# Chick Embryos in Shell-less Culture

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## Introduction

This method allows continuous observation of living chick embryos from day 3 to day 18 of incubation. Differentiation of organ systems and extraembryonic structures as well as pre-hatching behavior can be studied. Students can study developmental processes and also practice in vitro culture techniques. For advanced developmental biology courses, project-oriented studies of growth (Dunn and Boone, 1976), teratology (Pearson, 1983), calcium metabolism (Burke et al., 1979; Dunn et al, 1981; Narbaitz, 1979; Narbaitz and Jande, 1978; Tuan, 1980), angiogenesis (Castellot et al. 1986; Dobson et al., 1990; Sherer and Dostal, 1982), or osteogenesis can be devised.

Early attempts to grow chick embryos outside their shells (Boone, 1963; Quisenberry and Dillon, 1962) met with limited success. Bruce Dunn (1974), a high school student working in an improvised basement laboratory, devised a method for growing chick embryos in plastic slings. Working with Boone, Ramsey and Dunn improved shell-less embryo culture methods (Dunn and Boone, 1976; Ramsey and Boone, 1972). Castellot et al. (1982) simplified the method further by substituting disposable hot cups ("chick-in-a-cup") for plastic tripods.

Since Dunn's publication of his method in 1974, it has fascinated grade school children, won high school Science Fair Awards, and served as the experimental model for published scientific research.

In a world of rapidly changing technology and expensive equipment, the success of this method demonstrates that ingenuity and brains are more productive than button-pushing for good research.

## Materials

Fertile Chicken Eggs
48–72 hours of incubation (1 per student)
12- to 14-day-old embryos (1 per 4 students)

## Solutions

*For each group of students:*
Ethyl alcohol, 70%
Betadine solution
Saline, 0.85% (warm)

*To be shared by 20 students:*
Saline, 0.85% (2 liters)
Betadine skin cleanser, 4-oz bottles (2)
Ethyl alcohol, 70% (2 liters)

Ethyl alcohol, 95% (2 liters)
Ethyl alcohol, 100% (1 liter)
Toluene (500 ml)

*For bone staining:*
KOH, 1% (1 liter)
Working Alizarine red S solution (500 ml)

*For storing wet mounts:*
Clearing Solution A (200 ml)
Clearing Solution B (200 ml)
Clearing Solution C (200 ml)
Glycerin and thymol (200 ml)

## Equipment

*For individual groups*
Plastic bags to discard shells and eggs
Curved forceps
Straight forceps
Scissors
Sterile pasteur pipets
Glass rod
Styrofoam hot cups (1 per egg)
Disposable, sterile petri dishes (100 × 15 mm, 1 per 2 eggs)
Disposable petri dishes (60 × 15 mm,)
Plastic wrap
Rubber bands, 5.5"
150-ml sterile beakers
Sterile cotton balls
Finger bowls (about 4" in diameter)
Small foam rubber pad to set eggs on
Wax pencils or waterproof marking pens
Small screw-top bottles (1.5–2" in diameter such as baby food jars)
Trays, 9" × 12"

*To be shared by 20 students:*
UV light source (5)
Egg candlers (5)
Hot plate (1)
Thermometer (1)
Incubator (electric cabinet) for incubating at least 60 eggs at 38°C (1)
Large glass-topped incubator or oven-type incubator (preferably with inside glass door) (1)
Paper tape (1 roll)

## Procedure

*Preparation of culture chambers* (Total time: 35 minutes)

1. Cut 2 holes in the sides of styrofoam hot cups.
2. Cut an 8" long piece of plastic wrap and make a “sling” over the top of the hot cup. Secure sling in place with a rubber band (3–6 chambers/student).
3. Place on trays under UV lamp to sterilize for 20 minutes.

*Observation of living chick embryos and extraembryonic membranes and fixation of embryos for bone-staining* (Total time: 15 minutes)

1. While chambers are being sterilized (see above), candle 12-day-old embryos and discard dead or unfertilized eggs.
2. Crack shells and gently drop into a finger bowl filled with warm 0.85% saline.
3. Observe chorion, amnion, and yolk sac with extraembryonic circulation and external features of embryo.
4. Dissect away membranes and drop embryo into small screw-top bottle filled with 95% alcohol.

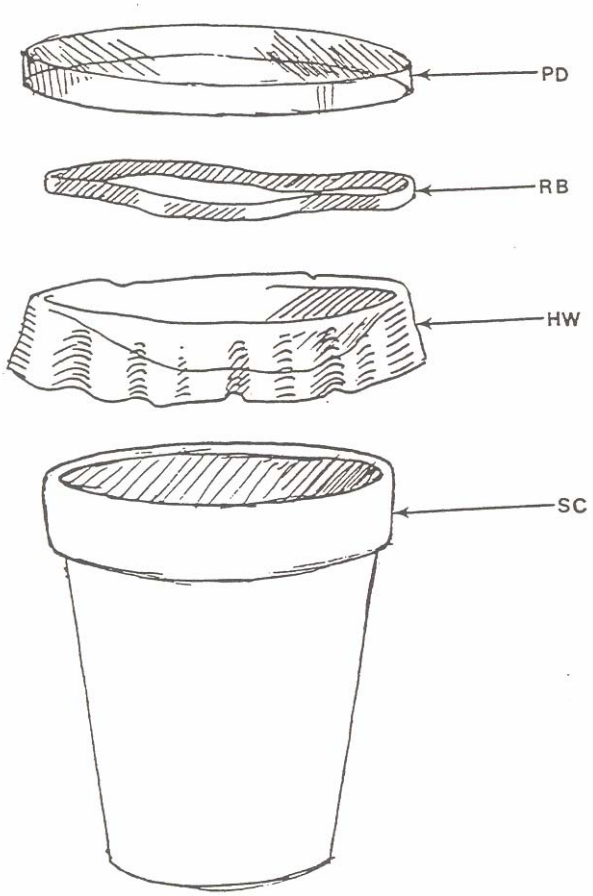


**Figure 1**. Construction of chick-in-a-cup culture chamber. Styrofoam hot cup (SC) supports a sling of plastic wrap (HW) held in place with a rubber band (RB) which is covered with a 100 × 15-mm petri dish cover (PD). Adapted from Dunn et al. (1981).

*Transfer of 72-hour-old chick embryos from shell to prepared sterile chambers*

(Total time: 45 minutes)

1. Candle 72-hour-old eggs, wash with Betadine solution and 70% ethyl alcohol and place on rubber pad for 2–3 minutes to allow chick to rotate to top of egg. Caution: Do not allow eggs to cool for more than 5 or 10 minutes or the embryo may die.
2. Wash hands, especially thumbs, with Betadine.
3. Gently crack the bottom side of the egg on the edge of the sterile glass beaker, pull shell open with both thumbs and gently drop egg contents into prepared chamber. The embryo should be on top of the yolk. If it is not and if it doesn't rotate to the top of the egg, use the wide, sterile ends of two pasteur pipets to gently rotate the embryo to the top surface. Discard any eggs in which the yolk is broken or the chick is not on top of the yolk.
4. Cover with the top of a 100 × 15-mm petri dish. Write the date and your name on the top of the petri dish and place immediately in a 38°C incubator.

*Bone staining (Humason, 1979) and preparation of wet embryos*

These procedures take short periods of time over a period of 5–7 days.

A – *Bone staining*

1. Remove embryos (12–19 days) from shell and membranes. Pluck feathers if present. Drop into jar of 95% ethyl alcohol for 1–2 days.
2. Discard alcohol and cover embryo with 1% KOH for 1 hour to 1 day (depending on age of embryo) until skeleton shows through muscles.
3. Discard KOH and cover embryo with Working Alizarine red S solution for 3–24 hours until bone is stained red.
4. Discard Alizarine solution and destain with 1% KOH for 5–10 minutes. Embryos can then be cleared and stored wet in bottles for display (follow procedure B below).

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B – *Clearing and storing of wet mounts*

1. Transfer through three clearing solutions (solutions A, B, and C): 24 hours each.
2. Transfer to pure glycerin with thymol added as a preservative.
3. Store in sealed tubes or bottles.