

# Eggshell Caps—A Method of Rearing Chick Embryos Visible Throughout Incubation

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To watch a living chick embryo develop is always interesting. To do this, we are usually content to follow the method of opening a number of eggs which have been incubated previously for the desired number of days, and to regard the exposed embryos as constituting a continuous series (see Miller and Blaydes).<sup>1</sup> However, many teachers of Biology have wished for a simple method of observing the same embryo day after day throughout its incubation. Such a method would impress the student with the continuous nature of development, which is a fundamental concept. It would focus attention upon the new features that develop day by day, thus impressing the student more strongly. Furthermore, it would reduce the number of eggs needed to demonstrate the entire course of their development and hatching.

The authors<sup>2</sup> have reported recently the successful incubation of chick embryos with the so-called eggshell cap method. It is so simple and easily followed that it should serve as a workable and interesting laboratory project in high school biology classes. In addition to the advantages listed above, it presents the growing chick still within its

<sup>1</sup> Miller, D. F., and G. W. Blaydes, *Methods and Materials for Teaching Biological Sciences*. N. Y.: McGraw-Hill Book Co., 1938, pages 402-403.

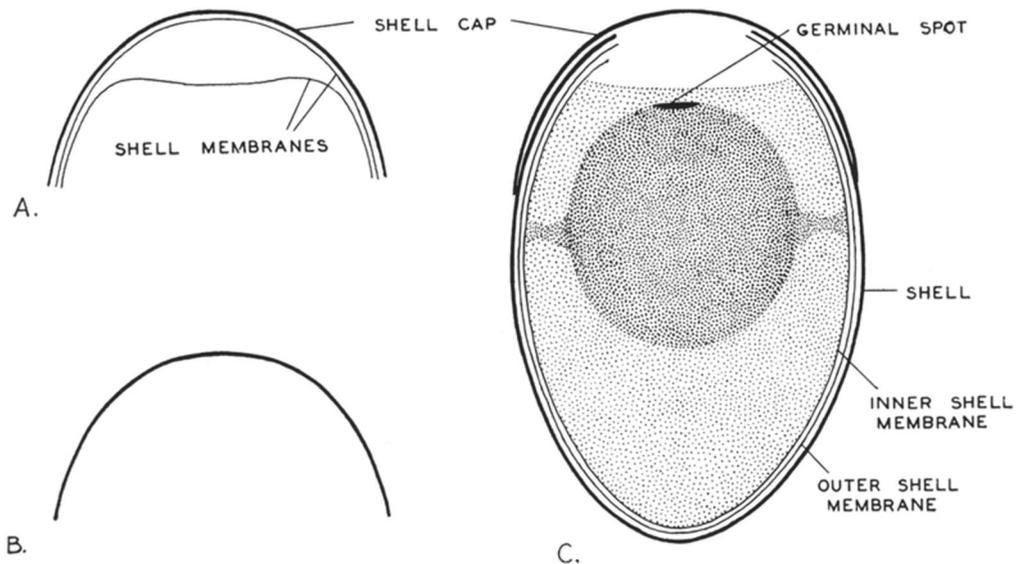
<sup>2</sup> Price, J. W., and E. V. Fowler. *Science*, 91: 271, 1940.

shell so that the normal interrelations between the organism and its environment are maintained.

This method involves the removal of the eggshell and both eggshell membranes, to expose the embryo and its extra-embryonic membranes directly beneath. The embryo may thus be observed directly and is subject to manual manipulation.

The method of preparation is simple: First, an eggshell cap is prepared by breaking an egg at the small end, emptying the contents and, with scissors, cutting the empty shell around its middle. The result is approximately a half eggshell with a cross-section similar to Fig. A. It is then dried and both shell membranes are removed, as in Fig. B. The eggshell cap taken from the large end of the egg consists then of the shell only. It is sterilized with alcohol, dried, and kept in a covered container until used.

Eggs to be incubated are opened prior to incubation. This point seems to be essential, to avoid injury to extra-embryonic membranes and blood vessels which is so likely to occur once they are formed. Holding the egg vertically, with the large end uppermost near a bright lamp, a light area marks the limits of the air cell in that region. After cleaning the eggshell with cotton moistened with alcohol, the outline of the air cell is marked with pencil. With tweezers a small opening is made above the air cell,



Figs. A, B, and C.

and by careful chipping, pieces of shell and membrane are removed to make a circular opening somewhat smaller in diameter than the air cell beneath. With sterile tweezers the inner shell membrane is next removed to the same extent, leaving the albumen exposed (see Fig. C). The previously prepared shell cap is now placed over the opening and the egg is ready for incubation. The shell cap is not sealed in any way.

After several days of incubation the level of albumen within has dropped and permits the further removal of shell. Care must be taken to avoid injuring the allantois. With a relatively large opening, later stages of development are clearly visible over the entire upper surface.

In the incubator such eggs must be held vertically. Holes may be made in strips of coarse screening to hold the eggs in a standard incubator tray. If the egg is permitted to roll over on its side, albumen will be lost. Held vertically, apparently no turning of the egg is necessary. Difficulty of hatching may be avoided by sprinkling the 18-day egg with water.

The incidence of infection of embryos has been extremely low, considering the fact that the shell cap is simply laid over the eggshell opening and may be removed daily for observation of the embryo. The overlapping margin of the eggshell and its cap is apparently an effective barrier to bacteria. The porous shell of the cap apparently permits adequate gaseous exchange between the living embryo and the exterior, yet prevents excessive desiccation.

In the absence of a commercial incubator, a satisfactory substitute may be made by using a glass-topped wooden box, modified somewhat from the diagram suggested by Miller and Blaydes,<sup>1</sup> p. 402, fig. 141. An ordinary store box of wood about 12" × 12" × 24" and lined with corrugated paper may be covered with two panes of glass which come together across the center above. Within, a lamp bulb is attached near the bottom of the box. A thermometer and a pan of water are placed within the box. The effective temperature near the eggs should be made to remain constant at approximately 101° F. and can be ad-

justed by increasing or decreasing the wattage of the bulb, changing its position with respect to the eggs, or by drawing apart the top panes of glass to allow greater circulation of air. To hold the eggs in an upright position, a sheet of  $\frac{1}{4}$ " mesh hardware cloth, with  $1\frac{1}{2}$ " holes cut to receive the eggs, may be placed as a horizontal shelf across the box above its center.

Given favorable conditions of temperature, humidity and circulation of air, fertile eggs should develop successfully and be observable throughout their incubation period when the eggshell caps are used as described above.

## HATCHING CHICKENS IN A LABORATORY

The course of study for high school biology in Washington includes these units: All Life Comes from Life, and Improvement in Plant and Animal Life Depends upon Variations and Heredity. Each unit is given approximately two weeks. During the study of these units eggs are hatched in an electric incubator costing less than \$10.00, purchased from a local mail order house. The incubator holds at least five dozen eggs.

Eggs are opened every day or so in order that the development of the embryo may be observed. It is best, but not necessary, to plan to use an egg for each class as each group prefers watching its own egg opened.

To open, an egg is placed in a pan of lukewarm water. The surface which rights itself above the water bears the embryo. With scissors, the shell is punctured at the large end, then cut lengthwise around the shell, taking care not to cut deeper than the underlying membrane. The upper portion of the shell is then removed.

The embryo of the opened egg may be kept alive for at least four hours by keeping it in a warm, moistened compartment. This is prepared by placing a small shallow container in warm water and inverting a large glass dish over all as cover. The small container supports the egg, and all is set in the incubator until the next class.

General directions for setting of the eggs and care of the incubator as well as early care of the chicks come with the incubator. However, all eggs should be set at the same time, for the incubator should not be opened from the eighteenth day to the time of hatching; escape of moisture is thus prevented. It is well to have the eighteenth day come on Saturday so that the class will not miss steps in the development of the embryo. Everyone wants to see a pipped egg. By all means, have enough eggs so that some will be sure to hatch. Candling is recommended so that odors from infertile eggs may be eliminated. Light eggs may be tested on the third or fourth day, dark eggs on the sixth or seventh. After the first day until the eighteenth, the eggs should be turned daily to keep the chick from sticking to the shell. After the eighteenth, the embryo is so large that it cannot change position easily in the shell and besides, moisture must be conserved.

Soon after the egg is placed in the incubator, the embryo, which is already in the early stages of germ-layer formation at the time of laying, resumes its growth. On the second day the heart beats, sending blood through a fine network of arteries and veins. By the third day, the gill clefts on the side of the neck reveal the fish-like plan of body. Limb buds, developing into miniature wings and legs, show well by the fifth day.

Feathers appear on the eighth day. Two weeks are necessary for formation