

~~58-17435~~  
~~00008~~

*Methods in Enzymology*

*Volume LII*

*Biomembranes*

*Part C: Biological Oxidations  
Microsomal, Cytochrome P-450, and Other  
Hemoprotein Systems*

EDITED BY

*Sidney Fleischer*

*Lester Packer*



*Methods in Enzymology*

*Volume LII*

*Biomembranes*

*Part C: Biological Oxidations  
Microsomal, Cytochrome P-450, and Other  
Hemoprotein Systems*

EDITED BY

*Sidney Fleischer*

DEPARTMENT OF MOLECULAR BIOLOGY  
VANDERBILT UNIVERSITY, NASHVILLE, TENNESSEE

*Lester Packer*

MEMBRANE BIOENERGETICS GROUP  
DEPARTMENT OF PHYSIOLOGY-ANATOMY  
UNIVERSITY OF CALIFORNIA, BERKELEY, CALIFORNIA

*Editorial Advisory Board*

Lars Ernster  
Ronald W. Estabrook  
Frank Gibson

Youssef Hatefi  
Martin Klingenberg  
David F. Wilson



ACADEMIC PRESS New York San Francisco London 1978

A Subsidiary of Harcourt Brace Jovanovich, Publishers

**COPYRIGHT © 1978, BY ACADEMIC PRESS, INC.**

**ALL RIGHTS RESERVED.**

**NO PART OF THIS PUBLICATION MAY BE REPRODUCED OR TRANSMITTED IN ANY FORM OR BY ANY MEANS, ELECTRONIC OR MECHANICAL, INCLUDING PHOTOCOPY, RECORDING, OR ANY INFORMATION STORAGE AND RETRIEVAL SYSTEM, WITHOUT PERMISSION IN WRITING FROM THE PUBLISHER.**

**ACADEMIC PRESS, INC.**

**111 Fifth Avenue, New York, New York 10003**

*United Kingdom Edition published by*  
**ACADEMIC PRESS, INC. (LONDON) LTD.**  
24/28 Oval Road, London NW1 7DX

**Library of Congress Cataloging in Publication Data**

Main entry under title:

Biomembranes.

(Methods in enzymology, v. 52)

Includes bibliographical references.

Pt. C has special title: Biological oxidations: Microsomal, cytochrome P-450, and other hemoprotein systems.

1. Cell membranes. 2. Cell fractionation.

3. Cell organelles. I. Fleischer, Sidney, ed.

II. Packer, Lester, ed. III. Series.

[DNLM: 1. Cell membrane. W1 ME9615K v. 31 / QH601 B6193]

QP601 M49 vol. 31-32 [QH601] 574.1'925'08s

ISBN 0-12-181952-3 (v. 52) [574.8'75] 54-9110

**PRINTED IN THE UNITED STATES OF AMERICA**

## Contributors to Volume LII

Article numbers are in parentheses following the names of contributors.  
Affiliations listed are current.

- CYRIL A. APPLEBY (16), *Division of Plant Industry, Commonwealth Scientific and Industrial Research Organization, City, Canberra, A.C.T.*
- TOSHIO ASAKURA (47), *Division of Hematology, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania*
- STEVEN D. AUST (30, 33), *Department of Biochemistry, Michigan State University, East Lansing, Michigan*
- JOHN A. BUEGE (30), *Instruments Products Division, E. I. du Pont de Nemours & Co., Inc., Wilmington, Delaware*
- M. D. BURKE (39, 42), *Department of Pharmacology, University of Aberdeen, Aberdeen, Scotland*
- WINSLOW S. CAUGHEY (45), *Department of Biochemistry, Colorado State University, Fort Collins, Colorado*
- DOMINICK L. CINTI (6), *Department of Pharmacology, University of Connecticut Health Center, Farmington, Connecticut*
- LYMAN W. CONDI (36), *The Toxicology Center, Department of Pharmacology, The University of Iowa, Iowa City, Iowa*
- MICHAEL CONNORS (8), *Department of Biochemistry, University of Connecticut Health Center, Farmington, Connecticut*
- MINOR J. COON (10, 20), *Department of Biological Chemistry, The University of Michigan Medical School, Ann Arbor, Michigan*
- DORIS CORCORAN (8), *Department of Biochemistry, Uniformed Services, University of the Health Sciences, Bethesda, Maryland*
- SYLVIA B. DAHL (10), *Department of Biological Chemistry, The University of Michigan Medical School, Ann Arbor, Michigan*
- GUSTAV DALLNER (5), *Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, Stockholm, Sweden*
- LEONORE M. DECARLI (37), *Alcohol Research Center, Bronx Veterans Administration Hospital and Mt. Sinai School of Medicine, The City University of New York, New York, New York*
- HECTOR F. DELUCA (41), *Department of Biochemistry, University of Wisconsin-Madison, Madison, Wisconsin*
- J. W. DEPIERRE (44), *Biochemical Institution, Arrhenius Laboratory, University of Stockholm, Stockholm, Sweden*
- JOHN DAVID DIGNAM (7), *Department of Biochemistry, University of Connecticut Health Center, Farmington, Connecticut*
- RICHARD E. EBEL (15, 23), *Department of Biochemistry and Nutrition, Virginia Polytechnic Institute and State University, Blacksburg, Virginia*
- JOHN A. EISMAN (41), *Department of Medicine, University of Melbourne, Repatriation General Hospital, Heidelberg, Victoria, Australia*
- HARRY G. ENOCH (18, 21), *Department of Biochemistry, University of Connecticut Health Center, Farmington, Connecticut*
- RONALD W. ESTABROOK (2, 22), *Department of Biochemistry, Southwestern Medical School, The University of Texas Health Science Center at Dallas, Dallas, Texas*
- L. L. FAN (32), *Department of Biochemistry, The University of Texas Health Science Center at Dallas, Dallas, Texas*
- PATRICK FLEMING (8, 21), *Department of Biochemistry, University of Connecticut Health Center, Farmington, Connecticut*
- IRWIN C. GUNSALUS (17), *Biochemistry Department, Roger Adams Laboratory, University of Illinois, Urbana, Illinois*
- FRANK, R. N. GURD (50), *Département of*

- Chemistry, Indiana University, Bloomington, Indiana*
- JAN-ÅKE GUSTAFSSON (40), *Department of Chemistry, Karolinska Institutet, Stockholm, Sweden*
- LOWELL P. HAGER (55), *Department of Biochemistry, University of Illinois, Urbana, Illinois*
- PAUL F. HALLENBERG (55), *Department of Biochemistry, Northwestern University Medical School, Chicago, Illinois*
- DAVID A. HAUGEN (10), *Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois*
- GERHARD HEINEMEYER (35), *Institut für Klinische Pharmakologie, Freie Universität Berlin, Berlin, West Germany*
- ALFRED G. HILDEBRANDT (35), *Institut für Klinische Pharmakologie, Freie Universität Berlin, Berlin, West Germany*
- BRIAN M. HOFFMAN (51), *Department of Chemistry, Northwestern University, Evanston, Illinois*
- JOHAN HÖGBERG (4), *Department of Forensic Medicine, Karolinska Institutet, Stockholm, Sweden*
- MARJORIE G. HORNING (34), *Institute for Lipid Research, Baylor College of Medicine, Houston, Texas*
- DONALD E. HULTQUIST (49), *Department of Biological Chemistry, The University of Michigan, Ann Arbor, Michigan*
- EIJI ITAGAKI (12), *Department of Chemistry, Faculty of Science, Kanazawa University, Ishikawa, Japan*
- C. R. JEFEOATE (27), *Department of Pharmacology, University of Wisconsin Medical School, Madison, Wisconsin*
- DONALD M. JERINA (28), *Laboratory of Chemistry, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland*
- K. A. M. JOHANNESSEN (44), *Biochemical Institution, Arrhenius Laboratory, University of Stockholm, Stockholm, Sweden*
- HENRY KAMIN (46), *Department of Biochemistry, Duke University Medical School, Durham, North Carolina*
- MASAYUKI KATAGIRI (12), *Department of Chemistry, Faculty of Science, Kanazawa University, Ishikawa, Japan*
- THOMAS KEEVIL (1), *Department of Chemistry, Southern Oregon State College, Ashland, Oregon*
- TOKUJI KIMURA (13), *Department of Chemistry, Wayne State University, Detroit, Michigan*
- YASUO KISHIMOTO (31), *Department of Neurology, The John F. Kennedy Institute, Baltimore, Maryland*
- WAYNE LEVIN (11, 19), *Department of Biochemistry and Drug Metabolism, Hoffmann-La Roche Inc., Nutley, New Jersey*
- CHARLES S. LIEBER (37), *Alcohol Research Center, Bronx Veterans Administration Hospital and Mt. Sinai School of Medicine, The City University of New York, New York, New York*
- ANTHONY Y. H. LU (11, 19), *Department of Biochemistry and Drug Metabolism, Hoffmann-La Roche Inc., Nutley, New Jersey*
- HOWARD S. MASON (1), *Department of Biochemistry, School of Medicine, University of Oregon Health Sciences Center, Portland, Oregon*
- BETTIE SUE SILER MASTERS (25, 32), *Department of Biochemistry, The University of Texas Health Science Center at Dallas, Dallas, Texas*
- SHOHEI MATSUZAKI (37), *Alcohol Research Center, Bronx Veterans Administration Hospital and Mt. Sinai School of Medicine, The City University of New York, New York, New York*
- R. T. MAYER (39), *The Veterinary Toxicology and Entomology Research Laboratory, Agriculture Research Service, U.S.D.A., College Station, Texas*
- KATSUYOSHI MIHARA (9), *Institute for Protein Research, Osaka University, Osaka, Japan*
- PETER MOLDEÚS (4), *Department of Foren-*

- sic Medicine, Karolinska Institutet, Stockholm, Sweden*
- ROBERT W. MOORE (33), *Department of Pathology, The University of Wisconsin, Madison, Wisconsin*
- M. S. MORÓN (44), *Biochemical Institution, Arrhenius Laboratory, University of Stockholm, Stockholm, Sweden*
- MATTHEW J. MURPHY (46), *Research Laboratory, Guinness Brewery, St. James Gate, Dublin, Eire*
- DANIEL W. NEBERT (24), *Developmental Pharmacology Branch, National Institute of Child Health and Human Development, Bethesda, Maryland*
- PETER J. O'BRIEN (43), *Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland, Canada*
- KUNIHICO OHNISHI (37), *Johnson Research Foundation, University of Pennsylvania, Philadelphia, Pennsylvania*
- DAVID H. O'KEEFE (15, 23), *Department of Biochemistry, The University of Texas Health Science Center at Dallas, Dallas, Texas*
- R. T. OKITA (32), *Department of Biochemistry, The University of Texas Health Science Center at Dallas, Dallas, Texas*
- NANETTE R. ORME-JOHNSON (26), *Department of Biochemistry, University of Wisconsin, Madison, Wisconsin*
- W. H. ORME-JOHNSON (26), *Department of Biochemistry, University of Wisconsin, Madison, Wisconsin*
- STEN ORRENIUS (4), *Department of Forensic Medicine, Karolinska Institutet, Stockholm, Sweden*
- JEFF H. PARCELLS (13), *Department of Chemistry, Wayne State University, Detroit, Michigan*
- JULIAN A. PETERSON (15, 23), *Department of Biochemistry, The University of Texas Health Science Center at Dallas, Dallas, Texas*
- L. L. POULSEN, (14), *Clayton Foundation Biochemical Institute and Department of Chemistry, The University of Texas at Austin, Austin, Texas*
- R. A. PROUGH (32, 39, 42), *Department of Biochemistry, The University of Texas Southwestern Medical School, Dallas, Texas*
- ANVER D. RAHIMTULA (43), *Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland, Canada*
- LARS REIMANN (54), *Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, Tennessee*
- IVAR ROOTS (35), *Institut für Klinische Pharmakologie, Freie Universität Berlin, Berlin, West Germany*
- T. MICHAEL ROTHGEB (50), *Department of Chemistry, Indiana University, Bloomington, Indiana*
- DENE RYAN (11), *Department of Biochemistry and Drug Metabolism, Hoffmann-La Roche Inc., Nutley, New Jersey*
- RYO SATO (9), *Institute for Protein Research, Osaka University, Osaka, Japan*
- BRENT A. SCHACTER (38), *Department of Medicine, University of Manitoba and The Manitoba Institute of Cell Biology, Winnipeg, Manitoba, Canada*
- JOHN B. SCHENKMAN (6), *Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut*
- DIANE M. SCHOLLER (51), *Department of Chemistry, Northwestern University, Evanston, Illinois*
- GREGORY R. SCHONBAUM (54), *Department of Biochemistry, St. Jude Children's Research Hospital, and University of Tennessee Center for the Health Sciences, Memphis, Tennessee*
- J. SIEDEGÅRD (44), *Biochemical Institution, Arrhenius Laboratory, University of Stockholm, Stockholm, Sweden*
- LOUIS M. SIEGEL (46), *Department of Biochemistry, Duke University Medical School, Durham, North Carolina*
- HELMUT SIES (3), *Institut für Physiologische Chemie, Physikalische Biochemie*

*und Zellbiologie der Universität München, München, Germany*

MICHAEL L. SMITH (45), *Department of Biochemistry, Colorado State University, Fort Collins, Colorado*

PHILIPP STRITTMATTER (8, 18, 21), *Department of Biochemistry, University of Connecticut Health Center, Farmington, Connecticut*

HENRY W. STROBEL (7), *Department of Biochemistry and Molecular Biology, The University of Texas Medical School at Houston, Houston, Texas*

KATSUKO SUHARA (12), *Department of Chemistry, Faculty of Science, Kanazawa University, Ishikawa, Japan*

SHIGEKI TAKEMORI (12), *Department of Environmental Science, Faculty of Integrated Arts and Sciences, Hiroshima University, Hiroshima, Japan*

AL TAPPEL (53), *Department of Food Science and Technology, University of California at Davis, Davis, California*

THOMAS R. TEPHLY (36), *The Toxicology Center, Department of Pharmacology, The University of Iowa, Iowa City, Iowa*

ROLF TESCHKE (37), *Alcohol Research Center, Bronx Veterans Administration Hospital and Mt. Sinai School of Medicine, The City University of New York, New York, New York*

DHIREN R. THAKKER (28), *Laboratory of Chemistry, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland*

MEI TJOE (35), *Institut für Klinische Phar-*

*makologie, Freie Universität Berlin, Berlin, West Germany*

N. E. TOLBERT (52), *Department of Biochemistry, Michigan State University, East Lansing, Michigan*

THEODORE A. VAN DER HOEVEN (10), *Department of Medicinal Chemistry, University of Maryland, Baltimore, Maryland*

GERALD C. WAGNER (17), *Biochemistry Departments, Roger Adams Laboratory, University of Illinois, Urbana, Illinois*

HANN-PING WANG (13), *Department of Chemistry, Wayne State University, Detroit, Michigan*

MING-YU R. WANG (51), *Department of Chemistry, Northwestern University, Evanston, Illinois*

MICHAEL R. WATERMAN (48), *Department of Biochemistry, The University of Texas Health Science Center at Dallas, Dallas, Texas*

ANN F. WELTON (33), *Department of Pharmacology, Hoffmann-La Roche Inc., Nutley, New Jersey*

J. WERRINGLOER (22, 29), *Department of Biochemistry, The University of Texas Health Science Center at Dallas, Dallas, Texas*

HARUHIKO YAGI (28), *Laboratory of Chemistry, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland*

D. M. ZIEGLER (14), *Clayton Foundation Biochemical Institute and Department of Chemistry, The University of Texas at Austin, Austin, Texas*

## Preface

A great deal of progress has taken place in biological oxidations and bioenergetics since "Oxidation and Phosphorylation" edited by Ronald W. Estabrook and Maynard E. Pullman (Volume X of "Methods in Enzymology") became available in 1967. To update this field five volumes on biomembranes (Volumes LII-LVI, Parts C-G, respectively) have been prepared, three dealing with biological oxidations and two with bioenergetics.

In this volume, Part C of "Biomembranes," subtitled "Biological Oxidations: Microsomal, Cytochrome P-450, and Other Hemoprotein Systems," we aim to bring together the new methodology that has accompanied the development of essentially a new field that has great relevance to molecular pharmacology, endocrinology, chemical carcinogenesis, and environmental toxicology.

We single out for special thanks the contributions of Dr. Ronald W. Estabrook of our Advisory Board for his extensive input in organizing this volume and for his wisdom in solving a number of problems that developed. Drs. M. J. Coon and I. C. Gunsalus have also provided valuable counsel in matters concerning this volume.

We are pleased to acknowledge the good counsel of the members of our Advisory Board for these five volumes. Special thanks are also due Drs. E. Carafoli, G. Palmer, H. Penefsky, and A. Scarpa for their helpful comments on our outlines for these volumes. We were very gratified by the enthusiasm and cooperation of the participants in the field of biological oxidations and bioenergetics whose advice, comments, and contributions have enriched and made possible these volumes. The friendly cooperation of the staff of Academic Press is gratefully acknowledged.

SIDNEY FLEISCHER  
LESTER PACKER



# METHODS IN ENZYMOLOGY

EDITED BY

Sidney P. Colowick and Nathan O. Kaplan

VANDERBILT UNIVERSITY  
SCHOOL OF MEDICINE  
NASHVILLE, TENNESSEE

DEPARTMENT OF CHEMISTRY  
UNIVERSITY OF CALIFORNIA  
AT SAN DIEGO  
LA JOLLA, CALIFORNIA

- 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*)
  - Preparation of Assay of Substrates
  - Special Techniques
- VII. Cumulative Subject Index

# Table of Contents

CONTRIBUTORS TO VOLUME LII . . . . .	xi
PREFACE . . . . .	xv
VOLUMES IN SERIES . . . . .	xvii

## Section I. General Overview

1. Molecular Oxygen in Biological Oxidations— An Overview	THOMAS KEEVIL AND HOWARD S. MASON	3
--	--------------------------------------	---

## Section II. Microsomal Electron Transport and Cytochrome P-450 Systems

2. Microsomal Electron-Transport Reactions: An Overview	RONALD W. ESTABROOK	43
--	---------------------	----

### A. Complex and Resolved Systems

3. The Use of Perfusion of Liver and Other Organs for the Study of Microsomal Electron- Transport and Cytochrome P-450 Systems	HELMUT SIES	48
4. Isolation and Use of Liver Cells	PETER MOLDEUS, JOHAN HÖGBERG, AND STEN ORRENIUS	60
5. Isolation of Microsomal Subfractions by Use of Density Gradients	GUSTAV DALLNER	71
6. Preparation of Microsomes with Calcium	JOHN B. SCHENKMAN AND DOMINICK L. CINTI	83
7. Purification and Properties of NADPH-Cyto- chrome P-450 Reductase	HENRY W. STROBEL AND JOHN DAVID DIGNAM	89
8. Purification of Cytochrome $b_5$	PHILIPP STRITTMATTER, PATRICK FLEMING, MICHAEL CONNORS, AND DORIS CORCORAN	97
9. Detergent-Solubilized NADH-Cytochrome $b_5$ Re- ductase	KATSUYOSHI MIHARA AND RYO SATO	102
10. Two Forms of Liver Microsomal Cytochrome P-450, P-450LM <sub>2</sub> , and P-450LM <sub>4</sub> (Rabbit Liver)	MINOR J. COON, THEODORE A. VAN DER HOEVEN, SYLVIA B. DAHL, AND DAVID A. HAUGEN	109

11. Purification of Cytochrome P-450 and P-448 from Rat Liver Microsomes	DENE RYAN, ANTHONY Y. H. LU, AND WAYNE LEVIN	117
12. Purification of Adrenal Cytochrome P-450 (Cholesterol Desmolase and Steroid 11 $\beta$ - and 18-Hydroxylase)	MASAYUKI KATAGIRI, SHIGEKI TAKEMORI, EIJI ITAGAKI, AND KATSUKO SUHARA	124
13. Purification of Adrenodoxin Reductase, Adrenodoxin, and Cytochrome P-450 from Adrenal Cortex	TOKUJI KIMURA, JEFF H. PARCELLS, AND HANN-PING WANG	132
14. Hepatic Microsomal Mixed-Function Amine Oxidase	D. M. ZIEGLER AND L. L. POULSEN	142
15. Purification of Bacterial Cytochrome P-450	DAVID H. O'KEEFFE, RICHARD E. EBEL, AND JULIAN A. PETERSON	151
16. Purification of <i>Rhizobium</i> Cytochromes P-450	CYRIL A. APPLEBY	157
17. Bacterial P-450 <sub>cam</sub> Methylene Monooxygenase Components: Cytochrome <i>m</i> , Putidaredoxin, and Putidaredoxin Reductase	IRWIN C. GUNSALUS AND GERALD C. WAGNER	166
18. Purification of Stearyl-CoA Desaturase from Liver	PHILIPP STRITTMATTER AND HARRY G. ENOCH	188
19. Purification and Assay of Liver Microsomal Epoxide Hydrase	ANTHONY Y. H. LU AND WAYNE LEVIN	193

## B. Reconstitution

20. Reconstitution of the Cytochrome P-450-Containing Mixed-Function Oxidase System of Liver Microsomes	MINOR J. COON	200
21. Incorporation of Microsomal Electron-Transfer Components into Liposomes: Considerations for Diffusion-Limited Reactions	PHILIPP STRITTMATTER, HARRY G. ENOCH, AND PATRICK FLEMING	206

## C. General Methods

22. The Measurement of Difference Spectra: Application to the Cytochromes of Microsomes	R. W. ESTABROOK AND J. WERRINGLOER	212
23. Dual-Wavelength Stopped-Flow Spectrophotometric Measurement of NADPH-Cytochrome P-450 Reductase	JULIAN A. PETERSON, RICHARD E. EBEL, AND DAVID H. O'KEEFFE	221

24. Genetic Differences in Microsomal Electron Transport: The <i>Ah</i> Locus	DANIEL W. NEBERT	226
25. The Preparation and Use of Antibodies as Diagnostic Biochemical Probes	BETTIE SUE SILER MASTERS	240
26. Detection and Quantitation of Free Cytochrome P-450 and Cytochrome P-450 Complexes by EPR Spectroscopy	NANETTE R. ORME-JOHNSON AND W. H. ORME-JOHNSON	252
27. Measurement of Substrate and Inhibitor Binding to Microsomal Cytochrome P-450 by Optical-Difference Spectroscopy	C. R. JEFEOATE	258
28. Analysis of Polycyclic Aromatic Hydrocarbons and Their Metabolites by High-Pressure Liquid Chromatography	DHIREN R. THAKKER, HARUHIKO YAGI, AND DONALD M. JERINA	279

#### D. Specific Assay Methods

29. Assay of Formaldehyde Generated during Microsomal Oxidation Reactions	JURGEN WERRINGLOER	297
30. Microsomal Lipid Peroxidation	JOHN A. BUEGE AND STEVEN D. AUST	302
31. Very Long Chain Fatty Acid $\alpha$ -Hydroxylase from Brain	YASOO KISHIMOTO	310
32. The Measurement of $\omega$ - and $\omega$ -1 Hydroxylation of Fatty Acids by Mixed-Function Oxidase Systems	R. A. PROUGH, R. T. OKITA, L. L. FAN, AND B. S. S. MASTERS	318
33. Detection of Hemoproteins in SDS-Polyacrylamide Gels	ROBERT W. MOORE, ANN F. WELTON, AND STEVEN D. AUST	324
34. Analysis of Drugs and Their Metabolites by Gas Chromatography-Mass Spectrometry-Computer Systems	MARJORIE G. HORNING	331
35. Hydrogen Peroxide in Hepatic Microsomes	ALFRED G. HILDEBRANDT, IVAR ROOTS, MEI TJOE, AND GERHARD HEINEMEYER	342
36. $\delta$ -Aminolevulinic Acid Synthetase-Sensitive Methods in Liver for Hemoprotein Biosynthesis	LYMAN W. CONDIE AND THOMAS R. TEPHLY	350
37. The Microsomal Ethanol Oxidizing System (MEOS)	CHARLES S. LIEBER, LEONORE M. DECARLI, SHOHEI MATSUZAKI, KUNIHIKO OHNISHI, AND ROLF TESCHKE	355

38. Assay of Microsomal Heme Oxygenase in Liver and Spleen	BRENT A. SCHACTER	367
39. Direct Fluorometric Methods for Measuring Mixed-Function Oxidase Activity	R. A. PROUGH, M. D. BURKE, AND R. T. MAYER	372
40. Steroid Hydroxylations Catalyzed by Cytochrome P-450	JAN-ÅKE GUSTAFSSON	377
41. Determination of Vitamin D Metabolites	JOHN A. EISMAN AND HECTOR F. DeLUCA	388
42. Fluorometric and Chromatographic Methods for Measuring Microsomal Biphenyl Hydroxylation	M. DANNY BURKE AND RUSSELL A. PROUGH	399
43. A Peroxidase Assay for Cytochrome P-450	PETER J. O'BRIEN AND ANVER D. RAHIMTULA	407
44. Radioactive Assay of Aryl Hydrocarbon Monooxygenase and Epoxide Hydrase	J. W. DEPIERRE, K. A. M. JOHANNESSEN, M. S. MORÓN, AND J. SEIDEGÅRD	412

### Section III. Other Hemoprotein Systems (See Vol. LIII for Cytochromes)

#### A. General

45. New Methods for Isolation and Characterization of Hemes	MICHAEL L. SMITH AND WINSLOW S. CAUGHEY	421
46. Siroheme: Methods of Isolation and Characterization	LEWIS M. SIEGEL, MATTHEW J. MURPHY, AND HENRY KAMIN	436

#### B. Hemoglobin and Myoglobin

47. Hemoglobin Porphyrin Modification	TOSHIO ASAKAURA	447
48. Spectral Characterization of Human Hemoglobin and Its Derivatives	MICHAEL R. WATERMAN	456
49. Methemoglobin Reduction System of Erythrocytes	DONALD E. HULTQUIST	463
50. Physical Methods for the Study of Myoglobin	T. MICHAEL ROTHGEB AND FRANK R. N. GURD	473
51. Metal-Substituted Hemoglobin and Other Hemoproteins	DIANE M. SCHOLLER, MING-YU R. WANG, AND BRIAN M. HOFFMAN	487

---

**C. Catalases and Peroxidases**

52. Peroxisomal Redox Enzymes	N. E. TOLBERT	493
53. Glutathione Peroxidase and Hydroperoxides	AL TAPPEL	506
54. Purification of Plant Peroxidases by Affinity Chromatography	LARS REIMANN AND GREGORY R. SCHONBAUM	514
55. Purification of Chloroperoxidase from <i>Caldariomyces fumago</i>	PAUL F. HALLENBERG AND LOWELL P. HAGER	521
AUTHOR INDEX . . . . .		531
SUBJECT INDEX . . . . .		558

**Section I**  
**General Overview**





## [1] Molecular Oxygen in Biological Oxidations—An Overview

By THOMAS KEEVIL and HOWARD S. MASON

In this overview of molecular oxygen in biological oxidations, we have surveyed the properties of the oxidases in an attempt to generalize on the mechanisms of oxygen use by living organisms. To do this, we assembled a complete catalog of 220 oxidases.<sup>1</sup> The oxidases are classified according to their prosthetic groups, and within prosthetic group classes according to reaction types and biological occurrence. We are then able to discuss some general questions, such as the following: (1) What prosthetic group structures account for the biological reactions of molecular oxygen? (2) How many different kinds of reaction of molecular oxygen are enzyme catalyzed, and are there any common properties among them? (3) Are there any relationships between active site types and reaction types? and (4) Do any generalizations regarding mechanism emerge from this survey?

Good comprehensive reviews of molecular oxygen enzymology have appeared recently.<sup>2-5</sup> Our goal in this short article is to give a brief overview that will provide some framework for the articles in this volume. We will show that, in the broadest sense, the enzymology of molecular oxygen arises from its strong electrophilic nature. The reaction types in which molecular oxygen participates are a consequence of the amount of electronic charge that oxidases and their substrates present to molecular oxygen. The reaction products are those that in model systems are produced by singlet oxygen, superoxide, hydrogen peroxide, atomic oxygen, and free hydroxyl radical.

<sup>1</sup> "Oxidases," here, is a collective term signifying the enzymes that catalyze reactions of molecular oxygen, rather than any specific type of reaction.

<sup>2</sup> P. D. Boyer, ed., "The Enzymes," 3rd ed., Vol. XII. Academic Press, New York, 1975.

<sup>3</sup> O. Hayaishi, ed., "Molecular Mechanisms of Oxygen Activation." Academic Press, New York, 1974.

<sup>4</sup> I. C. Gunsalus, T. C. Pederson, and S. G. Sligar, *Annu. Rev. Biochem.* **44**, 377 (1975).

<sup>5</sup> K. T. Yasunobu, H. F. Mower, and O. Hayaishi, eds., "Iron and Copper Proteins." Plenum, New York, 1976.