

**Advances  
in  
Lipid  
Research**

Volume 10

# **Advances in Lipid Research**

Volume 10

*Edited by*

**Rodolfo Paoletti**

*Institute of Pharmacology  
Milan, Italy*

**David Kritchevsky**

*The Wistar Institute  
Philadelphia, Pennsylvania*



1972

ACADEMIC PRESS • New York and London

COPYRIGHT © 1972, 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

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 63-22330

PRINTED IN THE UNITED STATES OF AMERICA

## LIST OF CONTRIBUTORS

Numbers in parentheses indicate the pages on which the authors' contributions begin.

CLAUDE F. BAXTER, *Neurochemistry Laboratories, Veterans Administration Hospital, Sepulveda, California* (261)

NICHOLAS R. DI LUZIO, *Department of Physiology, Tulane University School of Medicine, New Orleans, Louisiana* (43)

TRUDY FORTE, *Donner Laboratory, Lawrence Berkeley Laboratory, University of California, Berkeley, California* (1)

JAMES L. GAYLOR, *Section of Biochemistry and Molecular Biology and the Graduate School of Nutrition, Cornell University, Ithaca, New York* (89)

GENE KRITCHEVSKY, *Division of Neurosciences, City of Hope National Medical Center, Duarte, California* (261)

HAROLD J. NICHOLAS, *Institute of Medical Education and Research and Department of Biochemistry, St. Louis University School of Medicine, St. Louis, Missouri* (143)

ALEX V. NICHOLS, *Donner Laboratory, Lawrence Berkeley Laboratory, University of California, Berkeley, California* (1)

ROBERT B. RAMSEY, *Institute of Medical Education and Research and Department of Biochemistry, St. Louis University School of Medicine, St. Louis, Missouri* (143)

GEORGE ROUSER, *Division of Neurosciences, City of Hope National Medical Center, Duarte, California* (261)

FRED SNYDER, *Medical Division, Oak Ridge Associated Universities, Oak Ridge, Tennessee* (233)

AKIRA YAMAMOTO, *Second Department of Internal Medicine, Osaka University Medical School, Osaka, Japan* (261)

## PREFACE

This volume of *Advances in Lipid Research* is devoted to several special areas of lipid research which are becoming important; new frontiers of established areas of interest are also treated.

The first chapter discusses the application of electron microscopic techniques to the analysis of plasma lipoproteins, an entirely novel way of studying lipoprotein structure. The second article provides a new look at a topic that has piqued scientific interest for a long time—possible modification of reticuloendothelial function by lipids. Present awareness of the role played by lipids in membrane structure and function introduces new aspects to this field. The role of lipids in cellular, humoral, and immune responses is discussed in the second chapter. The third contribution summarizes current knowledge of the microsomal enzymes of sterol biosynthesis. With the extension of studies of lipid synthesis to tissue culture and with new methods of separation and identification of sterols, this paper should be useful to workers in a number of areas. The fifth chapter provides the latest information on one aspect (enzymatic synthesis and degradation) of glycerol lipids which contain ether bonds. This type of lipid has been found in an increasing number of tissues and cells, and its metabolic role is only now being delineated. Brain and nervous tissue research continues to expand with work covering normal development as well as genetic defects. Two chapters explore lipid neurochemistry in depth: the fourth chapter covers brain lipids (fatty acids, phospholipids, sphingolipids, galactosyl lipids, and sterols), whereas the sixth chapter is somewhat broader in scope and discusses lipids of the entire nervous system and their variation with age. Both articles contribute greatly to the knowledge of lipid neurochemistry.

RODOLFO PAOLETTI  
DAVID KRITCHEVSKY

## CONTENTS OF PREVIOUS VOLUMES

### Volume 1

The Structural Investigation of Natural Fats

*M. H. Coleman*

Physical Structure and Behavior of Lipids and Lipid Enzymes

*A. D. Bangham*

Recent Developments in the Mechanism of Fat Absorption

*John M. Johnston*

The Clearing Factor Lipase and Its Action in the Transport of Fatty Acids between the Blood and Tissues

*D. S. Robinson*

Vitamin E and Lipid Metabolism

*Roslyn B. Alfin-Slater and Rosemary Shull Morris*

Atherosclerosis—Spontaneous and Induced

*Thomas B. Clarkson*

Chromatographic Investigations in Fatty Acid Biosynthesis

*M. Pascaud*

Carnitine and Its Role in Fatty Acid Metabolism

*Irving B. Fritz*

Present Status of Research on Catabolism and Excretion of Cholesterol

*Henry Danielsson*

The Plant Sulfolipid

*A. A. Benson*

AUTHOR INDEX—SUBJECT INDEX

### Volume 2

Triglyceride Structure

*R. J. VanderWal*

## Bacterial Lipids

*M. Kates*

## Phosphatidylglycerols and Lipoamino Acids

*Marjorie G. Macfarlane*

## The Brain Phosphoinositides

*J. N. Hawthorne and P. Kemp*

## The Synthesis of Phosphoglycerides and Some Biochemical Applications

*L. L. M. van Deenen and G. H. deHaas*

## The Lipolytic and Esterolytic Activity of Blood and Tissues and Problems of Atherosclerosis

*T. Zemplényi*

## Evaluation of Drugs Active against Experimental Atherosclerosis

*Robert Hess*Comparative Evaluation of Lipid Biosynthesis *in Vitro* and *in Vivo**P. Favarger*

## AUTHOR INDEX—SUBJECT INDEX

## Volume 3

## The Metabolism of Polyenoic Fatty Acids

*E. Klenk*

## The Analysis of Human Serum Lipoprotein Distributions

*Alicia M. Ewing, Norman K. Freeman, and Frank T. Lindgren*

## Factors Affecting Lipoprotein Metabolism

*Angelo M. Scanu*

## The Action of Drugs on Phospholipid Metabolism

*G. B. Ansell*

## Brain Sterol Metabolism

*A. N. Davison*

## Lipases

*E. D. Wills*

## AUTHOR INDEX—SUBJECT INDEX

**Volume 4**

The Role of Lipids in Blood Coagulation

*Aaron J. Marcus*

Lipid Responses to Dietary Carbohydrates

*I. Macdonald*

Effects of Catecholamines on Lipid Mobilization

*Max Wenke*

The Polyunsaturated Fatty Acids of Microorganisms

*Robert Shaw*

Lipid Metabolism in the Bacteria

*W. J. Lennarz*

Quantitative Methods for the Study of Vitamin D

*Padmanabhan P. Nair*

Labeling and Radiopurity of Lipids

*Fred Snyder and Claude Piantadosi*

AUTHOR INDEX—SUBJECT INDEX

**Volume 5**

Fatty Acid Biosynthesis and the Role of the Acyl Carrier Protein

*Philip W. Majerus and P. Roy Vagelos*

Comparative Studies on the Physiology of Adipose Tissue

*Daniel Rudman and Mario Di Girolamo*

Ethionine Fatty Liver

*Emmanuel Farber*

Lipid Metabolism by Macrophages and Its Relationship to Atherosclerosis

*Allan J. Day*

Dynamics of Cholesterol in Rats, Studied by the Isotopic Equilibrium Methods

*F. Chevallier*

The Metabolism of Myelin Lipids

*Marion Edmonds Smith*



Brain Cholesterol: The Effect of Chemical and Physical Agents

*Jon J. Kabara*

The Analysis of Individual Molecular Species of Polar Lipids

*Ossi Renkonen*

Phase Diagrams of Triglyceride Systems

*J. B. Rossell*

AUTHOR INDEX—SUBJECT INDEX

## Volume 6

Practical Methods for Plasma Lipoprotein Analysis

*Frederick T. Hatch and Robert S. Lees*

The Lipids of *Mycoplasma*

*Paul F. Smith*

Lipid Quinones

*T. Ramasarma*

Comparative Pathogenetic Patterns in Atherosclerosis

*Robert W. Wissler and Dragoslava Vesselinovitch*

Chemistry and Metabolism of Bile Alcohols and Higher Bile Acids

*Takahiko Hoshita and Taro Kazuno*

Hydroxy Fatty Acid Metabolism in Brain

*David M. Bowen and Norman S. Radin*

Gas Phase Analytical Methods for the Study of Steroids

*E. C. Horning, C. J. W. Brooks, and W. J. A. Vanden Heuvel*

AUTHOR INDEX—SUBJECT INDEX

## Volume 7

Lipid Histochemistry

*C. W. M. Adams*

Control of Plasma and Liver Triglyceride Kinetics by Carbohydrate Metabolism and Insulin

*Esko A. Nikkilä*

Lipid Metabolism in Tissue Culture Cells

*George H. Rothblat*

## Carcinogenic Effects of Steroids

*Fritz Bischoff*

## The Chemical and Biological Properties of Heated and Oxidized Fats

*Neil R. Artman*

## AUTHOR INDEX—SUBJECT INDEX

## Volume 8

## Cholesterol Turnover in Man

*Paul J. Nestel*

## Arterial Composition and Metabolism: Esterified Fatty Acids and Cholesterol

*Oscar W. Portman*

## The Essential Fatty Acids

*Michael Guarnieri and Ralph M. Johnson*

## Lipids in Membrane Development

*Godfrey S. Getz*

## Plant Phospholipids and Glycolipids

*M. Kates*

## Metabolism of Long-Chain Fatty Acids in the Rumen

*Romano Viviani*

## Surface Chemistry of Lipids

*Dinesh O. Shah*

## AUTHOR INDEX—SUBJECT INDEX

## Volume 9

## Light and Electron Microscopic Radioautography of Lipids: Techniques and Biological Applications

*O. Stein and Y. Stein*

## The Origin of Hydrogen in Fatty Acid Synthesis

*Simonne Rous*

## Fatty Acid Biosynthesis in Aorta and Heart

*Arthur F. Whereat*

## Structure of Membranes and Role of Lipids Therein

*Frantz A. Vandenheuvel*

## Glycosphingolipids

*Herbert Weigandt*

## Biosynthesis of Pregnane Derivatives

*Shlomo Burstein and Marcel Gut*

## Lipid Composition of Vegetable Oils

*Enzo Fedeli and Giovanni Jacini*

## AUTHOR INDEX—SUBJECT INDEX

## CONTENTS

LIST OF CONTRIBUTORS .....	
PREFACE .....	xi
CONTENTS OF PREVIOUS VOLUMES .....	xiii

### Application of Electron Microscopy to the Study of Plasma Lipoprotein Structure

*Trudy Forte and Alex V. Nichols*

I. Introduction .....	1
II. Physical and Chemical Characterization of Plasma Lipoproteins .....	2
III. Various Techniques of Electron Microscopy Applied to Investigation of Lipoprotein Structure .....	4
IV. Concluding Remarks .....	37
References .....	38

### Employment of Lipids in the Measurement and Modification of Cellular, Humoral, and Immune Responses

*Nicholas R. Di Luzio*

I. Introduction .....	43
II. Problems Relative to the Evaluation of Reticuloendothelial Function ...	45
III. Employment of Lipids to Measure Reticuloendothelial Function <i>in Vivo</i> .	46
IV. <i>In Vitro</i> Employment of Lipids for Evaluation of Cellular and Humoral Events in Phagocytosis .....	48
V. Evaluation of Plasma Humoral Recognition Factor Activity in Human Subjects .....	53

VI. Employment of Lipids to Depress Phagocytic Activity of the Reticuloendothelial System .....	58
VII. Employment of Lipids to Stimulate Phagocytic Activity of the Reticuloendothelial System .....	67
VIII. Modification of Endotoxin Responses by Lipids .....	72
IX. Role of Intravenous Lipids in Modifying Infectious Processes .....	76
X. Influence of Intravenously Administered Lipids on the Immune Response .....	78
XI. Influence of Intravenously Administered Lipids on Transplantation Reactions .....	82
XII. Summary .....	85
References .....	86

## Microsomal Enzymes of Sterol Biosynthesis

*James L. Gaylor*

I. Introduction .....	89
II. Solubilization of Microsomal Enzymes .....	96
III. Other Aspects of Purification of Microsomal Enzymes .....	134
References .....	138

## Brain Lipids

*Robert B. Ramsey and Harold J. Nicholas*

I. Introduction .....	144
II. Fatty Acids .....	145
III. Galactosyl Mono- and Diglycerides .....	153
IV. Phospholipids: Introduction .....	153
V. Sphingolipids .....	166
VI. Cholesterol, Methyl Sterols, Trace Isoprenoids, and Other Trace Lipids .....	185
VII. Myelin and Other Subcellular Fractions of the Brain .....	196
VIII. Effect of Undernutrition of Lipids of the Brain .....	198
IX. Lipids and the Developing Brain .....	202
X. Enzymes of Significance to Brain Lipids .....	206
XI. Proteolipids .....	206
XII. Lipids in Cell-Enriched Fractions of the Brain .....	207
XIII. Metabolic Diseases of the Brain .....	209
XIV. Summary and Conclusions .....	218
References .....	219

## Enzymatic Systems That Synthesize and Degrade Glycerolipids Possessing Ether Bonds

*Fred Snyder*

I.	Introduction .....	233
II.	Nomenclature and Structural Formulas .....	235
III.	Methods of Analysis .....	239
IV.	Biosynthesis .....	241
V.	Degradation .....	250
	References .....	256

## Lipids in the Nervous System of Different Species as a Function of Age: Brain, Spinal Cord, Peripheral Nerve, Purified Whole Cell Preparations, and Subcellular Particulates: Regulatory Mechanisms and Membrane Structure

*George Rouser, Gene Kritchevsky, Akira Yamamoto,  
and Claude F. Baxter*

I.	Introduction .....	262
II.	Assessment of Methods for Accurate, Precise, Quantitative Lipid Analysis and the Reporting of Lipid Class Composition Data .....	263
III.	Human Nervous System Lipid Composition Changes with Age .....	276
IV.	Animal Nervous System Lipid Composition Changes with Age and Comparison to Humans .....	292
V.	Conclusions Derived from Studies of Lipid Composition of Isolated Whole Cell and Subcellular Particulate Preparations of the Nervous System .....	307
VI.	Membrane Biosynthesis and the Regulatory Mechanisms of Lipid Metabolism That Determine Membrane Lipid Class Composition .....	332
VII.	Membrane Structure .....	343
	References .....	350

AUTHOR INDEX .....		361
--------------------	--	-----

SUBJECT INDEX .....		380
---------------------	--	-----

# Application of Electron Microscopy to the Study of Plasma Lipoprotein Structure<sup>1</sup>

TRUDY FORTE AND ALEX V. NICHOLS

*Donner Laboratory, Lawrence Berkeley Laboratory,  
University of California, Berkeley, California*

I. Introduction .....	1
A. Definition of Lipoproteins .....	1
B. Scope of This Review .....	2
II. Physical and Chemical Characterization of Plasma Lipoproteins .....	2
III. Various Techniques of Electron Microscopy Applied to Investigation of Lipoprotein Structure .....	4
A. Fixation and Shadow-Casting Technique .....	4
B. Fixation and Embedding Technique .....	8
C. Freeze-Fracturing and Etching Technique .....	12
D. Negative Staining Technique .....	15
IV. Concluding Remarks .....	37
References .....	38

## I. Introduction

### A. DEFINITION OF LIPOPROTEINS

Plasma lipoproteins are macromolecules composed of proteins and lipids. They perform the important function of transporting complex lipids (triglycerides, phospholipids, unesterified cholesterol, and cholesteryl esters) in the plasma. The transported lipids are biologically important in the energy metabolism of tissues and in the structure of cell membranes.

The physical, chemical, and immunological properties of the various lipoprotein classes and their proteins (apolipoproteins) have received much attention in the past few years and several reviews have recently been published (Fredrickson *et al.*, 1967; Schumaker and Adams, 1969;

<sup>1</sup> This work was supported by Research Grants HE 12710-02 and HE 10878-05 from the National Heart and Lung Institute, U. S. Public Health Service, Bethesda, Maryland, and by the U. S. Atomic Energy Commission.

Zilversmit, 1969; Margolis, 1969; Scanu, 1965, 1969; Nichols, 1969). At this time, however, there is no comprehensive survey of available data on the microscopical visualization of plasma lipoproteins. Investigation of the various classes of plasma lipoproteins with the electron microscope has proceeded rapidly in the past few years, and in the present review we will examine the contribution of diverse electron microscopical approaches to our understanding of their morphology.

## B. SCOPE OF THIS REVIEW

The present review will focus on the morphological properties of lipoproteins as shown by various electron microscopic techniques including fixation and shadowing, fixation and sectioning, freeze-etching, and negative staining. The main emphasis will be on the isolated lipoprotein fractions since such fractions are physically and chemically well defined. The lipoprotein fractions discussed are mainly those from human plasma; however, where it pertains, the review will consider plasma lipoproteins from other mammalian species. Since lipoproteins isolated from plasma and serum appear to be identical in their chemical and physical properties, the terms "plasma" and "serum" will be used interchangeably.

The aim of this review is twofold: (1) to point out the capabilities as well as the limitations of the various electron microscopic techniques in the analysis of lipoprotein structure, and (2) to stimulate further studies on morphological aspects of lipid-protein interactions in plasma lipoproteins as well as in model systems containing specific lipids and proteins.

## II. Physical and Chemical Characterization of Plasma Lipoproteins

Based on many studies, four major classes of human plasma lipoproteins have been characterized by preparative and analytical ultracentrifugation and by electrophoresis. These classes are chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL). Each of these classes can, furthermore, be ultracentrifugally fractionated into subclasses on the basis of their density. According to their electrophoretic migration on paper, the HDL are designated  $\alpha$ -lipoproteins, LDL are designated  $\beta$ -lipoproteins, and VLDL are designated pre- $\beta$ -lipoproteins. The chylomicrons do not migrate in the electrical field and remain at the origin.

The operational classification and compositional properties of the major classes of plasma lipoproteins are summarized in Table I.



Table 1: CLASSIFICATION AND PROPERTIES OF THE MAJOR CLASSES OF PLASMA LIPOPROTEINS

	Chylomicrons	Very low density lipoproteins (VLDL)	Low density lipoproteins (LDL)	High density lipoproteins (HDL)
Preparative ultracentrifugal density classification <sup>a</sup>	d < 0.95 gm/ml	d 0.95–1.006 gm/ml	d 1.006–1.063 gm/ml	d 1.063–1.21 gm/ml
Analytic ultracentrifugal flotation rate classification <sup>b</sup>	$S_f > 400$	$S_f$ 20–400	$S_f$ 0–20	$F_{1.20}$ 0–9
Paper electrophoretic migration classification	Chylomicrons	pre- $\beta$	$\beta$	$\alpha$
Average composition <sup>c</sup>				
Phospholipid	7	19	22	24
Unesterified cholesterol	2	7	8	2
Cholesteryl esters	5	13	37	20
Triglyceride	84	51	11	4
Protein	2	8	21	50
Major protein (apolipoprotein) constituents <sup>d</sup>	Probably include: apoLP-ser apoLP-glu apoLP-ala <sub>1</sub> apoLP-ala <sub>2</sub>	apoLDL apoLP-ser apoLP-glu apoLP-ala <sub>1</sub> apoLP-ala <sub>2</sub>	apoLDL	apoLP-glnI <sup>e</sup> apoLP-glnII/

<sup>a</sup> d. values designate density ranges of the lipoprotein classes as isolated from plasma by sequential preparative ultracentrifugation.  
<sup>b</sup>  $S_f$  values are ultracentrifugal flotation rates expressed as Svedbergs ( $10^{-13}$  cm/sec/dyne/gm) in a solution of density 1.063 gm/ml at 26°C (1.748 molal NaCl).  $F_{1.20}$  values are flotation rates expressed in Svedbergs in a solution of density 1.20 gm/ml (Ewing *et al.*, 1965).  
<sup>c</sup> Major compositional constituents are listed; where available, content of nonesterified fatty acids was included in compositional composition but not listed in table (Hatch and Lees, 1968; Oncley and Harvie, 1969).  
<sup>d</sup> Protein constituents termed apolipoproteins (apoLP) are designated by their carboxyl terminal amino acids, abbreviated as follows: ala (alanine), glu (glutamine), glu (glutamic), ser (serine) (Fredrickson, 1969; Gotto *et al.*, 1971; Nichols *et al.*, 1972; Levy *et al.*, 1971; Eisenberg *et al.*, 1972).  
<sup>e</sup> Corresponds to ApoA-I of Kostner and Alaupovic (1971); R-thr of Shore and Shore (1969); Fraction III of Scannu *et al.* (1969); and Band C of Rudman *et al.* (1970).  
<sup>f</sup> Corresponds to ApoA-II of Kostner and Alaupovic (1971); R-glu of Shore and Shore (1969); Fraction IV of Scannu *et al.* (1969); and Band D of Rudman *et al.* (1970).