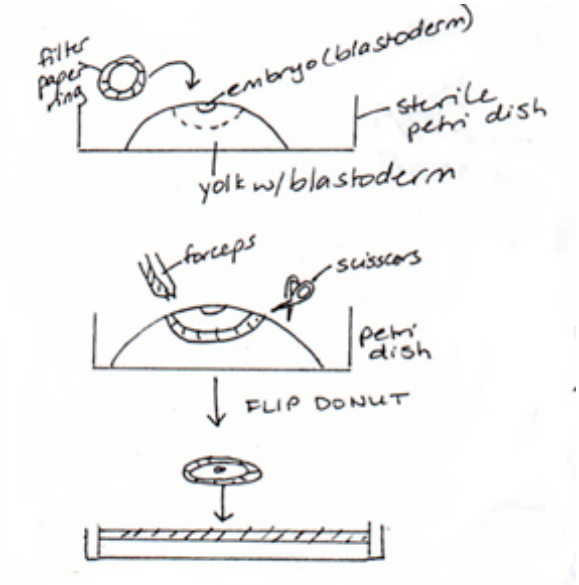

Figure 1. Isolation of 2-day chick embryo using filter paper ring.

6. Incubate in 37° incubator overnight. Check for evidence of development the next day, and thereafter.

7a. For fate mapping, apply a very small drop of Nile blue sulfate or 0.5 mg/ml DiI to the remains of Hensen's node in the posterior of the embryo using a small glass rod or drawn-out capillary pipet (Figure 3).

7b. To see if growth factors can increase the amount of blood formation, add a small square of filter paper soaked in bFGF or VEGF below the filter paper ring (Figure 3).

8. 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 down the drain with cold water.

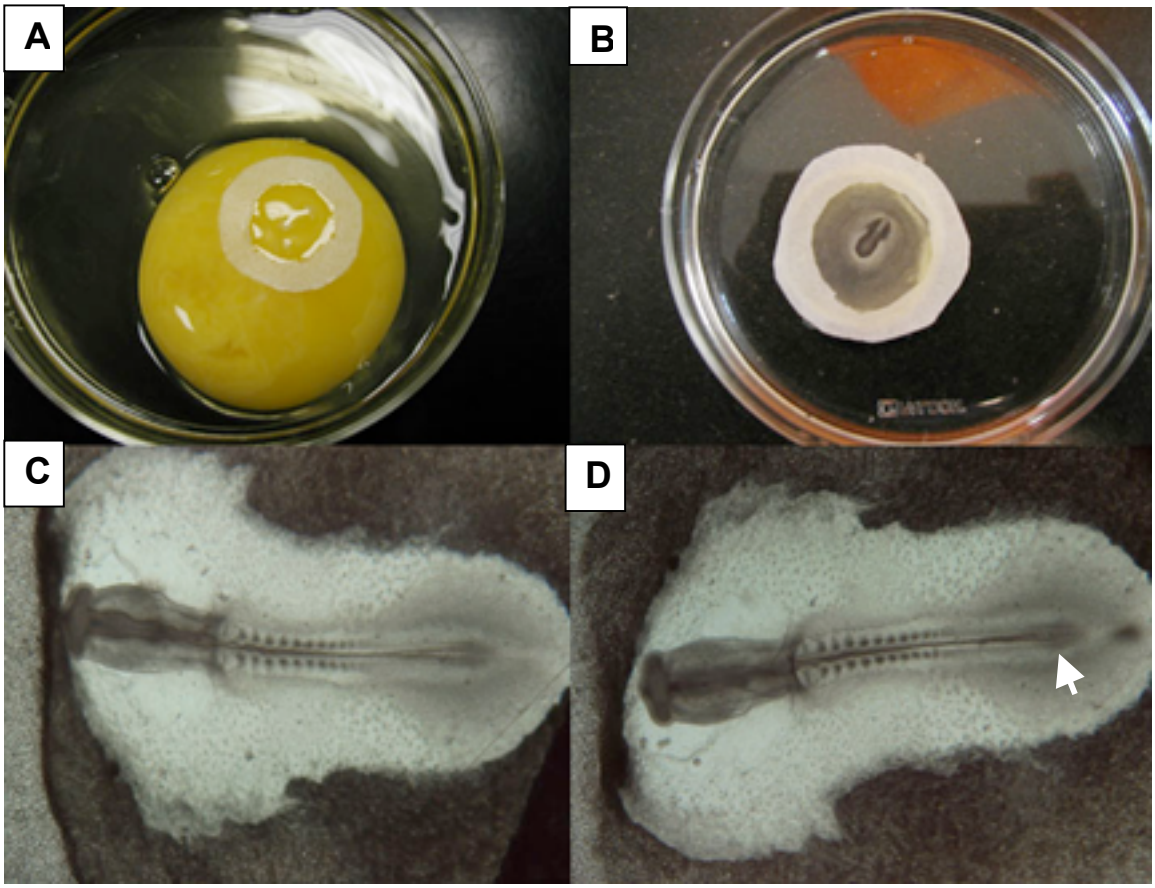

Figure 2. (A) Egg cracked into dish and filter ring placed around embryo. (B) Embryo removed to Petri dish with Howard's Ringer's solution. (C and D) Isolated 2-day chick embryos (HH stage 11). Arrow points to the remnants of Hensen's node and the primitive streak.

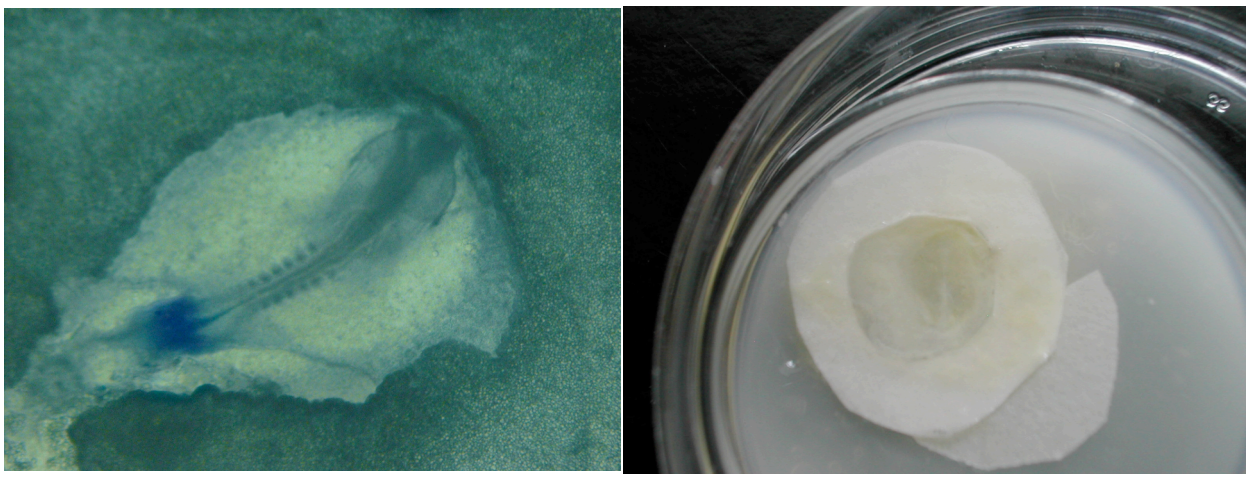

Figure 3. (A) Nile blue dye applied to the posterior of a 2-day chick embryo (HH stage 8) in culture on albumin-agar plate. (B) Two-day chick embryo in culture on albumin-agar plate with filter paper soaked in bFGF.

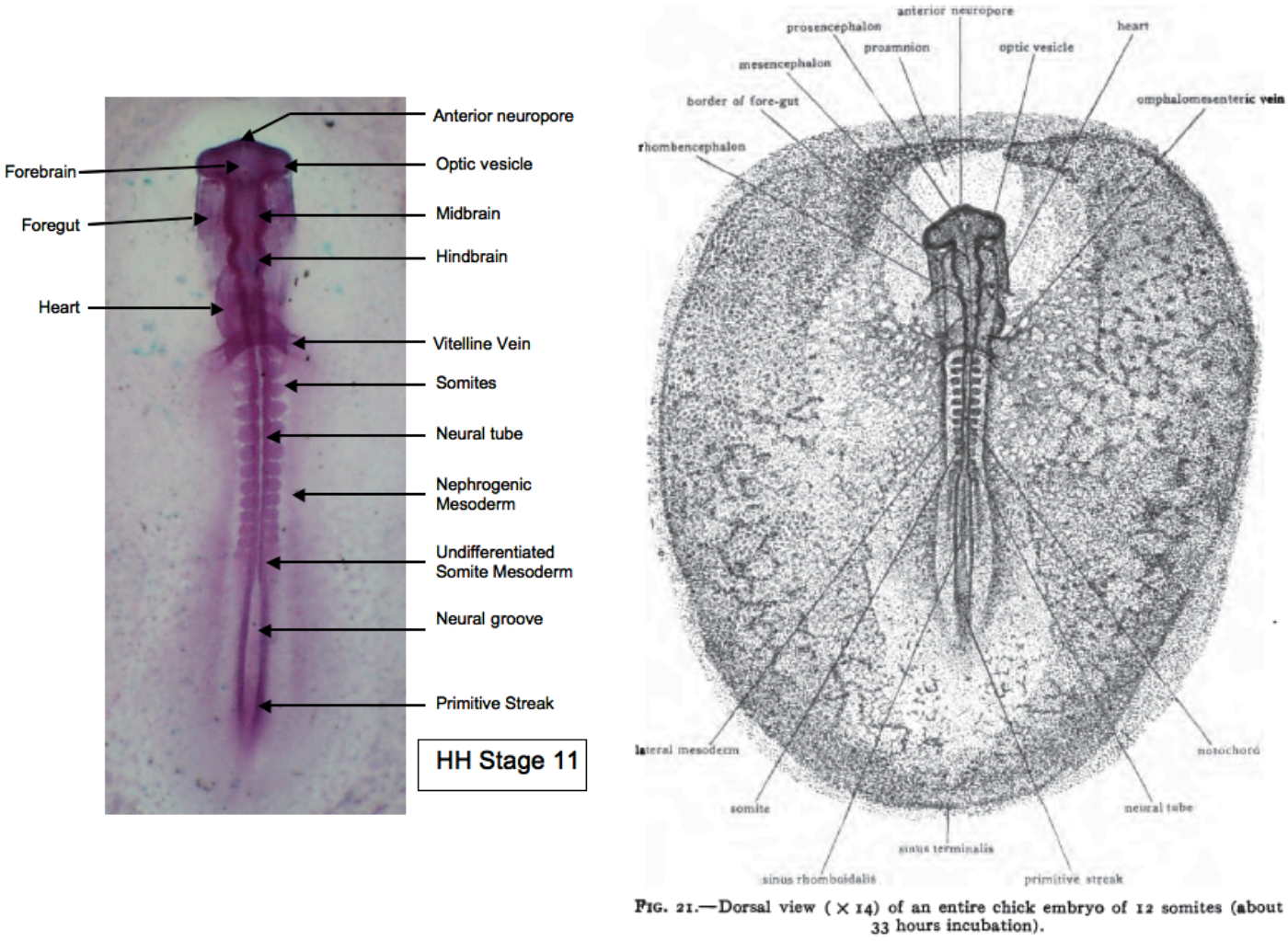

Figure 4. Photograph (ventral view) of fixed and stained HH stage 11 chick embryo and drawing (dorsal view) of comparable "33 hour" chick (from Patten, 1920).

References

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

Biroc, S., 1987. *Developmental Biology, a Laboratory Course with Readings*, Macmillan, p.48

Gilbert, S. 2010. *Developmental Biology*, 9th ed. Sinauer Associates, Inc., MA, pp. 285-298.

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

Patten, Bradley, 1920, *Embryology of the Chick*, P. Blakiston's Son & Co., Philadelphia, PA, p. 61.

Prep List

Dissecting microscopes
60 & 100 mm Petri dishes
transfer pipets
kimwipes
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)
    1 half-curved iris forceps
scissors for cutting plastic pipets and filter paper
filter paper circles (sterile)
Howard Ringer's solution (sterile)
2-day chick eggs incubated at 37°
(Note: it helps to prevent the yolks from breaking if you allow them to cool at room temperature for 30 minutes before using)
Whatmann 3MM filter paper
bFGF (1.5 ug/ml) or VEGF (0.5 ug/ml) in DMEM, PBS or Howard's
DiI (Molecular Probes) stock 5 mg/ml in 100% ethanol, dilute 1/10 in 0.3M sucrose
    (Note: spin in microfuge before use)
Copies of Hamburger & Hamilton staging series

Growth factor-soaked filter paper

1. Soak 3 x 3 mm squares of Whatmann 3MM in 3 mg/ml Hydrocortisone-acetate and air-dry. Coated filter paper can be stored.
2. Dilute bFGF (1.5 ug/ml) or VEGF (0.5 ug/ml) in 4 ml DMEM or Howard's. Soak filter paper squares for 30 minutes before use.

Albumin-agar plates (from Biroc, 1987)

1. Autoclave 200 ml beaker with stir bar Wipe eggs with 70% ethanol. Collect 100 ml albumin by separating egg whites from approximately 5 eggs into sterile beaker (discard yolks). Stir at room temperature to break up clumps. Warm to 45°C in water bath. Optional: Can substitute Just Whites™.
2. Autoclave 1.3 g agar, 1.3 g glucose and 33 ml Howards' Ringers solution in 100 ml flask for 15 minutes. Cool to 45°C in water bath.
3. Combine agar and egg whites with stirring. Transfer between flask and beaker to aid combination. Turn water bath up to 48°C to keep agar from solidifying while pouring plates.
4. Cut tip off of transfer pipet. Transfer approximately 3 ml to 35 mm petri dishes. Avoid transferring bubbles.
5. Store in bags or sealed with parafilm in refrigerator. Use within 2 weeks.

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