Benchmark Papers
in Biochemistry V. 3

MOLECULAR PROCESSES IN VISION

Edited by
Edwin W. Abrahamson
and
Sanford Eugene Ostroy

Hutchinson Ross Publishing Company

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A BENCHMARK® Books Series

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Stroudsburg, Pennsylvania

Benchmark Papers in Biochemistry, Volume 3
Library of Congress Catalog Card Number: 80-29543
ISBN: 0-87933-372-3

83 82 81 1 2 3 4 5
Manufactured in the United States of America.

LIBRARY OF CONGRESS CATALOGING IN PUBLICATION DATA
Main entry under title:
Molecular processes in vision.
(Benchmark papers in biochemistry; 3)
Includes indexes.
1. Vision—Addresses, essays, papers. 2. Molecular biology—Addresses, essay, papers. I. Abrahamson, Edwin W. II. Ostroy, Sanford Eugene, 1939– . III. Series.
QP475.M64 599.01'823 80-29543
ISBN 0-87933-372-3

Distributed world wide by Academic Press,
a subsidiary of Harcourt Brace Jovanovich,
Publishers.

Benchmark Papers in Biochemistry

Series Editor: Herbert C. Friedmann
The University of Chicago

Volume

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SERIES EDITOR'S FOREWORD

Welcher Lebendige,
Sinnbegabte
Liebt nicht vor allen
Wundererscheinungen
Des verbreiteten Raums um ihn
Das allerfreuliche Licht—
Mit seinen Strahlen und Wogen,
Seinen Farben,
Seiner milden Allgegenwart
Im Tage.

[*Hymns to the Night*, Novalis, 1800]

The terms *light* and *vision* conjure up emotions of beauty, of living, of mystery, that recede into the earliest experiences of mankind. The biblical act of creation is associated with the command: Let there be Light. The ancient Greek notion of fire as one of the four elements encompasses the experience of light both poetically and rationally. We are especially endowed to experience light. Cicero maintained that "The sense of sight is the keenest of all our senses." *Molecular Processes in Vision* is a record of recent progress in the rational and experimental approach toward an understanding of the phenomenon of vision. The study of vision benefits from the attitudes of physicists, physiologists, anatomists, and biochemists. It is difficult to decide whether one should be more impressed by the complexity of the phenomena studied or by the ingenuity of the approaches used to discover and elucidate them. The papers gathered here and the various masterful comments by Drs. Abrahamson and Ostroy benefit not only specialists in the field of vision, but no doubt will, in addition, incite the interest of scientists in the various contributing disciplines.

HERBERT C. FRIEDMANN

PREFACE

This collection of papers was assembled primarily for the scientist who wishes to obtain an overall view of ongoing research and of basic data in the field of vision. In the selection we have tried to maintain a historical perspective and yet provide the most recent and informative papers. We have begun at the level of the single molecule, the chromophore that absorbs light, and tried to maintain a continuity of description with appropriate commentary from the molecular through the cellular to the retina level and, in one instance, to cerebral registration.

Those familiar with the field will, no doubt, regret the omission of many key, classical papers; but space limitations and the desire to present a wide and current view of the field governed our choice. We regret that more papers could not be included and have depended on the Editorial Commentaries to cite significant omitted papers and to summarize their results. No attempt has been made to provide complete reviews in each of the areas considered; but rather our aim has been to highlight the major data and ideas.

We would like to thank the many people who provided us with their data and who offered suggestions and help. In particular, we thank Corinne Abrahamson, Gene Anderson, Mark Bitensky, Dean Bok, Deborah Farber, Gordon Fain, Betty Gick, Paul Hargrave, Wayne Hubbell, Aaron Lewis, Paul Liebman, Richard Lolley, Nancy Manguini, Zippy Ostroy, David Pepperberg, and Sheila Sullivan. We give special thanks to Meegan J. Wilson whose efforts helped make this volume possible.

E. W. ABRAHAMSON
SANFORD E. OSTROY

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PART VI: ELECTROPHYSIOLOGICAL EVENTS IN THE VERTEBRATE RETINA

INTRODUCTION

The study of vision, in its totality, involves many disciplines. It is not yet possible to present a unified view of this subject in the language of a single discipline—for example, molecular biology—and, indeed, there is some question whether it will ever be possible to do so. Nevertheless, many scientists who think in molecular terms have a more or less tacit faith that it is possible; and, certainly, it is the driving force of this faith in an eventual systematic molecular description of biology, that is chiefly responsible for the tremendous strides made in this direction.

In choosing papers for this volume, we had in mind primarily those physicists, chemists, biochemists, and biologists whose principal language is molecular. Although a number of the papers reprinted here are in languages other than the molecular, e.g., Part VI on electrophysiology, the papers have been chosen to stimulate the interest of the molecular scientist and to provide a current, comprehensive view of the area.

A convenient way of viewing the overall process of vision is in terms of size–order–time domains. Thus we have a relatively small polyene chromophore (molecular weight = 290 daltons), which upon absorption of light, undergoes a simple molecular change (i.e., isomerization) and in the process transfers a substantial fraction of the absorbed light energy to a covalently attached macromolecular glycoprotein (35,000–40,000 daltons). The individual steps in this initial process span a time domain from 10^{-15} to 10^{-9} seconds, and, so far as they are understood, can best be described in the quantum mechanical language of spectroscopy and radiationless transitions. Having thus been energized, the glycoprotein undergoes several intra- and intermolecular processes involving considerable entropic changes on a time scale from microseconds to milliseconds. These chemical and conformational changes are described in the somewhat less precise language of biophysical chemistry, i.e., chemical kinetics, thermodynamics, and statistical mechanics.

Changes in the macromolecular protein are coupled in the same millisecond time domain to processes at the organelle level of the photoreceptor cell, i.e., the disk membrane. At present, we do not have an adequate molecular language to describe this coupling. The language used here is operational, employing concepts such as transmitters, pumps, receptor sites, channels, pores, and carriers. Enzyme systems are the chief mediators of these coupling processes. While enzymes serve to preserve the molecular character of our description, little is known of the molecular structure and local disposition of these enzymes. Coupling between the disk organelle and the enclosing plasma membrane of the cell occurs in roughly the same time domain and is described in the same operational language, together with the language used for macroscopic description of the electrical responses of the cell, e.g., potentials and conductances.

When we leave the receptor cell for the extracellular domain of the whole retina and the brain, we are in a complex labyrinth of neural cells and our discussion is dominated by the language of electric circuitry. Elements of a molecular description, however, are retained in reference to enzyme systems and receptor sites.

The process of visual transduction and cerebral registration terminates slightly beyond the millisecond time domain. But there are also slower processes in vision: pigment regeneration and cell adaptation occur in minutes, and the much slower renewal of the receptor cell outer segment takes place on a circadian time scale.

It is appropriate that we begin this collection of papers on vision with the 1967 Nobel lecture of George Wald (Paper 1), whose work over the past 40 years has largely determined the guidelines along which the studies of the molecular basis of vision have developed. This lecture provides an excellent historical survey of the main facts of development up to 1967. Studies over the past decade have changed few of the fundamentals of the picture presented in the lecture, although they have filled in many details of the structure, composition, and dynamics of visual photoreceptors that were either obscure or unanticipated at the time.

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Reprinted from pages 260–280 of *Les Prix Nobel en 1967*, Stockholm: The Nobel Foundation, 1969, 298pp.

THE MOLECULAR BASIS OF VISUAL EXCITATION

by GEORGE WALD

Nobel Lecture, December 12, 1967

I have often had cause to feel that my hands are cleverer than my head. That is a crude way of characterizing the dialectics of experimentation. When it is going well, it is like a quiet conversation with Nature. One asks a question and gets an answer; then one asks the next question, and gets the next answer. An experiment is a device to make Nature speak intelligibly. After that one has only to listen.

As a graduate student at Columbia University, I was introduced to vision by Selig Hecht in a particularly provocative way. Hecht was one of the great measurers of human vision, like Aubert, König and Abney before him. But he was not content merely to measure. He wanted to understand what lay behind the measurements, what was going on at the molecular level in vision.

There a door was opened for him while still a graduate student at Harvard, by the great Swedish physical chemist Svante Arrhenius. Hecht has told me of the excitement with which he read Arrhenius's new book, "Quantitative Laws in Biological Chemistry (1)." It offered the hope of translating accurate measurements on whole organisms into the simple kinetics and thermodynamics of chemical reactions in solution.

In this vein Hecht applied his measurements and those of earlier workers to constructing a general conceptual model for the photoreceptor process. A photosensitive pigment, S, was dissociated by light into products, P + A, one of them responsible for excitation. In turn P + A, or a variant, P + B, recombined to regenerate S. In continuous light these opposed reactions achieved a pseudo-equilibrium, a photostationary state, that underlay the steady states of vision in constant illumination (2).

I left Hecht's laboratory with a great desire to lay hands on the molecules for which these were symbols. That brought me to Otto Warburg in Dahlem, where I found vitamin A in the retina (3). There were good reasons to look for it there, as I found out later, and that is the way I wrote my paper. Dietary night blindness, a condition already known in ancient Egypt, had been shown in Denmark during World War I to be a symptom of vitamin A deficiency (4); and Fridericia and Holm (5) and Tansley (6) had shown that vitamin A deficient rats synthesize less rhodopsin than normal animals. But vitamins were still deeply mysterious, and at that time one hardly expected them to participate directly in physiological processes. I think this was the first instance of so direct a connection, though Warburg and Christian were already analyzing the first yellow enzymes (7), and shortly their chromophore riboflavin would prove to be vitamin B_2 (8).

After that, things happened quickly. I went to Karrer in Zürich, who with Morf and Schöpp had the year before established the structure of vitamin A (9), to complete its identification in the retina. Then I went on to Meyerhof in Heidelberg, to do something else; but with a shipment of frogs that had gone astray, I found retinene, an intermediate in the bleaching of rhodopsin, on the way to vitamin A (10). Years later Ball, Goodwin and Morton in Liverpool showed that retinene is vitamine A aldehyde (11). At Morton's suggestion the names of all these molecules have recently been changed, in honor of the retina, still the only place where their function is understood. Vitamin A is now retinol, retinene is retinal (Fig. 1); there is also retinoic acid.

That early Wanderjahr in the laboratories of three Nobel laureates—Warburg, Karrer, Meyerhof—opened a new life for me: the life with molecules. From then on it has been a constant going back and forth between organisms and their molecules—extracting the molecules from the organisms, to find what they are and how they behave, returning to the organisms to find in their responses and behavior the greatly amplified expression of those molecules.

A basic characteristic of the scientific enterprise is its continuity. It is an organic growth, to which each worker in his time brings what he can; like Chartres or Hagia Sofia, to which over the centuries a buttress was added here, a tower there. Hecht's work was most intimately bound up with that of men who had worked generations before him: Hermann Aubert in Breslau (12), Arthur König in Berlin (13), Abney in England (14). Now I entered into such a relationship with Willy Kühne of Heidelberg. Kühne had taken up rhodopsin immediately upon Franz Boll's discovery of it in 1877 (15), and in two extraordinary years he and his co-worker Ewald learned almost everything known about it for another half-century (16). It was largely on the basis of Kühne's observations that I could conclude that rhodopsin is a protein, a carotenoid-protein such as Kuhn and Lederer had just shown the blue pigment of lobster shells to be (17), that in the retina, under the influence of light, engages in a cycle of reactions with retinal and vitamin A (10, 18).

I owe other such debts to past workers in far-off places. Köttgen and Abelsdorff had found the visual pigment from eight species of fish to have difference spectra displaced considerably toward the red from the rhodopsins of frogs, owls and mammals (19). Trying to check this observation at Woods Hole, I was surprised to find the same rhodopsin-retinal-vitamin A cycle in fishes there as frogs (20). It turned out that Köttgen and Abelsdorff had worked entirely with *freshwater* fishes. On turning to them, I found another visual pigment, porphyropsin, engaged in a cycle parallel with that of rhodopsin, but in which new carotenoids replace retinal and vitamin A (21). On the basis of these observations, it was suggested that the substance that replaces vitamin A in the visual system of freshwater fishes be called vitamin A_2 (22). In what follows I shall call it retinol$_2$, and its aldehyde retinal$_2$. These substances differ from their analogues in the rhodopsin cycle only in possessing an added double bond in the ring (Fig. 1) (23).

Shortly afterward it emerged that such familiar euryhaline and hence