A Simplified Procedure for Breaking Diapause in Grasshopper Eggs

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In a recent article (*1*) a method for breaking diapause in eggs of the grasshopper, *Melanoplus differentialis,* was described. The greater part of the chorion was removed with sodium hypochlorite from eggs which had been in diapause for several weeks. After careful washing and drying, the eggs were then treated for ½ hr with xylol. A simpler method for accomplishing the same purpose, and one in which no sodium hypochlorite is used, has now been devised. As would be expected, there is less spoilage than with the earlier method, for the eggs are exposed to a single toxic material instead of to two.

In this modified procedure the eggs,[1] with the chorion intact, are dried thoroughly and placed in a dish of xylol. Under a dissecting microscope each egg is then grasped lightly near its middle between the tips of a pair of forceps, and pressure just sufficient to crack the chorion is applied. Scarcely more than a touch is required to do this, for the chorion—providing the eggs have been dried properly beforehand—becomes extremely brittle in xylol. Following this, each egg, if grasped gently near one end, will usually slip out of its chorion. If this does not occur readily, the crack in the chorion may be enlarged with a needle. The actual procedure is easier to carry out than to describe.

After its removal from the chorion, each egg, still encased in its tough, resistant, and transparent chitinous cuticle, is transferred to another dish of xylol and allowed to remain there for 30 min. Recent experiments indicate that a shorter exposure may be sufficient, but one of 30 min does no apparent harm. Next, the eggs are removed from the xylol and dried rapidly on several changes of filter paper. They are then placed in a Petri dish on another piece of filter paper which has been soaked with distilled water.[2] During the first 24 hrs or so of incubation at 25° C, enough fluid is left in the dish so that the hydropyle of each egg is just submerged. Most of the liquid is then drained off and the filter paper kept only slightly dampened during the rest of the incubation period. If too little moisture is supplied, the eggs will not start to develop, and if too much is given, they will burst.

During the first day following treatment with xylol the eggs absorb water and increase markedly in size. At the same time the embryos, which during diapause have a dense, opaque, whitish appearance, enlarge and become noticeably more transparent. This is due, no doubt, to the uptake by their cells of a portion of the water which has just entered the egg. Soon the lateral body walls of the embryos begin to show rhythmic contractions, and on the third or fourth day the first eggs undergo blastokinesis. Hatching begins from 15 to 17 days later. If the amount of water surrounding the eggs has been properly controlled, all or a very high percentage of the eggs may be expected to hatch.

[1] Each egg is first examined in water and with a dissecting microscope to be certain that it is in good condition and that it contains an embryo with partially pigmented eyes.

[2] If molds cause trouble, 0.15% Moldex solution may be used in place of water.

Reference

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A Rapid Method for Celloidin Impregnation of Undecalcified Bone[1]

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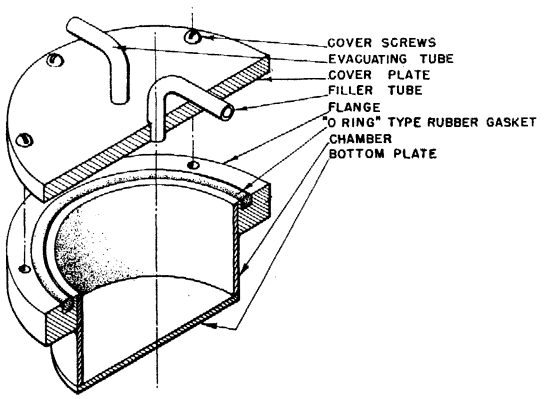
While working with Au^{198}, which has a half-life of 2.7 days, great difficulty was experienced in obtaining autoradiographs of bone because of its relatively rapid rate of decay. Routine celloidin embedding of bone (undecalcified) requires 7–10 days, which made it impractical to obtain autoradiographs if tracer conditions were to be maintained. For these reasons the following method was developed.

The bone specimen was placed in the embedding dish and enclosed in the apparatus as pictured (Fig. 1). A



BONE SPECIMEN IMPREGNATING BOMB

FIG. 1

vacuum of 5 mm Hg was obtained with a Hivac air pump and continued for 60 min. Celloidin was then introduced by means of the negative pressure, flowing through the central inlet and into the dish. Pressure was then applied (15 lbs.) overnight.

By this method satisfactory embedding was secured and excellent undecalcified 4-μ sections of bone were made.

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