REGENERATION IN VERTEBRATES

Edited by

CHARLES S. THORNTON

A Report from THE DEVELOPMENTAL BIOLOGY CONFERENCE SERIES, 1956

PAUL WEISS, *Organizer and General Chairman*

Held under the auspices of

THE NATIONAL ACADEMY OF SCIENCES—NATIONAL RESEARCH COUNCIL

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THE UNIVERSITY OF CHICAGO PRESS

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The Library of Congress Catalog Card

SYMPOSIUM ON REGENERATION IN VERTEBRATES, *Brown University, 1956.*

Regeneration in vertebrates, edited by Charles S. Thornton. [Chicago] University of Chicago Press [1959]

xi, 107 p. illus. 24 cm. (The Developmental biology conference series, 1956)

Condensed version of the proceedings of the conference.
Bibliography: p. 99–107.

1. Regeneration (Biology) 2. Vertebrates. I. Thornton, Charles Stead, 1910– ed. II. Title. (Series)

QH499.S94 1956 596 59–8869

Library of Congress

THE UNIVERSITY OF CHICAGO PRESS, CHICAGO 37
Cambridge University Press, London, N.W. 1, England
The University of Toronto Press, Toronto 5, Canada

© 1959 *by The University of Chicago. Published 1959*
Composed and printed by THE UNIVERSITY OF CHICAGO PRESS
Chicago, Illinois, U.S.A.

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The Developmental Biology Conference Series, 1956

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Paul Weiss
ORGANIZER AND GENERAL CHAIRMAN

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Preface to the Series

Development and growth have usually been studied rather piecemeal: as embryology, or plant physiology, or nutrition, or oncology; as seriation of stages of chick embryos, as cell division in fish eggs or plant root tips, as growth curves of children, as hormone response of plumage, as spread of a fungus, as repair of a broken bone or the swelling of a diseased spleen; by observation, measurement, comparison, chemical alteration, excision, transplantation, or sheer speculation. Yet, in reality, all these are merely isolated aspects of one broad continuous spectrum of phenomena, varied manifestations of the same basic principles and elementary processes—multiplication of organic mass (growth); diversification of that mass (differentiation); pattern formation (morphogenesis); progressive change (maturation and aging); and the repair or reproduction of patterns after disturbance (regulation and regeneration).

This unity of subject matter has received renewed emphasis in the "Developmental Biology Conference Series of 1956," a record of which is now presented in ten volumes, including the present report. The series consisted of co-ordinated and interdisciplinary conferences, symposia, and workshops, organized under the sponsorship of the Biology Council of the Division of Biology and Agriculture, National Academy of Sciences–National Research Council, with the generous financial support of governmental agencies, industrial organizations, and private foundations and donors (see list at end of Preface).

The Conference Series brought together experts from the fields of anatomy, biochemistry, biometry, biophysics, botany, cytology, embryology, endocrinology, genetics, histology, immunology, microbiology, neurology, nutrition, oncology, pathology, physiology, radiology, and zoölogy, from the United States and abroad, less for a display of most recent technical advances than for a concerted examination and evaluation of the contributions of these various specialties to the elucidation of focal issues of developmental biology. Fresh orientation and new ideas could be expected to emerge from this pool of critically distilled knowledge by the intersection of formerly unrelated trends of thought or by the discovery of

common cores in formerly unrelated sets of data. Nearly three hundred American and fifty-four foreign scientists (from nineteen countries) joined in this task.

To serve the outlined objective, the meetings had to be ruled by the key words: *perspective* and *relevance*. All participants were admonished to present only such itemized information, conclusions, demonstrations, criticisms, illustrations, questions, and quotations as promised to throw light on the *issue* under discussion; that is, to confine themselves to comments of "strategic" or "catalytic" pertinence, not merely adding to the bulk of information, but contributing to clarification, order, harmonization, and comprehensibility. Pertinent comments, however, were welcomed regardless of whether they referred to data so new as to be still largely unknown; so old as to have been widely forgotten; so specialized or technical as to have received limited currency among "outsiders"; so theoretical as to have escaped the practitioners; or so "self-evident" as to have evaded critical scrutiny. Each participant was expected to draw from his store of special knowledge points that might help correct misinterpretations, indicate the feasibility of new approaches, and, above all, reveal existing gaps of knowledge and understanding.

It is evident that a group exercise of this complexion, with free give-and-take, could not possibly "cover the ground" in any of the selected topics within the given time limits and without sacrificing spontaneity, informality, and depth of penetration. Often a few key issues, profoundly analyzed and critically elucidated, proved far more enlightening than a hurried bird's-eye view of a large field. In cases in which workers from different disciplines were only vaguely acquainted with one another's stock-in-trade and vocabulary, the time and effort spent describing even elementary facts in order to provide a common ground for communication proved very worthwhile indeed. In other instances, where the facts were familiar but their interpretation was controversial, it seemed preferable to let argumentation take precedence over the recital of facts.

The foregoing remarks are intended to explain the discursive nature of these volumes. In conferences which combine basic and clinical interests, technical and theoretical approaches, molecular and organismic concepts, botanical and zoölogical subjects, biochemical and morphological aspects, it is imperative to place the reconciliation and synthesis of viewpoints above all other considerations.

In line with this general precept, each conference chairman was to open his meeting with a brief keynote address, staking out the major

problems for discussion. By phrasing questions, rather than stating theses, he was to set the stage for free, though not necessarily unpremeditated, participation. Most of the conferences were closed meetings, with attendance confined to the invited panel members. In a few cases, auditors were admitted. Only the symposia at Brown University, which were cosponsored by the International Union of Biological Sciences, were open to the general public.

For the purpose of publication, an experienced scientist familiar with the subject matter was appointed as official reporter and editor for each conference, to attend all sessions without taking part in the discussions. From the sound-tape recordings of the proceedings and his or her own notes, each editor then produced a condensed version of the conference. These accounts constitute the substance of this series of publications.

The individual reports vary greatly in form, depending on the topic and organization of each conference, as well as on the personal predilections of the editor. Only in one instance has the dialogue style been kept, and even so, only after considerable pruning. In other cases, an entire conference has been reported as a third-person account, reordering the text rather liberally into a logical sequence by combining related fragments; in this process of synthesis, an editor assumed the full prerogatives of an author. Most of the reports, however, range somewhere between these two extremes, abstracting the major comments of the various participants without obliterating their identity, yet resorting to verbal quotations infrequently or not at all. Some participants furnished their own rewritten versions of the factual presentations, and these were in most cases inserted in the text as such. In all cases, the participants were given an opportunity to check their respective contributions, either in the original transcript or later in the condensed and revised text.

The lack of uniformity reflects the informal spirit of the meetings and accents the main theme of the Conference Series: that developmental biology is currently in a state of flux, fitting no rigid mold and shaping its own course as it gains momentum by the growth and confluence of its many tributaries. It is hoped that the publication of this series will add to that momentum, as did the conferences themselves.

To each of the participants and, above all, to the chairmen and editors we owe a deep debt of gratitude. To the following donors of funds, we reiterate our appreciation for generous assistance: Atomic Energy Commission, U.S. Departments of the Air Force, Army, and Navy (Medical Services); Office of Naval Research; Fulbright Fellowship Program; Na-

tional Institutes of Health; National Science Foundation; International Union of Biological Sciences; American Cyanamid Company; Diamond Alkali Company; Merck and Company, Inc.; Chas. Pfizer and Company, Inc.; Rohm and Haas Company; E. R. Squibb and Sons; American Cancer Society; and Rockefeller Foundation. Special thanks are due to Dr. Russell B. Stevens, executive secretary of the Biology Council, for carrying the major load in the recording of the conferences; and to Mrs. Geraldine A. Norton, administrative assistant, for her effective help with the preparations and practical details of the meetings.

PAUL WEISS

NEW YORK CITY
May 1958

SYMPOSIUM ON

Regeneration in Vertebrates

HELD JULY 23–24, 1956, AT BROWN UNIVERSITY
Providence, Rhode Island

Arranged by E. G. BUTLER
Princeton University,
Princeton, New Jersey

Presiding chairmen:

B. I. BALINSKY
University of the Witwatersrand, Union of South Africa

F. E. LEHMANN
University of Bern, Switzerland

Reporter and editor CHARLES S. THORNTON
Kenyon College, Gambier, Ohio

Participants:

D. T. CHALKLEY, *University of Notre Dame, Notre Dame, Indiana* (Present address: *National Cancer Institute, Bethesda, Maryland*)

HOWARD HOLTZER, *University of Pennsylvania, Philadelphia, Pennsylvania*

O. E. SCHOTTÉ, *Amherst College, Amherst, Massachusetts*

MARCUS SINGER, *Cornell University, Ithaca, New York*

L. S. STONE, *Yale University, New Haven, Connecticut*

H. A. L. TRAMPUSCH, *University of Amsterdam, Holland*

Discussion leaders:

E. G. BUTLER, *Princeton University, Princeton, New Jersey*

J. J. KOLLROS, *State University of Iowa, Iowa City, Iowa*

R. W. REYER, *University of Pittsburgh, Pittsburgh, Pennsylvania*

A. STEFANELLI, *University of Rome, Rome, Italy*

C. S. THORNTON, *Kenyon College, Gambier, Ohio*

PAUL WEISS, *Rockefeller Institute, New York, New York*

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Introduction

E. G. BUTLER
Providence, Rhode Island

During the last decade there has been a greatly renewed interest in one of the oldest subjects in experimental zoölogy—regeneration in vertebrates. An increasing number of workers has entered the field, new materials and techniques have been employed, and, I think it is fair to say, no other period has witnessed greater advances. However, many of the basic factors underlying regenerative activity continue to remain obscure. It seems highly desirable that we now make an assessment of some of the recent advances in our understanding of the problems concerned, to see where we stand and to chart further programs of study.

Clearly, in a symposium occupying only two half-days it will be impossible to cover the field in a truly comprehensive manner. It is necessary—and undoubtedly it is desirable—to concentrate on those structures which have been most extensively subjected to experimental analysis and to give attention to those methods of approach which have proved most fruitful. This is the basis on which this symposium has been organized.

Anyone working in the field of vertebrate regeneration, regardless of the structure or structures with which he deals, soon discovers that he must not only take into account the local features of regenerative activity but likewise give attention to the organismic influences that are involved. These two aspects of regeneration have long been recognized as inseparable, and together they constitute, with respect to any structure, what may properly be referred to as a "regenerating system." For purposes of investigation, however, it is at times necessary to concentrate first on one part of the system and then on the other. Likewise, in this symposium we shall deal more specifically in the first session with the cellular basis of regenerative activity, with respect particularly to the eye, the tail, and the limb. We shall then progress in the second session to consider in some detail such major organismic influences as those exerted by the nerves and by the various hormones of the body. Finally, we shall take into account a technique which has been widely used in

recent years, that of irradiation—a technique which has proved to be a valuable tool, especially in furthering our understanding of the relationships between the local and organismic aspects of the "regenerating system."

I wish to thank the main speakers who are taking part in this symposium and also those who are serving as discussion leaders. Particularly, I wish to express the pleasure we all feel in having as participants in the symposium so many of our European colleagues whose work we know so well and regard so highly.

· I ·

Regeneration of the Retina, Iris, and Lens[1]

L. S. STONE

Yale University School of Medicine

For many years I have used the eye of the vermilion spotted newt, *Triturus viridescens,* as a tool for studying the organization and development of the visual mechanism and for uncovering some of the fundamental factors governing regeneration. For the brief time at my disposal I shall confine my discussion largely to the findings of my own experiments, both published and unpublished, dealing with the eye of this adult newt. My attention will be centered upon the regeneration of the iris, lens, and neural retina, for, with this salamander, experiments can be devised to show that all three structures will regenerate from the same source, namely, the retina pigment epithelium. These cells are unique as an outstanding example of a specialized cell which can be followed from stage to stage as it changes its morphology and function to give rise to several totally different structures. I have often referred to this in previous publications. For the most complete and thorough survey of the regeneration of the lens I refer you to the recently published excellent review by Reyer (1954).

REGENERATION OF NEURAL RETINA FROM RETINA PIGMENT CELLS

Regeneration of the neural retina from retina pigment epithelium can be demonstrated very easily in the amphibians, especially in the urodeles (Stone, 1947, 1950*a, b, c*). It also has been shown to occur in anurans by a graduate student of mine, Dr. Katherine van Aken Smith in 1950 (unpublished) and recently by Sato (1953) in transplants of retina pigment cells in tadpole eyes of *Rana temporaria.*

Long ago—in fact, in 1924—I first observed the degeneration of the neural retina in transplanted eyes of the adult newt within 3 weeks after

[1] These investigations were aided by research grants (B-23 C7) from the National Institute of Neurological Diseases and Blindness of the National Institutes of Health, U.S. Public Health Service, and the Medical Fluid Research Fund of the Yale University School of Medicine.

operation. This was followed by regeneration of a new neural retina from the surviving retina pigment cells. Vision returned as soon as a newly regenerated optic nerve reached the brain. Since normal or abnormal vision depended upon the orientation of the graft (Stone, 1944, 1947, 1950*a*), the adult salamander eye proved to be an important tool for studying the functional organization in the retina, a subject which I shall not have time to include in this discussion.

We can also observe very clearly the regeneration of the neural retina from retina pigment cells by first removing the entire intact neural retina through a large opening in the dorsal wall of the eye (Stone, 1950*b*, Fig. 1) and then following the early changes in the denuded retina pigment cells as they proliferate non-pigmented cells that eventually differentiate into a functioning retina. Also fully differentiated neural retina membranes will develop from retina pigment epithelium implanted into the eye chamber (Stone, 1950*b*) or into the body cavity (unpublished). Later we shall point out in this discussion that the retina pigment cells can still give rise to entirely different functioning structures under appropriate conditions.

LENS REGENERATION FROM THE DORSAL IRIS EPITHELIUM

But before we discuss the role of the retina pigment cells further, I should like to review the formation of lenses from the iris epithelium. Replacement of a lost lens by a budding process of the epithelium along the free margin of the dorsal iris in *Triturus* newts has been well known since it was first reported by Colucci in 1891. I have studied this phenomenon in salamanders extensively and have pointed out that it does not occur in many species of amphibians and that it has not been shown in other vertebrates (Stone and Sapir, 1940). Much confusion has arisen in the literature because all lens material has not been removed by some investigators at the time of the experiment. This has been due partly to the fact that the entire lens cannot be excised with the same ease in all species.

Recently we recorded an extensive study of the normal variations in the early stages of lens regeneration from the dorsal iris in the eye of the adult newt after simple lentectomy. Several hundred cases were preserved at close intervals during the first 2 months after operation, and many *toto* mounts of dissections were made and illustrated along with the histologic picture (Stone and Steinitz, 1953*a*, Figs. 3–22, *A*). They not only epitomize the chief features of lens regeneration in certain newt eyes from the early depigmentation of the dorsal iris to the detachment of the lens regenerates at the end of a month but also call attention to

the occasional variations in the rate of regeneration and the amount of iris tissue contributing to the new lens.

Grafts taken from the mid-dorsal iris, including the pupillary margin, show the highest potential for lens regeneration. As one approaches 9 and 3 o'clock, lens regeneration in the iris graft approaches zero (Stone, 1952, Figs. 2 and 3). The entire ventral half of the iris possesses no lens-forming cells.

In studying lens regenerates from grafts, it is essential to know that slight wounds along the pupillary margin of the dorsal iris usually do not affect regeneration (Stone, 1954*a*, Fig. 1). However, if a wound is not perfectly healed before early lens regeneration begins, a partially double lens may result (Stone, 1954*a*, Fig. 5, *A*). On the other hand, one must keep in mind that fresh wounds along the pupillary margin of a mid-dorsal iris graft at the time of operation may seriously affect lens regeneration from the transplant. This can be illustrated in grafts of mid-dorsal iris tissue from which the pupillary margin has been removed. When transplanted immediately to lensless host eyes, small defective lenses develop from the grafts (Stone, 1954*a*, Fig. 2). If the pupillary margin is allowed to heal 4 days before transplantation to a lentectomized host eye, lens regeneration from the mid-dorsal iris graft is well defined and almost as rapid as the one from the host dorsal iris.

FORMATION OF MULTIPLE LENSES

Normally, only one lens regenerates from the free margin of the dorsal iris after lentectomy. However, more than one lens can be induced to develop by simply substituting a segment of mid-dorsal iris with a piece of non-lens-forming ventral iris in a lentectomized eye (Stone, 1953, Fig. 5). This separates two potential lens-forming areas, each of which gives rise to a lens. Implanted plastic membranes splitting the iris into segments can produce the same effects (unpublished).

It must be borne in mind that much of the dorsal iris besides the pupillary margin also possesses lens-forming cells. This can be shown experimentally by inserting plastic membranes in the regions of the dorsal iris to produce permanent accessory pupils (Stone, 1954*b*). Along their margins lens development can be released. These lenses are gradually smaller as the accessory openings approach the ora serrata. They also arise only from the dorsal iris region in accessory pupils lying in the temporal and nasal portions of the iris. Therefore, in lens regeneration we find a lens-forming area spreading from a central point, as in the case of limb reduplication.

A number of investigators have implanted pieces of mid-dorsal iris

into lentectomized eyes, but only one lens develops from one portion of the graft, namely, the original pupillary margin (Stone, 1952). However, we know from our experiments that the other borders of the graft also possess lens-forming cells. One can, for example, force the upper ciliary region of the mid-dorsal iris graft to give rise to a lens if the pupillary margin is cut away and the graft of appropriate size is rotated 180° and implanted in an eye already prepared for it (Stone, 1954*a*, Fig. 3). The ciliary border of the transplant then becomes part of the pupillary margin in the host eye and gives rise to a lens. Even then in some cases a lens will develop from host iris on either side of the transplant.

INHIBITION OF LENS REGENERATION BY LIVING LENS TISSUE

I should point out at this time that lens regeneration is inhibited when the dorsal iris is in the presence of a normal living lens, even though the latter is derived from the eye of another species of salamander (Stone, 1953). I have also reported that the presence of a regenerating lens does not produce any inhibiting effect until its outer capsule is completely formed. At this time it is 25–30 days of age and is ready to detach from its source of origin, the dorsal iris (Stone, 1952, Fig. 6). These regenerates, though small, are as effective in inhibiting lens regeneration as is the original lens. Ordinary mechanical forces of the normal lens play no active inhibitory role, for the substitution of wax lenses, glass beads, and spherical masses of tissue for the original lens have no inhibiting effect upon lens regeneration (Stone, 1953).

The importance of the iris-lens relationship to lens regeneration is shown in the following two contrasting experiments. In the first of these an impermeable plastic membrane, Pliofilm, was implanted so that the dorsal iris was isolated in a separate chamber from that occupied by the normal lens (Stone, 1953, Fig. 8). The dorsal iris was then no longer bathed by the aqueous humor circulating around the lens. Under these conditions a lens developed from the dorsal iris just as well as in complete lentectomy. Plastic membranes of Dupont No. 600, permeable to water-soluble substances, produce the same effects (unpublished).

Related to these results are those of the second experiment, which produced complete inhibition of lens regeneration. Into lentectomized eyes aqueous humor from eyes containing normal lenses was injected daily for 20, 40, 60, and 90 days. Lens regeneration took place only after the cessation of the injection of aqueous humor (Stone and Vultee, 1949; Stone, 1953, Fig. 9). Aqueous humor from the same or different species of salamander eye is equally effective (unpublished). Daily injections of

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