

Current Topics in Membranes and Transport

VOLUME 20

MOLECULAR APPROACHES TO EPITHELIAL TRANSPORT

Guest Editors

**James B. Wade
Simon A. Lewis**

ACADEMIC PRESS

Current Topics in Membranes and Transport

Edited by

Arnost Kleinzeller

Department of Physiology

*University of Pennsylvania School of
Medicine*

Philadelphia, Pennsylvania

Felix Bronner

Department of Oral Biology

*University of Connecticut Health
Center*

Farmington, Connecticut

VOLUME 20

Molecular Approaches to Epithelial Transport

Guest Editors

James B. Wade

Department of Physiology

*Yale University School of Medicine
New Haven, Connecticut*

Simon A. Lewis

Department of Physiology

*Yale University School of Medicine
New Haven, Connecticut*

Volume 20 is part of the series (p. xix) from the Yale Department of Physiology under the editorial supervision of:

Joseph F. Hoffman

Department of Physiology

*Yale University School of Medicine
New Haven, Connecticut*

Gerhard Giebisch

Department of Physiology

*Yale University School of Medicine
New Haven, Connecticut*

1984



ACADEMIC PRESS, INC.

(Harcourt Brace Jovanovich, Publishers)

Orlando San Diego San Francisco New York London
Toronto Montreal Sydney Tokyo São Paulo

**COPYRIGHT © 1984, 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.
Orlando, Florida 32887**

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

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 70-117091

ISBN 0-12-153320-4

PRINTED IN THE UNITED STATES OF AMERICA

84 85 86 87 9 8 7 6 5 4 3 2 1

Contributors

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

- William P. Alles**, Department of Physiology, Yale University School of Medicine, New Haven, Connecticut 06510 (87)
- Daniel Biemesderfer**, Department of Physiology, Yale University School of Medicine, New Haven, Connecticut 06510 (161)
- Chris Clausen**, Department of Physiology and Biophysics, Health Sciences Center, State University of New York, Stony Brook, New York 11794 (47)
- Malcolm Cox**, Renal Electrolyte Section, Medical Service, Philadelphia Veterans Administration Medical Center, and Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104 (271)
- M. E. M. Da Cruz**, Laboratório Nacional de Engenharia e Tecnologia Industrial, Lisbon, Portugal (245)
- Troy E. Dixon**, Department of Medicine, Northport Veterans Administration Hospital, Northport, New York 11768 (47)
- Darrell D. Fanestil**, Division of Nephrology, Department of Medicine, University of California San Diego, La Jolla, California 92093 (259)
- Bliss Forbush III**, Department of Physiology, Yale University School of Medicine, New Haven, Connecticut 06510 (161)
- E. Frömter**, Max-Planck-Institut für Biophysik, 6000 Frankfurt 70, Federal Republic of Germany (27)
- Michael Geheb**,¹ Renal Electrolyte Section, Medical Service, Philadelphia Veterans Administration Medical Center, and Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104 (271)
- David B. P. Goodman**, Department of Pathology and Laboratory Medicine, Hospital of the University of Pennsylvania, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104 (295)
- Victoria Guckian**,² Department of Physiology, Yale University School of Medicine, New Haven, Connecticut 06520 (217)
- Michael C. Gustin**, Department of Biochemistry, University of Wisconsin, Madison, Wisconsin 53706 (295)
- Doris A. Herzlinger**, Department of Anatomy and Cell Biology, Downstate Medical Center, State University of New York, Brooklyn, New York 11203 (181)
- T. Hoshiko**, Department of Physiology, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106 (3)

¹Present address: Department of Medicine, Wayne State University, Harper-Grace Hospital, Detroit, Michigan 48201.

²Present address: Department of Physiology, University of Maryland School of Medicine, Baltimore, Maryland 21201.

- Ivan Emanuilov Ivanov**, Department of Cell Biology, New York University School of Medicine, New York, New York 10016 (199)
- L. Kampmann**, Max-Planck-Institut für Biophysik, 6000 Frankfurt 70, Federal Republic of Germany (27)
- Michael Kashgarian**, Department of Pathology, Yale University School of Medicine, New Haven, Connecticut 06510 (161)
- Ralph J. Kessler**, Division of Nephrology, Department of Medicine, University of California San Diego, La Jolla, California 92093 (259)
- R. Kinne**,³ Department of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, New York 10461 (245)
- Ingeborg Koeppen**,⁴ Department of Physiology, Yale University School of Medicine, New Haven, Connecticut 06510 (217)
- G. Kottra**,⁵ Max-Planck-Institut für Biophysik, 6000 Frankfurt 70, Federal Republic of Germany (27)
- Simon A. Lewis**, Department of Physiology, Yale University School of Medicine, New Haven, Connecticut 06510 (87)
- J. J. Lim**,⁶ Max-Planck-Institut für Biophysik, 6000 Frankfurt 70, Federal Republic of Germany (27)
- J. T. Lin**, Department of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, New York 10461 (245)
- Alicia A. McDonough**, Department of Physiology and Biophysics, University of Southern California School of Medicine, Los Angeles, California 90033 (147)
- George K. Ojakian**, Department of Anatomy and Cell Biology, Downstate Medical Center, State University of New York, Brooklyn, New York 11203 (181)
- Lawrence G. Palmer**, Department of Physiology, Cornell University Medical College, New York, New York 10021 (105)
- Chun Sik Park**, Division of Nephrology, Department of Medicine, University of California San Diego, La Jolla, California 92093 (259)
- Heide Plesken**, Department of Cell Biology, New York University School of Medicine, New York, New York 10016 (199)
- Uzi Reiss**, Laboratory of Molecular Aging, National Institute on Aging, National Institutes of Health, Gerontology Research Center, Baltimore City Hospitals, Baltimore, Maryland 21224 (235)
- Michael J. Rindler**, Department of Cell Biology, New York University School of Medicine, New York, New York 10016 (199)
- B. C. Rossier**, Institut de Pharmacologie de l'Université de Lausanne, CH-1011 Lausanne, Switzerland (125)
- David D. Sabatini**, Department of Cell Biology, New York University School of Medicine, New York, New York 10016 (199)
- Bertram Sacktor**, Laboratory of Molecular Aging, National Institute on Aging, National Institutes of Health, Gerontology Research Center, Baltimore City Hospitals, Baltimore, Maryland 21224 (235)

³Present address: Max-Planck-Institut für Systemphysiologie, D-4600 Dortmund 1, Federal Republic of Germany.

⁴Present address: Anatomisches Institut der Universität Heidelberg, D-6900 Heidelberg, Federal Republic of Germany.

⁵Present address: Department of Internal Medicine, Semmelweis Medical University, 1088 Budapest, Hungary.

⁶Deceased.

James B. Wade,⁷ Department of Physiology, Yale University School of Medicine, New Haven, Connecticut 06510 (217)

N. K. Wills, Department of Physiology, Yale University School of Medicine, New Haven, Connecticut 06510 (61)

⁷Present address: Department of Physiology, University of Maryland School of Medicine, Baltimore, Maryland 21201.

Preface

In recent years the issues in epithelial transport research have gradually evolved toward the molecular level. As recently as ten years ago, the predominant problem was to determine exactly what electrolytes and nonelectrolytes particular epithelia absorb or secrete. Once armed with an understanding of the macroscopic transport processes, researchers asked whether the movement of substances was through the cells (i.e., active) or between the cells (i.e., passive) flowing along favorable electrical and/or chemical gradients. In turn, using electrical or chemical methods, the question was raised at which step in transcellular transport was energy in the form of ATP required. As an example, in transepithelial sodium transport, sodium enters the cell passively down a net electrochemical gradient and is actively extruded into the blood via an ATP-requiring transport protein (the Na^+/K^+ -ATPase). One of the most recent topics being addressed is the mechanism involved in the regulation of these electrolyte and nonelectrolyte transport proteins (synthesis and/or activation), and whether such proteins move substances by a channel-type configuration or a carrier configuration.

It is obvious that the more classic approaches to studying epithelial transport are inadequate by themselves to address these questions fully. It is the purpose of this book to outline and illustrate, by example, some recently developed approaches that can provide important new insight into epithelial transport mechanisms.

Part I of this volume is devoted to the electrical methodology used to address questions such as the following: Does sodium entry across the apical membrane of tight epithelia occur by a channel or carrier mechanism? Do hormones increase the number of transport proteins or the ability of a single protein to carry more ions per unit time? During stimulation of ion transport, are quiescent channels activated, or are cytoplasmic vesicles containing the transport protein mobilized on a certain signal and inserted into the membrane? The methods to be used consist of impedance analysis to measure changes in membrane area associated with stimulation of transport and fluctuation analysis to evaluate alterations in channel density.

Part II provides a wide range of examples of how antibodies to epithelial membrane proteins can be useful. Antibodies are clearly a powerful tool for evaluating the biosynthesis of transport proteins such as Na^+/K^+ -ATPase and

provide a means whereby membrane proteins can be identified and localized in epithelia.

Part III of the volume describes biochemical approaches to characterizing epithelial transport systems. These chapters illustrate approaches being taken for isolation and identification of transport proteins. In addition, these studies show how substrate protection can be utilized to identify chemical groups associated with important sites of a transport system.

We would like to acknowledge the generous financial support of Abbott Laboratories, North Chicago, Illinois; Hoffmann-LaRoche Inc., Nutley, New Jersey; ICI Americas, Inc., Wilmington, Delaware; Miles Laboratories, Inc., Elkhart, Indiana; C. F. Searle & Co., Chicago, Illinois; and the Upjohn Company, Kalamazoo, Michigan, for the Eighth Conference on Membrane Transport Processes sponsored by the Department of Physiology at Yale University School of Medicine which provided a basis for this volume. We also wish to thank Marie Santore for her invaluable assistance in organizing that meeting.

JAMES B. WADE

SIMON A. LEWIS

Yale Membrane Transport Processes Volumes

Joseph F. Hoffman (ed.). (1978). "Membrane Transport Processes," Vol. 1. Raven, New York.

Daniel C. Tosteson, Yu. A. Ovchinnikov, and Ramon Latorre (eds.). (1978). "Membrane Transport Processes," Vol. 2. Raven, New York.

Charles F. Stevens and Richard W. Tsien (eds.). (1979). "Membrane Transport Processes," Vol. 3: Ion Permeation through Membrane Channels. Raven, New York.

Emile L. Boulpaep (ed.). (1980). "Cellular Mechanisms of Renal Tubular Ion Transport": Volume 13 of *Current Topics in Membranes and Transport* (F. Bronner and A. Kleinzeller, eds.). Academic Press, New York.

William H. Miller (ed.). (1981). "Molecular Mechanisms of Photoreceptor Transduction": Volume 15 of *Current Topics in Membranes and Transport* (F. Bronner and A. Kleinzeller, eds.). Academic Press, New York.

Clifford L. Slayman (ed.). (1982). "Electrogenic Ion Pumps": Volume 16 of *Current Topics in Membranes and Transport* (A. Kleinzeller and F. Bronner, eds.). Academic Press, New York.

Joseph F. Hoffman and Bliss Forbush III (eds.). (1983). "Structure, Mechanism, and Function of the Na/K Pump": Volume 19 of *Current Topics in Membranes and Transport* (F. Bronner and A. Kleinzeller, eds.). Academic Press, New York.

James B. Wade and Simon A. Lewis (eds.). (1984). "Molecular Approaches to Epithelial Transport": Volume 20 of *Current Topics in Membranes and Transport* (A. Kleinzeller and F. Bronner, eds.). Academic Press, New York.

Contents

Contributors, xiii

Preface, xvii

Yale Membrane Transport Processes Volumes, xix

PART 1. FREQUENCY DOMAIN ANALYSIS OF ION TRANSPORT

CHAPTER 1. Fluctuation Analysis of Apical Sodium Transport

T. HOSHIKO

- I. Introduction, 3
- II. Mechanics of Fluctuation Analysis, 4
- III. Epithelial Sources of Fluctuations, 7
- IV. Fluctuations in Apical Sodium Conductance, 11
- V. Model Predictions, 14
- VI. Conclusion, 23
- References, 24

CHAPTER 2. Impedance Analysis of *Necturus* Gallbladder Epithelium Using Extra- and Intracellular Microelectrodes

J. J. LIM, G. KOTTRA, L. KAMPMANN, AND E. FRÖMTER

- I. Introduction, 27
- II. Data Analysis Technique, 29
- III. Equivalent-Circuit Models of *Necturus* Gallbladder Epithelium, 32
- IV. Transepithelial and Apparent Intracellular Transfer Functions under Control Conditions, 36
- V. Impedance Loci under Current-Induced Changes of Lateral Space Width, 40
- VI. Conclusions, 45
- References, 45

CHAPTER 3. **Membrane Area Changes Associated with Proton Secretion in Turtle Urinary Bladder Studied Using Impedance Analysis Techniques**

CHRIS CLAUSEN AND TROY E. DIXON

- I. Introduction, 48
- II. Methods, 52
- III. Results, 53
- IV. Discussion, 58
- References, 59

CHAPTER 4. **Mechanisms of Ion Transport by the Mammalian Colon Revealed by Frequency Domain Analysis Techniques**

N. K. WILLIS

- I. Overview of the Basic Features of the Rabbit Descending Colon, 62
- II. Impedance Analysis, 67
- III. Current Fluctuation Analysis, 72
- IV. Summary, 83
- References, 84

CHAPTER 5. **Analysis of Ion Transport Using Frequency Domain Measurements**

SIMON A. LEWIS AND WILLIAM P. ALLES

- I. Introduction, 87
- II. Black Box Analysis of Epithelial Transport, 88
- III. Dissociation of Membrane Properties, 89
- IV. Questions for Analysis in the Frequency Domain, 91
- V. Methods for Frequency Analysis, 91
- VI. Impedance Analysis, 92
- VII. Fluctuation Analysis, 96
- VIII. Summary, 102
- References, 103

CHAPTER 6. **Use of Potassium Depolarization to Study Apical Transport Properties in Epithelia**

LAWRENCE G. PALMER

- I. Introduction, 105
- II. Uses of K^+ Depolarization, 106
- III. Basis for the K^+ -Depolarization Technique, 108
- IV. Evaluation of Depolarization, 110
- V. Conclusions, 118
- References, 119

PART II. USE OF ANTIBODIES TO EPITHELIAL MEMBRANE PROTEINS**CHAPTER 7. Biosynthesis of Na^+ , K^+ -ATPase in Amphibian Epithelial Cells**

B. C. ROSSIER

- I. Introduction and General Background, 125
- II. Purification of Na^+ , K^+ -ATPase, 128
- III. Preparation and Characterization of Immunological Probes, 129
- IV. Biosynthesis of Na^+ , K^+ -ATPase in Amphibian Epithelial Cells, 133
- V. Hormonal Control of Na^+ , K^+ -ATPase Synthesis in Amphibian Epithelial Cells, 134
- VI. Possible Sites of Action of Hormones on the Control of Synthesis and Expression of Na^+ , K^+ -ATPase: A Model, 138
- VII. Concluding Remarks, 140
- References, 141

CHAPTER 8. Use of Antibodies in the Study of Na^+ , K^+ -ATPase Biosynthesis and Structure

ALICIA A. McDONOUGH

- I. Introduction, 147
- II. Methodology: Immune Precipitation and Solid Phase Assay, 148
- III. Applications: Biosynthesis and Structure, 151
- IV. Problems Associated with Antibody Detection of Membrane Proteins, 155
- References, 159

CHAPTER 9. Encounters with Monoclonal Antibodies to Na^+ , K^+ -ATPaseMICHAEL KASHGARIAN, DANIEL BIEMESDERFER,
AND BLISS FORBUSH III

- I. Introduction, 161
- II. Immunization Procedures, 162
- III. Screening Procedures, 164
- IV. Generation, Purification, and Characterization of Monoclonal Immunoglobulin, 170
- V. Morphologic Application of Monoclonal Antibodies to Membrane Surface Proteins, 172
- VI. Summary, 178
- References, 179

CHAPTER 10. Monoclonal Antibodies as Probes of Epithelial Cell Polarity

GEORGE K. OJAKIAN AND DORIS A. HERZLINGER

- I. Production of Monoclonal Antibodies against MDCK Cell Surface Proteins, 182

- II. Distribution of Polarized Cell Surface Proteins, 184
- III. Development and Maintenance of Epithelial Cell Polarity, 187
- IV. Demonstration of Nephron Segment-Specific Proteins, 189
- References, 195

CHAPTER 11. Immunolabeling of Frozen Thin Sections and Its Application to the Study of the Biogenesis of Epithelial Cell Plasma Membranes

IVAN EMANUILOV IVANOV, HEIDE PLESKEN, DAVID D. SABATINI,
AND MICHAEL J. RINDLER

- I. Introduction, 199
- II. Production of Ultrathin Frozen Sections, 201
- III. Indirect Immunolabeling, 206
- IV. Embedding and Staining of Frozen Thin Sections, 210
- V. Concluding Remarks, 212
- References, 214

CHAPTER 12. Development of Antibodies to Apical Membrane Constituents Associated with the Action of Vasopressin

JAMES B. WADE, VICTORIA GUCKIAN, AND INGEBORG KOEPPEN

- I. Introduction, 218
- II. Vasopressin Action, 219
- III. Antibody Assay Strategy, 221
- IV. Characterization of Antibodies, 225
- V. Concluding Remarks, 231
- References, 231

CHAPTER 13. Molecular Modification of Renal Brush Border Maltase with Age: Monoclonal Antibody-Specific Forms of the Enzyme

BERTRAM SACKTOR AND UZI REISS

- I. Introduction, 235
- II. Purification of Maltase, 236
- III. Production of Antibodies to "Young" and "Old" Enzyme, 237
- IV. Characterization of Antibodies, 238
- V. Conclusions, 241
- References, 242

PART III. BIOCHEMICAL CHARACTERIZATION OF TRANSPORT PROTEINS

CHAPTER 14. Sodium-D-Glucose Cotransport System: Biochemical Analysis of Active Sites

R. KINNE, M. E. M. DA CRUZ, AND J. T. LIN

- I. Introduction, 245
- II. The Possible Polymeric Structure of the Sodium-D-Glucose Cotransport System, 247
- III. Characterization of the Functionally Active Sites, 249
- IV. Summary and Conclusions, 255
- References, 256

CHAPTER 15. Probing Molecular Characteristics of Ion Transport Proteins

DARRELL D. FANESTIL, RALPH J. KESSLER, AND CHUN SIK PARK

- I. Introduction, 259
- II. A Phosphate-Binding Proteolipid, 260
- III. Functional Residues of the Na^+ Channel, 263
- IV. How Does Amiloride Inhibit the Na^+ Channel?, 267
- V. Summary, 269
- References, 269

CHAPTER 16. Aldosterone-Induced Proteins in Renal Epithelia

MALCOLM COX AND MICHAEL GEHEB

- I. Introduction, 272
- II. Subcellular Site of Action of Aldosterone, 272
- III. Aldosterone-Induced Proteins: Detection, 276
- IV. Aldosterone-Induced Proteins: Characterization by Two-Dimensional Polyacrylamide Gel Electrophoresis, 279
- V. Summary, 287
- References, 289

CHAPTER 17. Development of an Isolation Procedure for Brush Border Membrane of an Electrically Tight Epithelium: Rabbit Distal Colon

MICHAEL C. GUSTIN AND DAVID B. P. GOODMAN

- I. The Need to Isolate Brush Border Membrane from Tight Epithelia, 295
- II. Approach to Problems of Brush Border Membrane Isolation in Rabbit Descending Colon, 296
- III. Ouabain-Insensitive K^+ -Dependent Phosphohydrolase, 302
- IV. Critical Evaluation of Isolation Technique—Future Utility, 303
- References, 304

Index, 307**Contents of Previous Volumes, 311**

Part I

Frequency Domain Analysis of Ion Transport

Chapter 1

Fluctuation Analysis of Apical Sodium Transport

T. HOSHIKO

*Department of Physiology
Case Western Reserve University School of Medicine
Cleveland, Ohio*

I.	Introduction	3
II.	Mechanics of Fluctuation Analysis	4
	A. Sources of Fluctuations	4
	B. Spectral Properties of Fluctuations	6
III.	Epithelial Sources of Fluctuations	7
	A. Noise Equivalent Circuit of Epithelium	7
	B. Distortion of Apical Current Fluctuations	9
	C. Parameter Estimation	10
IV.	Fluctuations in Apical Sodium Conductance	11
	A. Time Constant of the Apical Sodium Channel	11
	B. Blocker-Induced Conductance Fluctuations	11
V.	Model Predictions	14
	A. Two-State Model	14
	B. Three- and Four-State Models	20
VI.	Conclusion	23
	References	24

I. INTRODUCTION

Fluctuation analysis has been used in the study of nerve and black lipid membranes for several years now, but it is only fairly recently that much has been accomplished in epithelial membranes using this technique. The complexity of epithelial membranes has been perhaps the primary deterrent. Four years ago I described some of the very early results (Hoshiko and Moore, 1978) as did Lindemann and Van Driessche (1978). Lindemann has subsequently reviewed work to 1980 (Lindemann, 1980). Since then two types of models for interpreta-