

Sidney P. Colowick and Nathan O. Kaplan

Methods in ENZYMOLGY

Volume X

Oxidation and Phosphorylation

Edited by

Ronald W. Estabrook

Maynard E. Pullman

Methods in
ENZYMOLGY

X

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Volume X

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METHODS IN ENZYMOLOGY

EDITORS-IN-CHIEF

Sidney P. Colowick Nathan O. Kaplan

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Ronald W. Estabrook

DEPARTMENT OF BIOPHYSICS AND PHYSICAL BIOCHEMISTRY
JOHNSON RESEARCH FOUNDATION
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Preface

Though important advances have been made in the last 15 years toward an understanding of the mechanism of energy-coupled respiration, the details of this major mitochondrial function remain largely unknown. The nature and volume of the experimental work on mitochondria reported in the past few years reflect the fact that not only has the enzymologist broadened his approach to this problem, but that this field has attracted from other areas of biology increasing numbers of investigators including electron microscopists, cell physiologists, and geneticists. The diversity of approaches employed by investigators interested in electron transport, oxidative phosphorylation, and other aspects of mitochondrial physiology indicated that this was an appropriate time to compile a methodological reference for this area of biochemistry.

The aim of this volume is to provide not only the specialist and the advanced student but also investigators from other areas of research with a *single authoritative* source for the vast and often difficult to retrieve and evaluate methodology associated with mitochondrial research. The accounts of procedures, written by leading investigators personally experienced with the methods, are intended to be sufficiently detailed and comprehensive to insure reproducibility without having to refer to original papers. Alternate methods are mentioned, and when possible an effort has been made to evaluate the more commonly used procedures.

The scope of coverage ranges from the commonly used techniques of measuring P:O ratios and the isolation of mitochondria to more specialized techniques, such as the application of electron microscopy to the study of mitochondria or the measurement of heat exchange occurring during electron transport. A chapter was devoted to the application of inhibitors and uncouplers to the study of electron transport and oxidative phosphorylation and includes the source, specificity and optimal concentrations required. While the major portion of the volume is devoted to mitochondrial respiratory enzymes and associated factors for oxidative phosphorylation, a number of newer procedures related to the properties and the purification of microsomal as well as bacterial respiratory pigments have been included. Related methods and techniques which have been included in previous volumes of this series have not been repeated here unless it was felt that recent developments represented significant improvements over the earlier described methods. Nevertheless references to these articles appear at the beginning of each section of this volume where they would normally have appeared.

We would like to express our gratitude to all of the contributors for their cooperation in making this volume possible. We are grateful to

Drs. B. Chance, D. E. Green, H. Lardy, A. L. Lehninger, E. Racker, and E. C. Slater for their advice and constructive criticism in the organization of the original outline for this volume. We also wish to thank Mr. Philip Blackwood, Mrs. Edith Casper, and Mrs. Phyllis Pullman for the excellent secretarial assistance they provided. Finally, we wish to acknowledge the warm cooperation and friendly patience of the staff of Academic Press.

RONALD W. ESTABROOK
MAYNARD E. PULLMAN

April, 1966

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EDITED BY

Sidney P. Colowick and Nathan O. Kaplan

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- I. Preparation and Assay of Enzymes
- II. Preparation and Assay of Enzymes
- III. Preparation and Assay of Substrates
- IV. Special Techniques for the Enzymologist
- V. Preparation and Assay of Enzymes
- VI. Preparation and Assay of Enzymes (*Continued*)
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- VII. Cumulative Subject Index

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