Liposome Technology

Volume II

Incorporation of Drugs, Proteins, and Genetic Material

Editor
Gregory Gregoriadis, Ph.D.

Liposome Technology

Volume II

Incorporation of Drugs, Proteins, and Genetic Material

Editor
Gregory Gregoriadis, Ph.D.
Clinical Research Centre
Middlesex, England

CRC Press, Inc.
Boca Raton, Florida

Library of Congress Cataloging in Publication Data
Main Entry under title:

Liposome technology.

Bibliography: v., p.
Includes indexes.
Contents: v. 1. Preparation of liposomes -- v. 2. Incorporation of drugs, proteins, and genetic material -- v. 3. Targeted drug delivery and biological interaction.
1. Drugs--Vehicles. 2. Liposomes. I. Gregoriadis, Gregory.
RS201.V43L55 1983 615'.191 83-7088
ISBN 0-8493-5316-5 (v. 1)
ISBN 0-8493-5317-3 (v. 2)
ISBN 0-8493-5318-1 (v. 3)

Direct all inquiries to CRC Press, Inc., 2000 Corporate Blvd., N.W., Boca Raton, Florida, 33431.

International Standard Book Number 0-8493-5316-5 (Vol. 1)
International Standard Book Number 0-8493-5317-3 (Vol. 2)
International Standard Book Number 0-8493-5318-1 (Vol. 3)

Library of Congress Card Number 83-7088
Printed in the United States

PREFACE

Targeting of drugs through carrier systems that would, ideally, ignore the normal part of the body and home to diseased areas in need of treatment, has been for the most part of this century only a little more credible than the philosophers' stone. Recently, however, parallel but separate developments have began to transform drug targeting from a striking intellectual concept to a powerful exact science. First, a wide array of biological molecules are now recognized to possess ligands with specific affinity for respective receptors on the cell's surface. Second, the technical breakthrough in the production of monoclonal antibodies has made available in large quantities immunoglobulins that are highly specific for individual antigens. Such molecules form the basis of the modern approach to receptor (including cell surface antigens in a wider sense) mediated drug delivery. Further, they are being instrumental to a third development namely, the emergence of sophisticated artificial carriers exemplified by liposomes.

Liposomes are unusually versatile, notably in size, surface charge, lipid composition, the plethora of pharmacologically active molecules they can accommodate in both the aqueous and lipid phase and permeability to entrapped molecules. This not only has led to a number of ingenious techniques for the preparation of liposomes with practical advantages, it has also helped to adjust the system to a multitude of uses in cell biology, pharmacology, immunology, genetic engineering, and therapeutic and preventive medicine. A significant advance has also been made by our ability to control the fate of liposomes and the effect of their contents in vivo. This was achieved through knowledge of factors in the body that influence liposomal behavior. Modification of the structure of liposomes in a variety of ways enables us to harness such behavior to suit particular needs. The drug-carrier potential of liposomes has now acquired a new important dimension through the use of targeted molecules. Receptor-recognizing antibodies, glycoproteins, and glycolipids anchored onto the surface of liposomes are able to mediate uptake of the liposomal moiety and its drug contents by target cells. There are clear indications that targeting of liposomes can occur in vivo, at least for cells to which the carrier has immediate access.

Although the role of liposomes in drug targeting has been discussed extensively in several reviews and books, there has been no comprehensive coverage of related methodology. This book constitutes the first attempt to put together all aspects of liposome technology as applied to medical sciences. Contributors were encouraged to place emphasis on methodology as experienced in their own laboratory and in certain cases, the same methodologies are described by more than one laboratory. This was a deliberate policy: several aspects of liposome technology are still not an exact science and personal experience coming from more than one source was thought likely to help everyone, especially those who have just entered the field. A typical chapter includes (1) an introductory section directly relevant to the author's subject with concise coverage of relevant literature; (2) a detailed methodology section presenting experiences from the author's laboratory and a few examples of actual application of the method presented; (3) a critical discussion to enable the reader to appreciate the advantages and disadvantages of the method and compare it with those developed by other workers. The fifty chapters contributed have been distributed logically into three volumes. Volume I deals directly with methods for the preparation of liposomes and auxiliary techniques. Volume II describes procedures for the entrapment of a number of drugs, including genetic material, into selected types of liposomes. These two volumes contain virtually all methods available for efficient drug entrapment in the presence or, preferably, absence of organic solvents. Finally, Volume III is devoted to the growing variety of techniques yielding targeted liposomes and to approaches of studying liposomal behavior in the biological milieu both in vitro and in vivo.

It has been a pleasure for me to edit this book and to come into close contact with the thoughts and experiences of so many liposomologists. It is hoped that in view of the great

and ever increasing number of workers in the field and the significance that liposomes have attained in a wide spectrum of disciplines, this multi-authored book in liposome technology will serve a useful purpose. I take this opportunity to thank all authors for their enthusiastic response to my invitation to contribute. I am also grateful to Mrs. M. Moriarty for editorial assistance and to CRC Press, Inc. personnel for their valuable help and truly professional cooperation.

Gregory Gregoriadis
June 1983

THE EDITOR

Gregory Gregoriadis, Ph.D., is a senior member of staff at the Medical Research Council's Clinical Research Centre, Harrow, Middlesex, England. He received his first degree in Chemistry from the University of Athens and his M.Sc. and Ph.D. in Biochemistry from McGill University. He has carried out research in as diverse fields as the metabolism of trace metals and the interaction of plasma glycoproteins with hepatic receptors. Since 1970 he has worked, published, and lectured extensively on the targeting of drugs via liposomes. Dr. Gregoriadis' interest in drug targeting is reflected in his founding in 1978 the Gordon Research Conference Series on "Drug Carriers in Biology and Medicine" of which he was the first Chairman and in 1981 the NATO Advanced Studies Institute Series "Targeting of Drugs" of which he is the Director. He has also been the editor of *Drug Carriers in Biology and Medicine* and the senior editor of *Liposomes in Biological Systems* and *Targeting of Drugs*.

CONTRIBUTORS

T. M. Allen, Ph.D.
Associate Professor
Department of Pharmocology
University of Alberta
Edmonton, Alberta, Canada

Carl R. Alving, M.D.
Department of Membrane Biochemistry
Walter Reed Army Institute of Research
Washington, D.C.

B. K. Bachhawat, Ph.D.
Department of Enzyme Engineering
Indian Institute of Chemical Biology
Calcutta, India

John D. Baldeschwieler, Ph.D.
Professor of Chemistry
California Institute of Technology
Pasadena, California

Jacques Barbet, Ph.D.
Section on Analytic Immunology
Centre d'Immunologie INSERM-CNRS
Marseille, France

Gillian M. Barratt, Ph.D.
Research Fellow
Department of Biochemistry
Charing Cross Hospital Medical School
London, England

Edward A. Bayer, Ph.D.
Department of Biophysics
Weizmann Institute of Science
Rehovot, Israel

Pierre Baudhuin, Ph.D.
Professor
Laboratoire de Chimie Physiologique
University of Louvain
Brussels, Belgium

Robert Blumenthal, Ph.D.
Senior Investigator
Laboratory of Mathematical Biology
National Cancer Institute
National Institutes of Health
Bethesda, Maryland

Vicente J. Caride, M.D.
Associate Clinical Professor
School of Medicine
Yale University; and
Director, Department of Nuclear Medicine
Hospital of St. Raphael
New Haven, Connecticut

Dennis Chapman, Ph.D.
Professor
Department of Biophysical Chemistry
Royal Free Hospital School of Medicine
University of London
London, England

Pierre Chatelain, Ph.D.
Laboratoire de Recherche
Continental Pharma
Mont-Saint-Guilbert, Belgium

Theodore C. Cree, Ph.D.
Research Associate
Department of Human Oncology
University of Wisconsin
Madison, Wisconsin

Amelia Cudd, Ph.D.
Centre de Biophysique Moléculaire
Centre National de la Recherche Scientifique
Orleans, Cedex, France

Jan Damen, Ph.D.
Division of Cell Biology
Netherlands Cancer Institute
Amsterdam, The Netherlands

Pijush K. Das, Ph.D.
Visiting Fellow
Developmental and Metabolic Neurology Branch
National Institutes of Health
Bethesda, Maryland

David W. Deamer, Ph.D.
Professor
Department of Zoology
University of California
Davis, California

Fabienne Defrise-Quertain, Ph.D.
Laboratoire de Chimie Physique des Macromolécules aux Interfaces
Université Libre de Bruxelles
Brussels, Belgium

Michel Delmelle, Ph.D.
Department of Atomic and Molecular Physics
University of Liege
Liege, Belgium

Paul Dragsten, Ph.D.
Proctor and Gamble, Inc.
Cincinnati, Ohio

Hiroo Endoh, Ph.D.
Research Member
Kureha Chemical Company
Tokyo, Japan

Eva Eriksson, Ph.D.
Research Scientist
Department of Biochemistry
Chemical Center
University of Lund; and Ferrosan
Malmö, Sweden

Hakan Eriksson, Ph.D.
Research Scientist
Department of Pure and Applied Biochemistry
Chemical Center
University of Lund
Lund, Sweden

Michael W. Fountain, Ph.D.
Senior Research Scientist
The Liposome Company
Princeton, New Jersey

Jürgen Freise, M.D.
Senior Research Scientist
Department of Internal Medicine and Gastroenterology
Medizinische Hochschule Hannover
Hannover, West Germany

Sven Frøkjaer, Ph.D.
Head, Physical-Chemistry Laboratory
Novo Research Institute
Bagsvaerd, Denmark

Nigel Gains, Ph.D.
Research Scientist
Department of Biochemistry
Swiss Federal Institute of Technology
Zurich, Switzerland

Prahlad C. Ghosh, Ph.D.
Research Associate
Department of Microbiology
Uniformed Services University of the Health Sciences
Bethesda, Maryland

Gregory Gregoriadis, Ph.D.
Head, Laboratory for Drug Targeting
Clinical Research Centre
Harrow, Middlesex, England

Pierre Guiot, Ph.D.
Research Worker
International Institute of Cellular and Molecular Pathology
Brussels, Belgium

Luke S. S. Guo, Ph.D.
Group Leader
Research and Development
Tago, Inc.
Burlingame, California

Robert L. Hamilton, Ph.D.
Senior Staff
Cardiovascular Research Institute
Associate Professor of Anatomy
University of California Medical Center
San Francisco, California

Yoshiyuki Hashimoto, Ph.D.
Professor and Chairman
Department of Hygienic Chemistry
Pharmaceutical Institute
Tohoku University
Sendai, Japan

Helmut Hauser, Ph.D.
Department of Biochemistry
Swiss Federal Institute of Technology
Zurich, Switzerland

Amalia S. Havaranis, Ph.D.
Technical Specialist
AMF-CUNO Microfiltration Division
Meriden, Connecticut

Pierre Henkart, Ph.D.
Immunology Branch
National Cancer Institute
National Institutes of Health
Bethesda, Maryland

Stuart M. Heywood, Ph.D.
Professor and Head
Department of Genetics and Cell Biology
University of Connecticut
Storrs, Connecticut

Ejnar L. Hjorth, B. Pharm., M.Sc.
Analytical Chemist
Department of Analysis
Bagsvaerd, Denmark

Anthony Huang, Ph.D.
Biology Division
Oak Ridge National Laboratory
Oak Ridge, Tennessee

Leaf Huang, Ph.D.
Associate Professor of Biochemistry
University of Tennessee
Knoxville, Tennessee

Karl J. Hwang, Ph.D.
Associate Professor
School of Pharmacy
University of Southern California
Los Angeles, California

Vilma K. Jansons, Ph.D.
Associate Professor of Microbiology
UMDNJ-New Jersey Medical School
Newark, New Jersey

D. S. Johnston, Ph.D.
Biochemistry and Chemistry Department
Royal Free Hospital Medical School
University of London
London, England

Stephen J. Kennel, Ph.D.
Biology Division
Oak Ridge National Laboratory
Oak Ridge, Tennessee

Christopher J. Kirby, Ph.D.
Senior Research Scientist
Division of Clinical Sciences
The Clinical Research Centre
Harrow, Middlesex, England

Richard Kirsh, Ph.D.
Department of Immunology
Smith Kline and French Laboratories
Philadelphia, Pennsylvania

Richard D. Klausner, Ph.D.
Laboratory of Biochemistry and Metabolism
National Institute for Arthritis, Metabolic, and Digestive Diseases
National Institutes of Health
Bethesda, Maryland

Thomas Koestler, Ph.D.
Department of Tumor Biology
Smith Kline and French Laboratories
Philadelphia, Pennsylvania

A. W. T. Konings, Ph.D.
Department of Radiopathology
University of Groningen
Groningen, The Netherlands

R. Lazo, B.S.
Department of Experimental Pathology
Roswell Park Memorial Institute
Buffalo, New York

Peter I. Lelkes, Ph.D.
Scientist
Laboratory of Cell Biology and Genetics
National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases
National Institutes of Health
Bethesda, Maryland

Lee D. Leserman, M.D., Ph.D.
Section on Biological Applications of Liposomes
Centre d'Immunologie INSERM-CNRS
Marseille, France

Paul F. Lurquin, Ph.D.
Associate Professor of Genetics
Program in Genetics and Cell Biology
Washington State University
Pullman, Washington

Patrick Machy, Ph.D.
Section on Biological Applications of Liposomes
Centre d'Immunologie INSERM-CNRS
Marseille, France

Richard L. Magin, Ph.D.
Assistant Professor of Electrical Engineering and Bioengineering
University of Illinois
Urbana, Illinois

Bo Mattiasson, Ph.D.
Associate Professor
Department of Pure and Applied Biochemistry
Chemical Center
University of Lund
Lund, Sweden

Inger Mattsby-Baltzer, Ph.D.
National Research Council Research Associate
Department of Membrane Biochemistry
Walter Reed Army Institute of Research
Washington, D.C.

E. Mayhew, Ph.D.
Cancer Research Scientist V
Roswell Park Memorial Institute; and
Associate Research Professor
State University of New York
Buffalo, New York

Toshiyuki Nagata, Ph.D.
Faculty of Science
Department of Biology
Nagoya University
Nagoya, Japan

Claude Nicolau, Ph.D.
Professor and Director
Centre de Biophysique Moléculaire
Centre National de la Recherche Scientifique
Orleans, Cedex, France

John T. O'Loughlin, Ph.D.
Postdoctoral Researcher
Marine Biological Laboratory
Woods Hole, Massachusetts

Mitree M. Ponpipom, Ph.D.
Senior Research Fellow
Membrane and Arthritis Research Department
Merck Sharp and Dohme Research Laboratories
Rahway, New Jersey

George Poste, D.V.M., Ph.D.
Vice President
Director of Research and Development
Smith Kline and French Laboratories; and
Research Professor
Department of Pathology and Laboratory Medicine
University of Pennsylvania
Philadelphia, Pennsylvania

Evelyn Ralston, Ph.D.
Laboratoire de Chimie Biologique
Universite Libre de Bruxelles
Gensie, Belgium

L. S. Rao, Ph.D.
Pharmaceutical Development
The Wellcome Foundation Ltd.
Dartford, Kent, England

J. M. Ruysschaert, Ph.D.
Associate Professor
Laboratoire de Chimie Physique des Macromolécules aux Interfaces
Universite Libre de Bruxelles
Brussels, Belgium

Brenda E. Ryman, Ph.D.
Professor
Department of Biochemistry
Charing Cross Hospital Medical School
London, England

Gerrit L. Scherphof, Ph.D.
Professor of Physiological Chemistry
State University
Groningen, The Netherlands

Judith Senior, B.Sc.
Research Biochemist
Clinical Research Centre
Harrow, Middlesex, England

T. Y. Shen, Ph.D., D.Sc.
Vice President, Membrane and Arthritis Research
Merck Sharp and Dohme Research Laboratories
Rahway, New Jersey

Shigeki Shichijo, Ph.D.
Department of Immunology
Kurume University
Kurume, Fukuoka, Japan

Harm Snippe, Ph.D.
Associate Professor of Immunology
Department of Immunology
Laboratory of Microbiology
State University of Utrecht
Utrecht, The Netherlands

Issac Soliemani
Department of Genetics and Cell Biology
University of Connecticut
Storrs, Connecticut

Henry D. Sostman, M.D.
Assistant Professor
Department of Diagnostic Imaging
Yale University School of Medicine
New Haven, Connecticut

George Strauss, Ph.D.
Professor of Chemistry
Rutgers, The State University of New Jersey
New Brunswick, New Jersey

Minoru Sugawara
Student
Department of Hygienic Chemistry
Tohoku University
Sendai, Japan

Glenn M. Swartz
Department of Membrane Biochemistry
Walter Reed Army Institute of Research
Washington, D.C.

Inga-Mai Tegmo-Larsson, Ph.D.
Visiting Assistant Professor
Department of Chemistry
Hobart and William Smith Colleges
Geneva, New York

Vladimir P. Torchilin,Ph.D.
Head, Laboratory of Enzyme Engineering
Institute of Experimental Cardiology
National Cardiology Research Centre
Moscow, U.S.S.R.

Nilden S. Tüzel*
Postgraduate Research Student
Department of Biochemistry
Charing Cross Hospital Medical School
London, England

W. J. Vail, Ph.D.
Professor and Chairman
Department of Biology
Frostburg State College
Frostburg, Maryland

Catherine Vakirtzi-Lemonias, Ph.D.
Senior Research Staff
Biology Division
Nuclear Research Center "Demokritos"
Greek Atomic Energy Commission
Attikis, Greece

Arend Jan van Houte, Ph.D.
Research Fellow
Department of Immunology
Laboratory of Microbiology
State University of Utrecht
Utrecht, The Netherlands

Hans Georg Weder, Ph.D.
Professor
Swiss Federal Institute of Technology
Zurich, Switzerland

* Present address: Department of Biochemistry, Royal Free Hospital Medical School, London, England.

John N. Weinstein, Ph.D.
Senior Investigator
Laboratory of Mathematical Biology
National Cancer Institute
National Institutes of Health
Bethesda, Maryland

Meir Wilchek, Ph.D.
Professor
Department of Biophysics
Weizmann Institute of Science
Rehovot, Israel; and National Institutes of Health
Bethesda, Maryland

Jan Wilschut, Ph.D.
Assistant Professor
Laboratory of Physiological Chemistry
University of Groningen
Groningen, The Netherlands

Ole Wørts, B. Pharm., M.Sc.
Research Manager
Department of Pharmaceutics
Novo Research Institute
Bagsvaerd, Denmark

Po-Shun Wu, Ph.D.
Postdoctoral Researcher
Division of Chemistry and Chemical Engineering
California Institute of Technology
Pasadena, California

Milton B. Yatvin, Ph.D.
Professor of Human Oncology and Radiology
University of Wisconsin
Madison, Wisconsin

Othmar Zumbuehl, Ph.D.
Assistant Professor
Swiss Federal Institute of Technology
Zurich, Switzerland

TABLE OF CONTENTS

Volume I

Volume II

Volume III