Proceedings of the Iowa Academy of Science

Volume 18 | Annual Issue Article 30

1911

# Notes on Methods for the Study of Amphibian Eggs and Larvae

Albert Kuntz

# NOTES ON METHODS FOR THE STUDY OF AMPHIBIAN EGGS AND LARVAE.[1]

BY ALBERT KUNTZ.

In the prepration of amphibian eggs and larvae for histological study, the technique employed is a factor of primary importance. The amphibian egg represents the final attempt on the part of nature to produce a large yolk-laden egg in which cleavage involves the entire ovum. As a result of this method of cleavage all the embroynic tissues, during the early stages of development, contain more or less yolk. Furthermore, the ventral region of the young embryo contains a large, compact yolk-mass which is not readily penetrated by the fixing and the clearing agent, and the embryonic tissues show a greater tendency to shrink than do the tissues of other vertebrate embryos.

In the preparation of this material it is essential to select a fixing agent which will penetrate the yolk readily, but which will not cause the tissues to shrink. In the process of dehydration, high per cent alcohols must not be employed longer than is absolutely necessary. A clearing agent must be selected which will clear the yolk, but which will not render the tissues unduly brittle. In the process of imbedding, thorough impregnation must be secured without subjecting the tissues for too long a time to high temperature.

In the course of an investigation of the development of the sympathetic nervous system in the Ambhibia,[2] the writer has had the opportunity to compare the results obtained from various methods of technique. Ambhibian eggs and embryos fixed and dehydrated in the usual manner show a great tendency to shrink. As a clearing agent, xylol is unsatisfactory because it does not clear the yolk readily and renders the tissues unduly brittle. The method which was found to yield the most satisfactory preparations of amphibian embryos for general microscopic study is a modification of the method described

[1]From the Laboratories of Animal Biology of the State University of Iowa.

[2]The development of the sympathetic nervous system in the Amphibia. Jour. Comp. Neur., vol 21, no. 4. pp. 397-416.

by Carnoy and Lebrun.[3] This method will be described somewhat in detail.

Ova and embryos which are still enclosed in their albumenous envelopes are fixed in Gilson's mercuro-nitric fluid[4] 1.5 hrs. or longer. The albumen is now sufficiently hardened that it can be incised to permit the egg or embryo to be squeezed out by gently applying pressure at the opposite pole. This operation must be made with care, as any distortion of the egg or embryo after fixation may separate or break the germ-layers; consequently, perfect sections cannot be obtained. Embryos which are free from their albumenous envelopes are fixed in Gilson's fluid 45 min. to 1 hr. If large enough to swim freely, they may to advantage be placed in water to which a few drops of chloroform have been added until they become quiescent, before being placed in the fixing fluid. This treatment prevents tearing of the delicate tissues along the neural tube and the notochord by muscular exertion after they have been partly penetrated by the fixing agent.

After fixation the embryos are washed thoroughly in water and dehydrated in the usual manner, care being taken not to leave them in 95% alcohol longer than 15 min. or in absolute alcohol longer than 5 or 10 min. From absolute alcohol they are brought into a mixture of absolute alcohol and chloroform in equal parts. After the embryos sink in this mixture they are brought into pure chloroform for an hour or longer. Paraffin is now added to about double the volume and the whole is placed in a thermostat at about 35° C. for 3 hrs. The embryos are now placed in pure paraffin in the paraffin bath for 15 to 30 min. After imbedding sections may be cut to any desired thickness and stained by any of the common methods.

Amphibian eggs or embryos successfully carried through the processes above outlined afford preparations which stain well and show no evidence of shrinking.

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[3]Lee. Microtomist's Vade-Mecum (sixth edition), p. 331.

[4]Gilsos's fluid: Nitric acid, 15 c. c.; Glacial acetic acid, 4 c. c.; Corrosive sublimate, 20 grs.; 60% alcohol, 100 c. c.; Distilled water, 880 c. c.