EXPERIMENTAL BIOPHYSICS

A SERIES OF ADVANCES

EDITED BY

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THE UNIVERSITY OF TEXAS
M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE
HOUSTON, TEXAS

VOLUME 2

1969

MARCEL DEKKER, New York and London

MARCEL DEKKER, INC.
95 Madison Avenue, New York, New York 10016

United Kingdom Edition
Published by
MARCEL DEKKER LTD.

LIBRARY OF CONGRESS CATALOG CARD NUMBER 66-29484

PRINTED IN THE UNITED STATES OF AMERICA

THEORETICAL AND

Theoretical and Experimental Biophysics

VOLUME 2

Preface

In the preface to Volume 1 of *Theoretical and Experimental Biophysics* I suggested that the term Biophysics, as applied to this series, will be interpreted in a broad sense, that is "the study or description of biological phenomena in terms of the contributing physical events." The articles included in this volume, I believe, illustrate such an approach.

"Biological Membranes" by Edward Korn is, for the space utilized, a remarkably comprehensive outline of the current state of knowledge of the topic from both the theoretical and experimental viewpoint. George Czerlinski's review discusses the theory and application of chemical relaxation (chemical kinetics following a fast or stepwise perturbation of conditions). This detailed description is by one of the pioneers in the field. The article by Peter Mitchell on chemosmotic coupling presents an original and intriguing approach toward a unified description of (bio)-chemical reactions in terms of the stoichiometric relationships, the energy transductions, and the movement of the reactants. Dr. Andrew Bajer has given a review of the significant technical and practical considerations in time-lapse microphotography, a field of increasing importance in quantitative studies of intracellular and cellular movement and growth kinetics. A discussion of the structure and arrangement of the nucleic acids in viruses, as presently understood, is given in the article by Heather Mayor; such descriptions are obviously necessary before a real understanding of the replicative or genetic processes of viruses can be achieved.

The reader who harbors either the motivation or qualification to find interest in all the contributions of this series is probably a rare duck. It is, however, the intent of the publisher and editor to select contributions such that each volume should contain at least one or two articles of interest to the randomly selected (enlightened?) biological scientist. There certainly is no dearth of topics to choose from. When the publisher first asked for suggestions I found it difficult to terminate a list of dozens that immediately came to mind; such is the price for participation in an evolving field such as biophysics.

February, 1969 A. C.

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Time-Lapse Microcinematography in Cell Biology

Andrew Bajer

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Heather D. Mayor

Biological Membranes

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I. INTRODUCTION

Two detailed theories of membrane structure have been proposed. One, the unit membrane theory (*1*), states that all membranes are fundamentally alike and consist of a bimolecular leaflet of phospholipid covered on both sides by a monomolecular layer of nonlipid, usually protein in the β-configuration. The forces holding the bimolecular leaflet together are considered to be van der Waals and other nonpolar interactions among the hydrocarbon portions of the long-chain fatty acids of the phospholipid molecules and other nonpolar lipids, principally steroids, that may be present. The polar groups of the phospholipids are visualized as oriented outwards and linked to the covering layer of protein by ionic bonds. This concept is derived from X-ray–diffraction and electron-microscopic studies of myelin and model systems, and from some of the properties of phospholipids in bulk and as monolayers and bilayers. The theory is extended to other biological membranes because myelin is continuous with the plasma membrane of the Schwann cell and the electron-microscopic image of that membrane is, at least superficially, similar to the image of most other membranes in fixed and positively stained preparations. The unit membrane theory is a refinement and extension of the paucimolecular

model of Davson and Danielli (*2*), which was based on early concepts of cell permeability and the surface tension, electrical properties, and chemical composition of membranes.

The alternative theory (*3*) states that all membranes are fundamentally alike and consist of vesicular or tubular systems which are composed of repeating units of lipoprotein macromolecules one layer thick. The structural subunits are viewed as identical to the functional subunits. This concept is derived mainly from electron microscopy of the inner membrane and cristae of mitochondria by the technique of negative staining and from extensive biochemical studies of the macromolecular complexes derived from these membranes that catalyze the reactions of electron transport. The concept is extended to other membranes in the belief, for which some support exists, that integrated metabolic pathways are membrane-associated phenomena and the assumption that they are catalyzed by macromolecular complexes that are in themselves the elements of the membrane continuum.

A third view, that any given membrane may exist in any one of several interchangeable configurations which may all be converted to the same stable form by the techniques used to study membrane structure, has its attractions; but since it is not readily susceptible to proof, it will not be considered further.

This review has been written from the position that the best present theory of membrane structure is the pauci-informational theory. This theory states that we have too little information about most membranes to justify any conclusions about their structures. Furthermore, even for the two most well-studied membrane systems there is still no unequivocal way to rationalize all the available morphological, chemical, and enzymatic data, and, therefore, extrapolations from these membranes to other membranes must be made with caution. For these reasons, the data for each membrane system have been summarized independently and few, if any, generalizations have been offered. Eventually, enough information must be acquired to allow reasonable postulations for the structure of all biological membranes; but to maintain that all membranes are essentially identical, at the present state of knowledge, requires a careful selection and omission of data which may seriously affect the design and interpretation of future experiments.

Limitations of time preclude the reading of every pertinent paper, and limitations of space preclude adequate discussion of every paper that has been read. In particular, the interesting and germane studies of the properties of lipids in bulk phase, in monolayers, and in bilayers are

reviewed only as they relate directly to the biological membranes under consideration. Fortunately, there are several recent reviews to which the reader can be referred (*4–7*). Elsewhere I have (*8*) briefly reviewed this same topic from a somewhat different viewpoint, emphasizing what we do not know about membranes and how to study them. It is hoped that this review presents a reasonable summary of what *is* known.*

II. MYELIN

The multilayered mebrane system that surrounds many nerves is probably the most studied and most reviewed (*1,6,9*) biological membrane. Its ordered structure has allowed examination by a variety of physical techniques, and its relative abundance and homogeneity simplifies the problems of isolation and purification for chemical analyses.

A. Chemical Data

For meaningful chemical data myelin must first be isolated in high purity. The techniques in most general use are the separation of an homogenate of white matter by centrifugation in sucrose gradients

TABLE I

The Molar Percentage of the Lipids of Myelin

	Human (*13*)	Bovine (*14*)	Rat (*15*)
Cholesterol	39	34	46
Cerebroside	14	10	17
Cerebroside sulfate	5	2	3
Sphingomyelin	5	3	5
Phosphatidyl ethanolamine	15	14	14
Phosphatidyl serine	5	6[a]	3
Phosphatidyl choline	13	8	8
Phosphatidyl inositides	2	—	1

Recent analysis of developing rat brain suggest that gangliosides and phosphatidic acid may also be components of myelin (*18,19*).

[a] Includes inositides.

(*10–12*). Analyses of myelin prepared from human, bovine, rat, and guinea pig are all similar (*13–16*). Human myelin, for example, contains about 20% protein and 80% lipid. This is the smallest percentage of protein of any membrane. None of the protein has been shown to be enzymatic.

* This review was completed in June, 1967 and partially revised in January, 1968.

In specific, alkaline phosphatase, 5′-nucleotidase and leucyl-naphthylamidase have been looked for and found to be absent (*17*). The compositions of the lipids are shown in Table I. These analyses are unusual for membranes with respect to the presence of cerebrosides and a concentration of cholesterol that is higher than that of all membranes other than the erythrocyte ghost. Plasmalogens account for 50% of the phosphatidyl

TABLE II

Percent Major Fatty Acids of Phospholipids of Human Myelin (*20*)

Carbon chain: unsaturated bonds	Phosphatidyl ethanolamine	Phosphatidyl serine	Phosphatidyl choline	Sphingomyelin
16:0	6.5	2.6	40.1	5.4
18:0	7.7	40.0	6.1	33.6
18:1	72.5	43.3	51.6	0.4
20:1	3.9	3.6		
20:4	1.6	4.7		
22:5	4.6	2.3		
23:0				1.4
24:0				8.0
24:1				40.0
25:0				1.8
25:1				3.6
26:1				2.4

ethanolamine, 1% of the phosphatidyl choline, and 1 to 10% of the phosphatidyl ethanolamine. The lipids of myelin are also unusual with respect to fatty acid composition (*20*). Only about 10% of the total fatty acids are polyunsaturated C_{20} to C_{24} compounds, and the overwhelming majority are the saturated and monounsaturated C_{18} acids, stearic and oleic (Table II). The fatty acids of the cerebrosides are particularly unusual in that they are predominantly α-hydroxy C_{22} to C_{26} saturated and monounsaturated fatty acids that occur only in myelin (Table III). It may well be that the unique stability of myelin and its function as an electrical insulator are a direct function of this unusual lipid composition.

B. Physical Studies

Polarized light microscopy and X-ray–diffraction analysis of fresh nerve revealed a regular repeat pattern of radially oriented molecules with a spacing of 180 to 185 A (*21,22*). By the same techniques artificial myelin forms prepared from the lipids extracted from natural myelin were shown

TABLE III

Percent Major Fatty Acids of Cerebrosides of Human Myelin (*20*)

Carbon chain: unsaturated bonds (*h* is α-hydroxy)	Cerebroside	Cerebroside sulfate
16:0	—	6.4
18:0	1.4	2.9
22:0	—	1.0
24:0	2.5	11.0
24:1	7.0	32.0
25:0	1.4	4.6
25:1	2.3	5.5
26:0	—	1.5
26:1	1.0	6.2
16*h*:0	—	1.6
20*h*:0	1.0	—
22*h*:0	10.0	3.2
23*h*:0	10.0	2.5
24*h*:0	26.6	10.0
24*h*:1	19.7	2.4
25*h*:0	3.7	5.5
25*h*:1	2.6	—
26*h*:0	1.6	6.2
26*h*:1	5.7	—

to be bimolecular leaflets with a repeat pattern of approximately 60 to 70 A. This led to the conclusion that the repeating structure of myelin consists of two bimolecular leaflets interspersed by a layer of protein (or, more rigorously, nonlipid) about 25 A thick. When Finean (*23*) observed that under certain conditions the repeat distance measured by X-ray diffraction could be halved, myelin could be considered as a repeating structure of bimolecular leaflets of lipids 50 to 55 A thick separated by layers of nonlipid 30 A thick. Alternate layers of nonlipid were thought to be different (the difference factor), thus explaining the larger repeat distance of the earlier X-ray data. From these data details of the orientation of the lipid in the bimolecular leaflet could not be deduced, but recently Finean and Burge (*24*) have attempted a Fourier analysis of the X-ray data. The electron-density maps thus obtained are interpreted in terms of a bimolecular leaflet in which the polar heads of the phospholipid molecules are oriented outwards.