A Simple Method for Explanting and Cultivating Early Chick Embryos *in Vitro*

NELSON T. SPRATT, JR.

Department of Biology,
The Johns Hopkins University

The *in vitro* technique, in spite of its usefulness in the study of many problems of embryonic development, has generally been considered far too difficult and complicated to be used in classroom experiments. This is probably true of the classical tissue culture technique with all of its special equipment and elaborate sterilization and blood-taking procedures. The following method, which has been designed for student use, dispenses with all of these but is just as effective and reliable in terms of the results it gives (Fig. 1). In the brief but complete procedure outlined below, no sterilization of equipment or solutions is necessary because of the bacteriolytic property of egg albumen used in the culture medium. Tap water may be used in place of distilled water. (When synthetic media, yolk-extract media, etc. containing no egg albumen are used, all glassware and instruments are dry sterilized and solutions are autoclaved.)

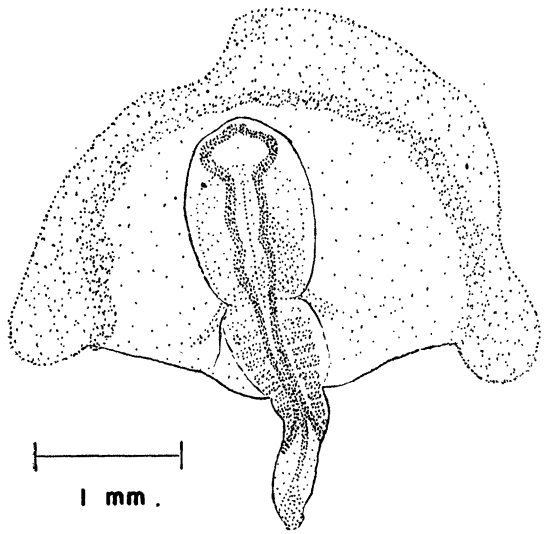

FIG. 1. Camera lucida drawing of a typical 10-hour-old, living explant of the anterior portion of a short head-process blastoderm which was transected about 0.4 mm. posterior to the node. This result was obtained after carrying out verbatim the method outlined for saline-agar-albumen media. With a little practice, the student can accumulate many similar cases of beautifully symmetrical and essentially normal morphogenesis.

Equipment. The following ordinary laboratory equipment is all that is necessary: 1 dozen Petri dishes (4 inches in diameter), 1 dozen watch crystals (2 inches in diameter), absorbent cotton (ca. 40 grams), 1 finger bowl (4½ inches in diameter), 2 Erlenmeyer flasks (ca. 500 cc.), 1 Erlenmeyer flask (ca. 125 cc.), 1 graduated cylinder (100 cc.), 1 wide-mouthed pipette (inside diameter, 3–4 mm.), 1 fine pipette (inside mouth diameter, ca. 1 mm.), 1 student dissecting set (2 needles, 1 pair of scissors, 1 pair of forceps), and 1 dissecting microscope.

Preparation of equipment. The glassware and dissecting instruments are thoroughly washed, rinsed in hot, running, tap water, and set aside to dry on a clean towel. The culture dishes (which have a large, humid, air space) are like those used by Fell and Robison (*1*). These are assembled by placing a moist cotton ring in each Petri dish, placing a watch crystal (concave side up) on this, and replacing the cover of the dish.

Preparation of culture medium. The final medium consists of two components which are made up separately and combined just before the medium is placed in the culture dishes. *Part I* (Ringer-albumen): The egg white from one unincubated egg (after separation from the yolk in the usual fashion) is added to 50 cc. of ordinary chick Ringer solution (0.9 per cent NaCl, 0.042 per cent KCl, 0.024 per cent $CaCl_2$) contained in a 500-cc. flask. The flask is stoppered and shaken vigorously for about 1 minute. *Part II* (Ringer-agar): 0.13–0.15 gram of agar (U.S.P. XI) is placed in the small flask along with 30 cc. of Ringer solution. The Ringer-agar is carefully brought to a boil over a Bunsen burner. A small flame is used, and the flask is frequently agitated to prevent the agar from sticking.

When the Ringer-agar has cooled down to about 40°–45° C., 20 cc. of the Ringer-albumen component (exclusive of the foamy portion) is added, and the flask is gently shaken to mix the two. Approximately 2 cc. of the medium is poured out into each watch crystal. The medium is allowed to gel (ca. 30 minutes–1 hour) before the Petri dishes are moved.

Operative procedure. Eggs containing the embryos to be cultivated[1] are opened into a finger bowl containing about 100 cc. of Ringer solution. The yolk is held steady with the forceps while a cut (ca. ¼ inch from the border of the blastoderm) is made through the vitelline membrane with the scissors and carried all the way around the blastoderm. The vitelline membrane with the blastoderm adhering to its underside is grasped at the cut edge and gently rolled back from the yolk. The blastoderm is then freed from the membrane by gently working a dissecting needle around its border (a fairly blunt needle is best and can be used to push or roll the blastoderm away from the membrane). It is then transferred in the wide-mouthed pipette to a Petri dish containing about 20 cc. of Ringer solution. Under a dissecting microscope, most or all of the yolky opaque area is trimmed off with the dissecting needles. When finer operations are performed on the embryo, it is important to use freshly sharpened needles.

Culture procedure. The embryo or parts of it to be cultivated are transferred in a wide-mouthed pipette to the surface of the culture medium, oriented as desired, and flattened out by gently sucking away the excess saline with a fine pipette. The blastoderm may be marked with carbon powder if desired (*2*). The Petri dish is covered and placed in the incubator.

With minor modifications,[2] the basic procedure outlined above is being used in the study of some of the problems of early embryonic nutrition, morphogenesis, localization of organ-forming areas, etc. The results of some of these studies will be reported in the near future.

References

1. FELL, H. B., and ROBISON, R. *Biochem. J.*, 1929, **23**, 767–784.
2. SPRATT, N. T. *J. exp. Zool.*, 1947, **104**, 69–100.

[1] Incubation at ca. 38° C. for 20–25 hours will furnish definitive primitive streak, head-process, head-fold, and early somite blastoderms. A simple incubator made out of a desk lamp and a cardboard carton can be used for the eggs as well as the cultures.

[2] For example, when glucose (100–800 mg. per cent, added to the Ringer-agar solution) is substituted for the albumen, some interesting effects on development can be observed.