governed ecologically by factors such as temperature, rainfall, and the chemical constituents of the environment. In some instances drying of the eggs appears necessary.

It may be of interest to note that in the collections of the University of Michigan Museum of Zoology there are some specimens of *Branchinecta coloradensis* taken from a hollow in a boulder. These were obtained on Sept. 14, 1916, at Estes Park, Colorado, at an elevation of 8,000 feet.

It is quite apparent that the phyllopods are zoogeographically unruly. Ecologically they are profoundly interesting, and they certainly merit, as Mr. C. H. Behre, Jr., says, a detailed study of their life history.

EDWIN P. CREASER
UNIVERSITY OF MICHIGAN

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A TECHNIQUE FOR FIXING AND REMOVING CHICK BLASTODISCS AND EMBRYOS FROM THE EGG SHELL

FOR some time I have been looking for a simple, rapid and effective method for removing blastodiscs and embryos from the egg shell which might be used both by the technician in making up quantities of whole mounts or serial sections, and one which can be readily acquired by students in the classroom who have had very little or no training in handling such delicate objects. The method outlined by Dr. M. F. Guyer in his "Animal Micrology" (p. 127) is very good, but in order to use it the worker must have had considerable experience. Because of this I have found it impracticable to use in the classroom when both time and material are limited.

The technique outlined by Dr. C. E. McClung in his "Handbook of Microscopic Technique" (p. 213) is the best I have so far been able to find, but in using the formal-nitric fixative recommended (three parts ten per cent. formalin to one part ten per cent nitric acid) I experienced difficulty in removing the vitelline membrane, and also in washing the blastodisc free from yolk. The length of time required by the McClung method for the fixative to act sufficiently to permit the removal of the blastodiscs or embryo was from fifteen to twenty minutes. During the past year and a half I have prepared several hundred whole mounts and serial sections of blastodiscs and embryos. By modifying the formal-nitric fixative suggested by Dr. McClung from three parts of 10 per cent. formalin and one part 10 per cent. nitric acid to four parts of 10 per cent. formalin and one part of 20 per cent. nitric acid, I have found that I can remove as many as sixty blastodiscs or embryos up to ninety-six hours' incubation within an hour and a half with the aid of an assistant, whereas the original method as outlined by Dr. McClung required from four to five hours to prepare the same number. Comparative studies of tissue fixed by the two different concentrations of the fixative did not reveal any differences in their effect upon cell structure.

The following is a summary of the procedure I followed:

(1) Incubate the egg to the desired stage.

(2) Remove the egg from the incubator to a finger-bowl filled with normal salt solution. (Seven to nine grams NaCl per liter of distilled water.)

(3) Using a pair of small forceps and curved scissors, remove the upper part of the egg shell to expose the blastodisc or embryo.

(4) Flood the blastodisc with several drops of the formal-nitric fixative. Remove the coagulated albumen and apply more of the fixative. Repeat this operation until the blastodisc is free from albumen. When all of the albumen is removed flood the blastodisc or embryo with the fixative and permit it to act for from one half to two minutes.

(5) Cut around blastodisc with curved scissors.

(6) By inserting a section lifter into the yolk under the blastodisc, lift the latter and transfer it to a Syracuse watch-glass filled with normal saline. Agitate the salt solution until the blastodisc is washed free from the yolk, and then transfer it to a watch-glass of fresh salt solution.

(7) Remove the vitelline membrane by grasping its free edge with forceps and move the membrane gently back and forth until it becomes free.

(8) Transfer the blastodisc, washed free from yolk and vitelline membrane, to a dish of Worchester's fluid.[1] (Nine parts ten per cent. formalin saturated with mercuric chloride to eleven parts glacial acetic acid.) It is desirable to place about a quarter of an inch of the fixative in a rather large flat-bottomed dish in order that the embryonic tissue being fixed will lay flat.

(9) Place the tissue in seventy per cent. alcohol containing iodine colored to that of port wine to remove the corrosive sublimate. (Three to ten hours.)

(10) Preserve in fresh seventy per cent. alcohol until ready for use.

MARK M. KEITH
DEPARTMENT OF ZOOLOGY,
UNIVERSITY OF MINNESOTA

[1] Fixatives other than Worchester's may be used if desired, but for total mount work Worchester's is preferable since it has a tendency to leave the tissue tough rather than brittle as do many fixatives.