How-To-Do-It

How to Culture Chicken Embryos in Petri Dishes

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Chicken embryos are used extensively in biology classes at all levels. They are a favorite for demonstrating embryonic development in vertebrates because of their large size, hardiness, and ready availability. The one drawback to chick embryos is that development proceeds within an opaque shell, which makes observation of the development of the living embryo difficult. This article describes a method which I have used successfully to allow direct observation of chick embryo development from three days to about seventeen days of incubation, by culturing the entire embryo in a petri dish.

The traditional procedure for the observation of the living chick embryo is to make a "window" in the shell, i.e., remove a small portion of the shell so that the embryo inside can be observed (Rugh 1962). This, however, is far from ideal since observation of the embryo is still difficult. Researchers have recently devised methods for culturing chick embryos in polyethylene bags ("sandwich bags") (Elliott and Bennett 1971) or petri dishes (Auerbach, *et al*., 1974). The Auerbach method has proved extremely useful in the classroom.

Materials Needed

The procedure is convenient since the only specialized equipment needed is an incubator that can be maintained at 37°C, and this is usually available in any biology laboratory. The chick embryos are placed in a petri dish culture after three days of incubation, since for unknown reasons survival is reduced if an earlier stage is used. This may be due to dehydration of the embryo if placed in culture prior to amnion development.

Procedure

Swab the egg with 70% alcohol and allow to air dry to reduce the chances of contamination. Although sterile procedures are not essential, care must be taken to ensure that anything coming in contact with the petri dishes or embryos is scrupulously clean. Leave the egg in a horizontal position for a minute, so that the embryo will rotate to the uppermost position. The egg contents are then carefully placed in a sterile 100 × 20 mm round petri dish (Falcon No. 1005). This is usually done by simply cracking the underside of the egg and gently dropping the contents into the petri dish just as one would do if frying eggs "sunny side up." Alternatively, one can remove the upper half of the shell with forceps, placing the albumin in the petri dish with a pasteur pipette, and then dumping the yolk gently into the petri dish along with the rest of the albumin.

The petri dish is then covered and placed inside a larger 150 × 25 mm petri dish (Falcon No. 1013) containing a few milliliters of distilled water for humidification. The larger outer dishes can be reused many times. The embryo is then placed in the incubator at 37°C and allowed to develop. Maintain adequate humidity by keeping a pan of water in the incubator. The petri dish containing the embryo may be removed at intervals for observation. As long as care is taken not to disturb the embryo, the petri dish lids can be removed and the embryo observed directly. Care should be taken not to keep an embryo out of the incubator too long since cooling will adversely affect the development of the embryo. Figure 1 illustrates the procedure and results. The formation of the extraembryonic membranes can be studied particularly well in this type of culture.

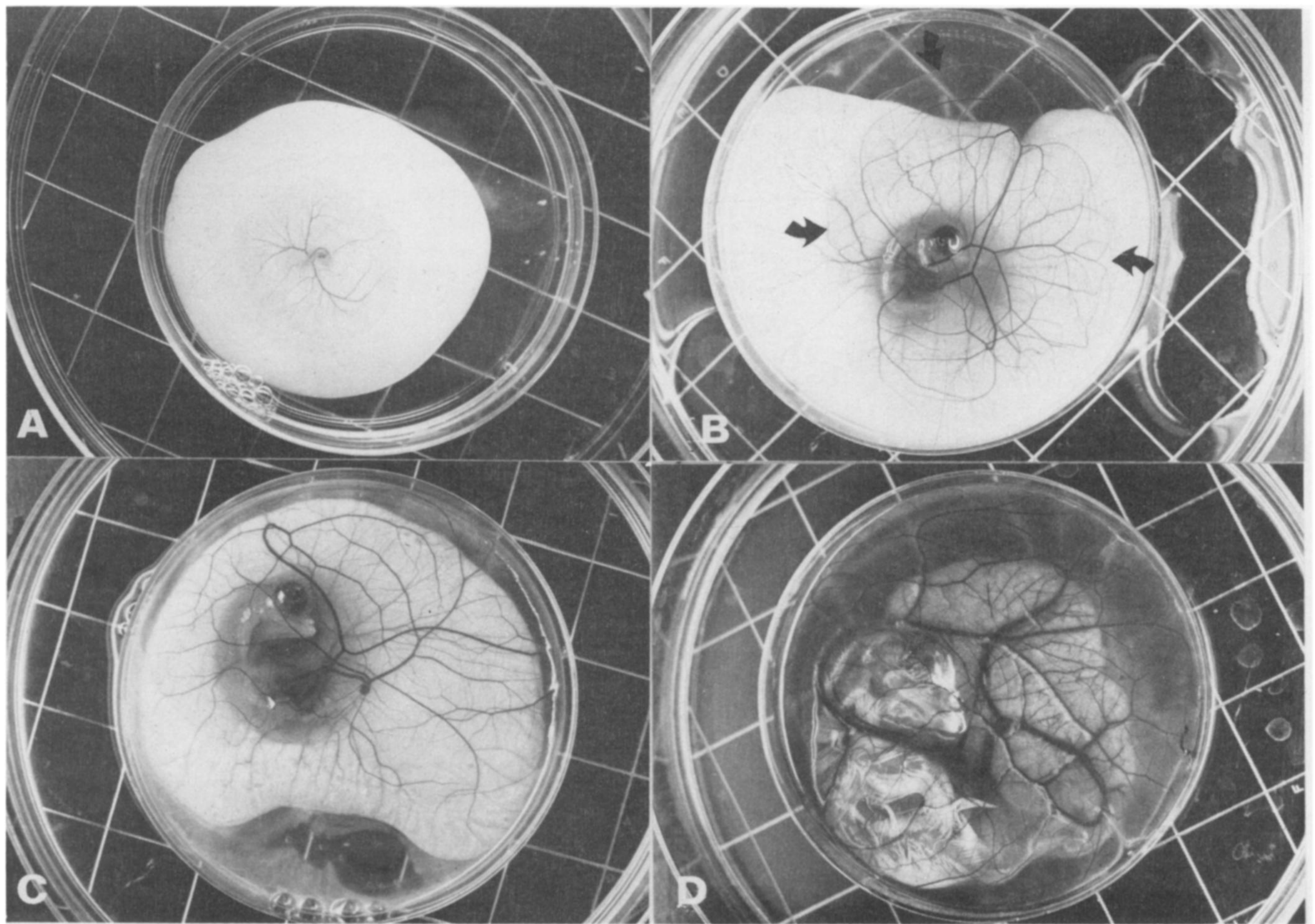

FIGURE 1. **(A) Chick embryo one day after being placed in petri dish (four days total incubation time). At this stage, the circulation of blood can be** studied effectively. The yolk sac and the vitelline circulation can be seen growing across the surface of the yolk. (B) Chick embryo six days after being placed in petri dish (nine days total incubation time). The yolk sac has grown outward to completely cover the yolk. The embryo is now completely contained within the amnion. The chorioallantoic membrane (arrows) can be seen spreading outward from the embryo. (C) Chick embryo eight days after being placed in petri dish (eleven days total incubation time). Chorioallantoic membrane has completely covered the upper surface of the yolk sac. (D) Chick embryo fifteen days after being placed in petri dish (eighteen days total incubation time). Embryos will not survive much beyond this stage.

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Results

The initial problem is getting the eggs into the petri dish without rupturing the yolks. Some students are extremely adept at this, others rather clumsy. I have found that a typical undergraduate class will get about half the embryos into culture without breaking the yolk. Typical results in my classes: about 50% of the embryos successfully placed in a petri dish will still be alive and growing at seven days and about 10% will survive for fourteen days. Since the embryos are placed in culture at three days' development, this represents seventeen days of incubation. For reasons which are not clear, all embryos will die before "hatching."

While this procedure can be used simply for observation of embryonic development, it can also be used for simple experiments as well. Once it develops, the chorioallantoic membrane (CAM) can be used as a graft site for CAM grafts. The petri dish culture allows continuous observation of the graft. The effect of adding extra albumin (save the thin albumin from the eggs whose yolks were ruptured) can also be studied. (In my experiments, this has slightly increased survival.) A variety of other experiments would also be possible. For example, by adding Locke's saline solution (Rugh 1962) with increased calcium chloride to the cultures, one could test the effect of extra calcium. (Is the reason that none of the embryos survive to hatching due to the absence of calcium which the embryo would normally get from the shell?)

Student Reactions

This procedure has proved extremely popular with students, who show much more enthusiasm for observation of these living embryos than for the study of fixed microscope slides.

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an important point can be made that bacteria can enter through the natural stomatal openings. Inoculate all leaflets of one or two leaves.

4. Using the same procedure, inoculate control plants with distilled water only.

5. Use a wooden pot marker to record the date, treatment, and student initials, and return to the growing area.

Third Week:

1. Examine the newly inoculated plants for symptoms of tomato speck. Compare these symptoms with the original plants. Compare inoculated leaves with those not inoculated.

2. Discuss how Koch's Postulates have been demonstrated (and what remains to be done to satisfy the fourth requirement.)

Variations on the Basic Exercise

The steps outlined above can be broadened slightly to produce a more systematic approach to the demonstration. Leaves from a healthy plant could also be sampled for the presence of the pathogen and a "blank" or control sample carried through. Healthy plants could be inoculated with non-pathogenic bacteria or with contaminants from the agar plates (although we have experienced surprisingly infrequent contamination).

The most obvious addition to the exercise is to re-isolate the pathogen from the newly infected tomato and compare it with the initial isolate, thereby satisfying the fourth postulate.

I have found, however, that the more basic steps detailed above are of sufficient length to adequately demonstrate the principles in question within a reasonable framework of time.

Conclusion

Working with tomatoes infected with *Pseudomonas tomato*, I have provided a laboratory exercise which can easily and in a reasonable period of time demonstrate the workings of Koch's Postulates. The exercise also provides an opportunity to practice aseptic techniques used in microbiological laboratories.

I would be happy to provide a bacterial culture to anyone who would like to try the procedure.

Acknowledgment—I would like to express my sincere appreciation to Dr. Randall C. Rowe, Associate Professor of Plant Pathology at the Ohio Agricultural Research and Development Center, for suggesting tomato speck as an appropriate disease for this exercise, for providing the original pathogen, and for his review of this paper.

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Letters

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or "models," one sees immediately why creationism cannot be part of science. The fundamental postulate of all creationists' "models" is a supernatural creative force. For more than 300 years, scientists have agreed that assumptions about the supernatural were not admissable as scientific postulates. All the postulates of the three major theories in evolution are assumptions about nature. The attempts to bring the supernatural back into science stem from a failure to understand the limited realm of science, and to sense vast realms of human experience outside of science. The proper teaching of science as systems of theories—hypothetico-deductive systems—could help remove some of this failure.

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