

## APPENDIX

### *METHODS OF PRESERVATION, ETC.*

FOR general purposes the eggs and embryos may be preserved in a saturated solution of picric acid in seventy per cent. alcohol to which a little sulphuric acid has been added (as in Kleinenberg's picro-sulphuric solution). The segmenting eggs or the early stages of the embryo surrounded by the jelly should be put directly into the fluid. Each egg should have, however, the outer jelly-coats cut off with a pair of scissors, and it is well to use an abundance of the preserving solution. Older embryos may be shelled out in the preserving fluid with *sharp* needles. After from three to five hours the eggs or embryos are transferred to seventy per cent. alcohol, which is changed several times; they should be kept for several days in eighty per cent. alcohol. In this alcohol (eighty per cent.) the inner egg-membrane slowly separates from the egg, and can be easily removed, after which the egg is preserved permanently in eighty-five per cent. to ninety per cent. alcohol. Corrosive-acetic solution gives good results with older embryos. For the early stages of fertilization and of extrusion of the polar bodies the following solution is to be recommended: one per cent. chromic acid, twenty-five parts; water, seventy parts; glacial acetic acid, five parts. Boiling water also gives good results.

Difficulty is often found in cutting the eggs on account of the brittleness of the yolk-portion; but if the following method is carefully followed, there will be no trouble in this regard. The preserved egg or embryo is put into absolute alcohol from two to five hours, turpentine two to three hours, soft paraffine a half-hour (change once), *hard paraffine* a half-hour. The melting-point of the hard paraffine should be from 56

to 58 degrees C. The egg must then be cut at a *temperature of seventy-five to eighty degrees Fahrenheit* (24 to 26 degrees C.); one often succeeds best if the microtome is placed in the sunlight during the cutting.

The segmentation-stages do not need to be stained. The older embryos stain well *in toto* in borax carmine or in hæmatoxylin on the slide. Fresh material cuts and stains better than that long preserved.

Formalin preserves eggs and jelly most admirably for demonstration. The segmentation-stages show particularly well when preserved (permanently) in this solution.