

A MANUAL OF EXPERIMENTAL EMBRYOLOGY

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THE UNIVERSITY OF CHICAGO PRESS
CHICAGO · ILLINOIS

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THE UNIVERSITY OF CHICAGO PRESS
CHICAGO 37, ILLINOIS

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Agent: THE CAMBRIDGE UNIVERSITY PRESS
LONDON

351 18
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SECOND IMPRESSION 1947

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COMPOSED AND PRINTED BY THE UNIVERSITY OF CHICAGO PRESS
CHICAGO, ILLINOIS, U.S.A.

INTRODUCTION

Experimental embryology has achieved a prominent position in modern biology; its classical experiments and concepts are, by now, an integral part of biological thinking. Yet few students of biology have an opportunity to obtain a firsthand acquaintance with its methods and materials. This is due partly to the difficulties of providing the embryonic material and partly to the technical difficulties of experimentation on living embryos. However, a large number of classical experiments do not require exceptional manual skill and are suitable as "classroom" experiments. Most of the experiments described in the following pages have been made by advanced undergraduate and graduate students in a one-semester course which has been offered at Washington University since 1935. One of the main assets of such a course is to bring the student into intimate contact with the living, developing organism; and the enthusiastic response of the students indicates clearly the demand for such an approach to biology.

This *Manual* emphasizes the intrinsic factors of morphogenesis, that is, origin of form and of organs. It includes regeneration but gives little consideration to histogenesis and growth. In the selection of the experiments we were guided by practical considerations. Only those elementary experiments were chosen which do not require a high degree of manual skill¹ and which can be done in the limited time of a three-hour laboratory period. We excluded all experiments which require expensive apparatus, such as a micromanipulator. Special attention was paid to the development of simple and inexpensive instruments for operations. Only such living material as can be collected in the field or purchased at relatively small expense is recommended. All experiments were devised in such a way that sectioning of the material is not essential for the study of the results. For instance, regenerated lenses in amphibian larvae can be studied by making them opaque and thus visible by fixation in formaldehyde; chorio-allantoic grafts of limbs can be cleared and stained *in toto* with methylene blue; etc.

Another consideration which determined the selection of the experiments was their analytical value, that is, their expediency in illustrating

¹ In the Appendix the experiments are arranged in groups according to the technical difficulties involved.

important principles of morphogenesis. The theoretical significance of the experiments has been strongly emphasized. I believe that a course in an experimental branch of biology not only should acquaint the student with new facts but should strengthen his power of reasoning and his logical acuity as well. He should be aware of what an experiment proves and of what it does not prove. Each experiment or group of experiments is preceded by a brief outline of its theoretical implications. These general remarks integrate the different problems handled in the *Manual*, but they are not to be considered as a substitute for lectures or for textbooks. On the contrary, it is hoped that they will stimulate collateral reading. The bibliographies serve the same purpose. They are not complete but give references only to those articles which are directly related to the experiment under consideration and to pertinent review articles. A special chapter on gastrulation in amphibians was added, because familiarity with this phase of development is indispensable for experimental work on early embryos.

The experiments are organized according to a logical plan. This is not necessarily the sequence in which they should be taken up in the laboratory. The planning of the course work will depend largely on the availability of living material. To facilitate a flexible schedule each experiment has been treated as a separate unit. The technical procedures are described for each experiment separately; they are not based on previous experience with other experiments. It is immaterial whether one starts with amphibians, with regeneration in planarians, or with chick experiments. The selection and arrangement of the exercises is left to the discretion of the instructor. A tentative schedule for a one-semester course will be found in the Appendix.

The instructions for technical manipulations are given in great detail. I hope that they will be useful not only for students but also for research workers in biology, experimental medicine, and related fields, who may find one or another of the techniques applicable to their own problems. The technical procedures have worked satisfactorily in our course, which does not mean that they cannot be further improved. I hope that the students will feel encouraged to develop their own initiative and resourcefulness in trying out new experiments and in improving the techniques and instruments.

The highly specialized technique of tissue culture has been omitted. The role of endocrines in morphogenesis is adequately presented in A. E. Adams' *Studies in Experimental Zoölogy* (1941), which contains all information necessary for experimentation in this field. Experiments on marine-animal eggs are dealt with in Just (1939).

I am indebted to many friends and colleagues. Many techniques described in the chapter on amphibians have been worked out in the laboratory of Dr. H. Spemann (Freiburg, Germany), with whom I was associated for many years. Dr. B. H. Willier and Dr. Mary Rawles generously made available their experiences with operations on the chick embryo. I am much obliged to Drs. L. G. Barth, G. Frankhauser, T. S. Hall, J. A. Moore, and C. Parmenter for personal communication of technical procedures. Grateful acknowledgment is made to all students and assistants who helped materially to improve the techniques and to revise the outlines. Dr. K. Gayer kindly read the manuscript and made many helpful suggestions.

I am grateful to Dr. R. G. Harrison for his kind permission to publish sketches of his unpublished stage series of *Ambystoma maculatum*. These sketches, as well as all other original drawings, were made by Miss S. E. Schweich. Acknowledgment is made to the Wistar Institute, Edwards Brothers, Incorporated, Akademische Verlags-Gesellschaft, and Springer's Verlag for permission to reproduce illustrations.

TEXTBOOKS OF EXPERIMENTAL EMBRYOLOGY

- BERTALANFFY, L. VON, and WOODGER, J. H. 1933. Modern theories of development. London: Oxford University Press.
- BRACHET, J. 1945. Embryologie chimique. Paris: Masson et Cie.
- CHILD, C. M. 1941. Patterns and problems of development. Chicago: University of Chicago Press.
- DALCO, A. M. 1938. Form and causality in early development. Cambridge, England: Cambridge University Press.
- DÜRKEN, B. 1932. Experimental analysis of development. Trans. New York: W. W. Norton & Co.
- HUXLEY, J. S., and DE BEER, G. R. 1934. The elements of experimental embryology. Cambridge, England: Cambridge University Press.
- KORSCHULT, E. 1927-31. Regeneration und Transplantation. 3 vols. Berlin: Borntraeger.
- MANGOLD, O. 1928. Das Determinationsproblem. I. *Ergebn. d. Biol.* Vol. 3.
- . 1929. Das Determinationsproblem. II. *Ibid.*, Vol. 5.
- . 1931. Das Determinationsproblem. III. *Ibid.*, Vol. 7.
- MORGAN, T. H. 1927. Experimental embryology. New York: Columbia University Press.
- NEDHAM, J. 1931. Chemical embryology. 3 vols. Cambridge, England: Cambridge University Press.
- . 1942. Biochemistry and morphogenesis. Cambridge, England: Cambridge University Press.
- SCHLEIP, W. 1929. Die Determination der Primitiventwicklung. Leipzig: Akademische Verlags-Gesellschaft.
- SPEMANN, H. 1938. Embryonic development and induction. New Haven: Yale University Press.
- WADDINGTON, C. H. 1936. How animals develop. New York: W. W. Norton & Co.
- . 1940. Organizers and genes. Cambridge, England: Cambridge University Press.
- WEISS, P. 1939. Principles of development. New York: Henry Holt.

LABORATORY MANUALS OF EXPERIMENTAL EMBRYOLOGY

- ADAMS, A. E. 1941. Studies in experimental zoölogy. 2d ed. Ann Arbor, Mich.: Edwards Bros., Inc.
- JUST, E. E. 1939. Basic methods for experiments on eggs of marine animals. Philadelphia: Blakiston.
- RUGH, R. 1941. Experimental embryology (a manual of techniques and procedures). New York: New York University Bookstore.

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PART I
EQUIPMENT AND INSTRUMENTS