

Red Cell Membrane Transport in Health and Disease

Springer-Verlag Berlin Heidelberg GmbH

I. Bernhardt · J. C. Ellory (Eds.)

Red Cell Membrane Transport in Health and Disease

With 103 figures and 31 tables.



Springer

Professor Dr. INGOLF BERNHARDT
Universität des Saarlandes
Naturwissenschaftlich-Technische Fakultät III
Arbeitsgruppe Biophysik
P.O.Box 151150
66041 Saarbrücken
Germany

Professor Dr. J. CLIVE ELLORY
University of Oxford
Laboratory of Physiology
Parks Road
Oxford OX1 3PT
United Kingdom

Cataloging-in-Publication Data applied for

A catalog record for this book is available from the Library of Congress.

Bibliographic information published by Die Deutsche Bibliothek

Die Deutsche Bibliothek lists this publication in the Deutsche Nationalbibliografie;

detailed bibliographic data is available in the Internet at <http://dnb.ddb.de>

This work is subject to copyright. All rights are reserved, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilm or in other ways, and storage in data banks. Duplication of this publication or parts thereof is permitted only under the provisions of the German Copyright Law of September 9, 1965, in its current version, and permission for use must always be obtained from Springer-Verlag. Violations are liable for prosecution under German Copyright Law.

ISBN 978-3-642-07920-7 ISBN 978-3-662-05181-8 (eBook)

DOI 10.1007/978-3-662-05181-8

©Springer-Verlag Berlin Heidelberg 2003

Originally published by Springer-Verlag Berlin Heidelberg New York 2003

Softcover reprint of the hardcover 1st edition 2003

<http://www.springer.de>

The use of general descriptive names, registered names, trademarks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

Product liability: The publisher cannot guarantee the accuracy of any information about dosage and application contained in this book. In every individual case the user must check such information by consulting the relevant literature.

57/3020 UW – Gedruckt auf säurefreiem Papier – 5 4 3 2 1 0

Preface

The red cell has been a focus for scientific and medical investigation since the earliest times. A higher erythrocyte sedimentation rate was associated with diseases (usually pyrexias) before the thermometer was invented. Furthermore, ever since the early observers Swammerdam and Leeuwenhoek saw discrete corpuscles in samples of blood using the first microscopes, there has been a significant scientific interest in the structure and function of red blood cells. The later discovery that red cells were not spherical, but biconcave discs introduced a scientific puzzle which is still not completely resolved today, and identified the need for a detailed knowledge of the plasma membrane composition and structure, and its interaction with the cytoskeleton. Important concepts like the lipid bilayer, together with its more recent refinement as asymmetric in phospholipid composition led to the identification of translocases involved in actively maintaining its composition. Understanding the mechanics of red cell deformation as these biconcave discs traverse capillaries was advanced by the pioneering work of Rand and Burton in the Sixties, and progressed by Evans, Skalak and others. Based on the bilayer-couple hypothesis, the shape changes that are possible for a human red cell from echinocyte to stomatocyte were described by Sheetz and Singer in the Seventies in terms of alterations in the individual halves of the bilayer. Certain clinical conditions are associated with obvious changes in red cell morphology. Although alterations in membrane lipid or protein can be responsible, mutant haemoglobins (Hb SS), or changes in other cytoplasmic constituents can also be responsible for dramatic alterations in red cell shape, size and lifetime. Another area of importance which has attracted much recent attention is the intraerythrocytic life of malaria, and the way *Plasmodium falciparum* affects red cell contents, function and membrane transport.

In fact red cell contents, and in particular the intracellular concentrations of cations and anions represents an enduring topic of scientific interest. Over a hundred years ago, Abderhalden made meticulous chemical analyses of red cell ionic composition, and established two important concepts. He identified the fundamental principle of a non-equilibrium distribution of ions across the membrane, i.e. a low intracellular sodium and high intracellular potassium concentration. He also identified differences in internal cation levels between species' red cells, a finding taken further in classical work on HK and LK sheep red cells by Kerr, then Evans and later Tosteson and Hoffman and in the work on carnivore red cells leading to the seminal work of Parker with dog red cells.

The obvious conclusion from the finding of non-equilibrium ion distribution across the red cell membrane was that the cell membrane was impermeable (e.g. Gürber 1895). However, van Slyke et al. in the Twenties showed that the red cell membrane has a very high chloride permeability, whilst amongst others Dean et al. and Eisenmann in the early Forties demonstrated a significant sodium and potassium permeability in the red cells of various species. With this information, and the consequent interest in membrane properties and intracellular composition, it was realised that the red cell represents a convenient model for studying membrane transport processes, since it was assumed to be an easily accessible homo-

geneous population of isolated cells. The further discovery of the resealed red cell ghost allowed the incorporation of selected molecules, and the alteration of ion concentrations. This allowed red cells to be used to investigate the stoichiometry and energetics of the Na^+/K^+ pump and for radioisotope flux studies to characterise a variety of transporters including glucose and amino acid carriers as well as KCC and NKCC, the Ca^{2+} -activated K^+ channel and band 3-mediated anion transport.

The enormous impact of molecular biology on cell physiology was initially less applicable for studying mammalian red cells which lack intracellular organelles, including a nucleus. However, using progenitor cells, or reticulocytes has allowed a molecular biological approach to red cells, and further, the value of the transgenic mouse in answering haematological questions will continue to grow. In this context, the variety of diseases causing anaemias, resulting from unstable haemoglobins, or metabolic alterations involving particular enzymes and pathways continue to attract considerable research effort and attention.

The rationale for the present volume evolved partially from the meetings of the respective Red Cell Clubs in Europe and the USA. Many of the doyens of the red cell world who have contributed to this volume represent a lifetime of knowledge and authority in the field. Additionally there are contributions from the younger generation of scientists who are mapping significant areas of knowledge and contributing new techniques and developments. As always it is difficult to be comprehensive in coverage with such a volume, but we are pleased with the breadth of contributions, and feel that significant ground has been covered. In terms of editorial input, we have exercised a light touch, which means there are differences in style and density between the various contributions. There are also some inconsistencies between American and Oxford English, and some elements of non-native English style may still exist. The editors have tried to produce a book which is consistent within its scientific content. One of us (IB), in addition, has laboured long and hard, and wrestled with the publishing template to achieve homogeneity as far as possible. In this task he has been assisted by Erwin Weiss in Saarbrücken, and by Maureen Paler in Oxford.

The aim of the present authors is to produce a red cell book which will inform and provide cover of the major biophysical and cell physiological aspects of red cell function. We must thank all the contributors for their input, and also Dr. Rogaschweski (Humboldt University Berlin) for the photograph on the cover of the book. Springer-Verlag have been active in helping to edit and process the text, and we thank Mrs. Cuneus for her assistance.

Finally we hope that everyone will find something interesting or surprising in one or other chapters of this book. We hope it may encourage scientists to continue to work on red cells, and solve some of the mysteries still present. In his paper "Questions for red blood cell physiologists to ponder for the millennium" (Blood cell, molecules and diseases (2001) 27:57-61), Joseph Hoffman posed many fascinating and important questions still to be resolved in the field of red cell physiology. Although it must be admitted that the present volume may not answer them directly, it is by stimulating interest in the subject of red blood cells that we will produce the research and hopefully the answers to his millennium questions.

Contents

Contributing Authors.....	XXIII
----------------------------------	--------------

1 Distribution and Movement of Membrane Lipids.....	1
1.1 Introduction.....	1
1.1.1 Lipid Composition, Structure and Motions	1
1.1.2 Measurements of Distribution and Movement of Lipids between the Membrane Bilayer Leaflets.....	3
1.1.3 Steady State Distribution of Lipids between the Membrane Bilayer Leaflets.....	4
1.1.4 Lipid Domains.....	5
1.2 Non-Mediated Passive Transbilayer Movements of Lipids	6
1.2.1 Phospholipids	6
1.2.2 Cholesterol and Other Neutral Lipids.....	7
1.2.3 Fatty Acids: Mediated or Non-Mediated Movement?.....	8
1.3 Mediated Passive Transbilayer Movements of Lipids	9
1.3.1 Ca^{2+} -Activated Bidirectional Movement of Lipids via the Phospho- lipid Scramblase	9
1.3.2 Movement of Anionic Lipids via the Anion Exchanger AE1	11
1.4 Mediated Outward Movement of Newly Synthesized Phosphatidyl- choline	12
1.5 Active Transbilayer Movements of Lipids	13
1.5.1 Inward Movements of Phosphatidylserine and Phosphatidyl- ethanolamine via the Aminophospholipid Translocase (APLT)	13
1.5.2 Outward Movements of Lipids via the Multidrug Resistance Protein MRP1 (ABCC1)	14
1.6 Disturbance of Distribution and Transbilayer Movements of Membrane Phospholipids in Pathology	15
1.7 Summary.....	16
References.....	17

2 Membrane Lipids and Proteins as a Basis of Red Cell Shape and its Alterations	27
2.1 Introduction.....	27
2.2 Early History.....	28
2.3 Experimental Changes of Resting Shape	28
2.3.1 Outline of Observations.....	28
2.3.2 Outline of the Possible Origins of Shape Changes	30
2.4 A Role for the Lipid Bilayer: the Bilayer Couple Concept of Shape Changes	33
2.4.1 Underlying Observations and Definition.....	33
2.4.2 Shape Changes Following Alterations of the Transbilayer Balance of Membrane Lipids.....	36

2.4.3 Shape Changes Following <i>in situ</i> Modification of Phospholipid Patterns.....	37
2.4.4 Shape Changes Arising from Experimentally Induced Transbilayer Redistribution of Endogenous Phospholipids	38
2.5 Shape Changes of Unresolved Origin.....	40
2.5.1 Depletion of ATP or Magnesium and Exposure to Vanadate	40
2.5.2 Ligands of Band 3 Protein.....	40
2.5.3 Effects of Glass Contact and of Serum Albumin	41
2.5.4 Phospholipid Symmetrization Following Field Pulse Exposure: a Tool for Testing the Requirement of Bilayer Asymmetry for Shape Changes of Unresolved Origin	41
2.6 From the Bilayer to a Quadrilaminar Membrane.....	43
2.6.1 Constituents of the Exo- and the Endofacial Membrane Surface Coat.....	43
2.6.2 Influence of the Exofacial Lamina: Shape Effects of Enzyme Treatment	45
2.6.3 Influence of the Endofacial Lamina: the Role of the Membrane Skeleton in Shape Transformations	45
2.6.4 Shape Effects of Cytoplasmic pH	46
2.6.5 Influence of Ion Gradients and Transmembrane Potential	47
2.6.6 Limitation of Shape Changes by the Membrane Skeleton	48
2.6.7 Membrane "Stabilization" by the Skeletal Network and its Alterations	49
2.7 Pathological Alterations of Red Cell Shape <i>in vivo</i>	49
2.7.1 Stomatocytosis Going Along with Alterations of Cell Volume	50
2.7.2 Shape Changes Due to Altered Properties of Haemoglobin.....	50
2.7.3 Lipid-Based Alterations of Cell Shape.....	50
2.7.4 Protein-Based Alterations of Red Cell Shape.....	51
2.8 Outlook	52
References	52

3 Human Red Cell Shape and the Mechanical Characteristics of the

Membrane	61
3.1 Introduction	61
3.2 Continuum Mechanics vs. Membrane Composition	62
3.3 Modes of Membrane Deformation.....	63
3.3.1 General Remarks.....	63
3.3.2 Isotropic or Biaxial Deformation	63
3.3.3 Shear or Uniaxial Deformation	63
3.3.4 Bending Deformation.....	63
3.4 Resting Shape of Membrane Patches.....	64
3.4.1 General Remarks.....	64
3.4.2 Surface Area.....	64
3.4.3 Aspect Ratio.....	65
3.4.4 Spontaneous Curvature	65
3.5 Membrane Stiffness	66
3.5.1 General Remarks.....	66

3.5.2 Isotropic Stiffness.....	66
3.5.3 Shear Stiffness.....	67
3.5.4 Bending Stiffness	67
3.6 Uniformity of the Membrane	69
3.6.1 General Remarks	69
3.6.2 Surface Area.....	69
3.6.3 Aspect Ratio	69
3.6.4 Single-Layer Based Spontaneous Curvature	69
3.6.5 Double-Layer Based Spontaneous Curvature.....	70
3.7 Resting Shapes of Red Cells	70
3.7.1 General Remarks	70
3.7.2 The Biconcave Discocyte	70
3.7.3 Echinocytes and Stomatocytes	71
3.7.4 Conclusions	72
3.8 Relative Importance of Single-Layer and Double-Layer Based Spontaneous Curvature.....	72
3.9 Non-Uniform Mechanical Properties of the Membrane	73
3.9.1 Spherical Echinocytes and Stomatocytes	73
3.9.2 Effects of Raising the Cytoplasmic Concentration of Calcium	74
Appendix	74
References.....	80
4 Passive Membrane Permeability for Ions and the Membrane Potential.....	83
4.1 Introduction.....	83
4.2 Specific Transport Systems for Monovalent Cations in the Mammalian Red Cell Membrane.....	83
4.3 The Low Ionic Strength (LIS) Effect.....	87
4.4 The $K^+(Na^+)/H^+$ Exchanger.....	91
4.5 General Consideration of the Residual and Leak Cation Fluxes.....	95
4.6 The Transmembrane Potential, Surface Potential, and the Electric Field in the Membrane.....	97
4.7 Conclusion	103
References.....	103
5 Na^+/K^+ Pump	111
5.1 Introduction.....	111
5.2 Development of the Concept of Active Transport	111
5.3 Early Characterization of the Na^+/K^+ Pump in Red Cells.....	112
5.4 The Enzymatic Basis of the Na^+/K^+ Pump	114
5.5 Red Cell Characteristics Favourable for the Study of the Na^+/K^+ Pump..	115
5.6 Transport of Na^+ and K^+ and the Reaction Mechanism of the Na^+/K^+ Pump.....	117
5.6.1 Na^+/K^+ Exchange	118
5.6.2 Na^+/Na^+ Exchange	120
5.6.3 K^+/K^+ Exchange	121
5.6.4 The Reaction Mechanism of the Na^+/K^+ Pump.....	123
5.7 The Quaternary Structure of the Red Cell Na^+/K^+ Pump	127

5.8 Unresolved Issues	129
5.8.1 Properties of the Third Na^+ Site.....	129
5.8.2 Selectivity of the Cation Binding Sites	131
5.8.3 Na^+/H^+ Exchange and K^+/H^+ Exchange.....	132
5.8.4 Na^+ Transport Coupled with Anion Transport.....	132
5.9 Conclusion.....	133
References	133
6 Ion Channels	139
6.1 Introduction	139
6.2 The Ca^{2+} -Activated K^+ Channel	139
6.3 The Voltage-Dependent Non-Selective Cation Channel.....	144
6.4 The Low Conductance K^+ Channel.....	146
6.5 The Anion-Selective Channel	146
6.6 Whole Cell Patch-Clamp Recordings	147
6.7 Conclusion.....	147
References	148
7 The Swelling-Sensitive Osmolyte Channel	153
7.1 Introduction	153
7.2 Swollen to the Same Extent, Red Cells can Adopt Different Regulatory Patterns	154
7.3 Red Cells will Adopt the Regulatory Pattern that Maintains Cell Homeostasis	156
7.4 Regulation of Swelling-Sensitive, Osmolyte Pathways.....	158
7.4.1 The Opening of Osmolyte Pathways is Controlled by Ionic Strength and is Insensitive to Cell Volume Expansion	159
7.4.2 A Single or Several Osmolyte Transport Pathways?.....	161
7.4.3 Opening of the Osmolyte Channel in Response to a Gradual and Slow Decrease in Medium Osmolality	162
7.5 Swelling-Sensitive Osmolyte Pathways: Association with Band 3 Protein	162
7.5.1 The Organic Osmolyte Pathway has the Functional Characteristics of an Anion Channel	162
7.5.2 The Organic Osmolyte Channel: a Pathway for Cations.....	163
7.5.3 The Swelling-Sensitive Osmolyte Channel is Associated with Band 3 Protein (AE1).....	164
7.6 Molecular Identification of the Swelling-Sensitive Osmolyte Channel...	165
7.7 Conclusion.....	167
References	168
8 $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ Cotransport	173
8.1 Introduction	173
8.2 The NKCC1 Protein	174
8.3 NKCC1 Antibodies.....	176
8.4 The NKCC1 Reaction Cycle.....	176
8.5 Breadth of Physiological Functions.....	181

8.6 Coordinate Regulation of Volume-Regulatory Transporters	182
8.7 Cell Volume Detection	183
8.8 Regulation of NKCC	185
8.9 Thermodynamic Considerations	186
8.10 Function of NKCC in Red Cells	186
References.....	190
9 K⁺-Cl⁻ Cotransport in Vertebrate Red Cells.....	197
9.1 Introduction.....	197
9.1.1 Historical Perspective.....	197
9.1.2 The LK Sheep Red Cell.....	198
9.1.3 Ionic Requirements and Transporter Kinetics.....	198
9.1.4 Molecular Identity of the K ⁺ -Cl ⁻ Cotransporter	199
9.2 Physiological Characteristics	200
9.2.1 Volume Dependence	200
9.2.2 H ⁺ Dependence	202
9.2.3 Urea	203
9.2.4 Oxygen	203
9.2.5 Hydrostatic Pressure.....	204
9.2.6 Temperature	204
9.2.7 Bicarbonate.....	205
9.3 Mechanism of Regulation.....	205
9.3.1 Protein Phosphorylation	205
9.3.2 Macromolecular Crowding, Ion Concentration or Mechano- reception?.....	207
9.3.3 Magnesium and Organic Phosphates.....	208
9.3.4 KCC and Redox Potential	209
9.4 Future Perspectives	210
9.4.1 The Cytoskeleton and Regulation of KCC	210
9.4.2 Diverse Functions of KCC in Red Cells and Other Tissues	211
References.....	212
10 The Band 3 Protein: Anion Exchanger and Anion-Proton Cotransporter	221
10.1 Introduction.....	221
10.2 Measuring Anion-Proton Cotransport.....	222
10.3 H ⁺ -Cl ⁻ Cotransport and its Relationship to Cl ⁻ /Cl ⁻ Exchange.....	225
10.4 Molecular Basis of the Relationship between Anion Exchange and Anion-Proton Cotransport.....	229
10.5 Chloride Equilibrium Exchange and Chloride-Proton Cotransport: Comparison of Experimental Evidence with Theoretical Predictions ...	231
10.6 H ⁺ -SO ₄ ²⁻ Cotransport and its Relationship to H ⁺ -Cl ⁻ Cotransport: General Features	237
10.7 The Relationship between H ⁺ -Cl ⁻ and H ⁺ -SO ₄ ²⁻ Cotransport: Specific Features.....	239
10.8 H ⁺ -SO ₄ ²⁻ Cotransport vs. H ⁺ -Cl ⁻ Cotransport: Discussion	242

10.9 Chemical Modification of the Cotransport Process	244
10.10 Summary	247
Appendix	248
References	250
11 Band 3 Mediated Transport	253
11.1 Functions of Band 3 and Related Proteins	253
11.2 Basic Characteristics	254
11.2.1 One-for-One Exchange	254
11.2.2 Divalent vs. Monovalent Anion Transport	255
11.2.3 Basic Transport Mechanism – Ping-Pong Model	255
11.2.4 Evidence for the Ping-Pong Model	259
11.3 Asymmetry of the System	260
11.3.1 Sidedness of Unloaded Transport Sites	260
11.3.2 Effects of Chloride on Sidedness of Transport Sites	261
11.3.3 Effects of Bicarbonate on Sidedness of Transport Sites	261
11.3.4 Consequences of Asymmetry for Fluxes under Physiological Conditions	262
11.4 Structure of AE1	263
11.4.1 The Cytoplasmic Domain (cdAE1)	263
11.4.2 Structure of the Membrane Domain Revealed by Electron Microscopy	264
11.4.3 Topology of the AE1 Membrane Domain	264
11.4.4 The First Eight TM Segments	265
11.4.5 Remaining C-Terminal Segments; Controversial Topologies	267
11.5 From Structure to Function: Information from Mutations and Chemical Labelling	268
11.5.1 Interpretation of Mutation and Chemical Modification Data	270
11.5.2 Carboxyl Group Modification: Putative Identification of Proton Binding Sites	271
11.5.3 Amino Group Modification by Disulphonic Stilbenes	272
11.5.4 Other Amino-Reactive Probes	275
11.5.5 Histidine Modification	275
11.5.6 Arginine Modification	276
11.5.7 Cysteine Modification	277
11.5.8 Functional Importance of Serine and Threonine	277
11.5.9 Protein Flexibility and Cross-Linking	277
11.5.10 Sensing of Changes in AE1 Conformation by Chemical Probes ..	278
11.6 Model for Anion Exchange	280
11.6.1 Residues that Cross the Permeability Barrier (PB) and/or Bind Substrates	280
11.6.2 Evidence for Anion Access Channels in AE1	283
11.6.3 Separate Binding Sites for Halides and Oxyanions	284
11.6.4 Structural Nature of the Transporting Conformational Change (TCC)	286
11.6.5 Relation to AE1 Structure	287

11.6.6 Relation to Structure and Mechanism of Other Membrane Transport Proteins	288
References.....	289
12 Amino Acid Transport.....	303
12.1 Introduction.....	303
12.2 Why do Red Cells Need Amino Acid Transporters?	304
12.3 A Comment on Methodology	306
12.4 Transport System Nomenclature.....	306
12.5 Identification of Red Cell Amino Acid Transport Systems	307
12.6 Kinetic Studies.....	307
12.7 System gly	309
12.8 Band 3.....	309
12.9 The Heterodimeric Amino Acid Transporters (L, asc, y ⁺ L).....	309
12.9.1 System L.....	310
12.9.2 System y ⁺ L	311
12.10 System ASC.....	312
12.11 System y ⁺	312
12.12 System N.....	312
12.13 System T	313
12.14 System glu	313
12.15 Physiological Transport Rates for Amino Acids	313
12.16 Alterations of Amino Acid Transport in Disease States	315
12.17 Conclusion	316
References.....	316
13 Equilibrative Nucleoside Transport Proteins.....	321
13.1 Introduction.....	321
13.2 Biochemical Studies of Red Cell Nucleoside Transport Proteins.....	322
13.2.1 Identification of the Red Cell Nucleoside Transporter as a Band 4.5 Protein.....	322
13.2.2 Production of Nucleoside Transporter Antibodies	324
13.2.3 Purification of the Human and Pig Red Cell Nucleoside Transporters	324
13.2.4 Tryptic Cleavage Studies.....	325
13.3 cDNA Cloning of ENT Nucleoside Transport Proteins.....	325
13.3.1 cDNA Cloning and Heterologous Expression of Recombinant Human and Rat ENT1	325
13.3.2 cDNA Cloning and Heterologous Expression of Recombinant Human and Rat ENT2.....	327
13.3.3 Other ENT Family Members.....	328
13.4 Molecular Properties of Recombinant Mammalian ENT Proteins	329
13.4.1 ENT Membrane Topology	329
13.4.2 Chimeric Studies	330
13.4.3 Identification of an Exofacial Cysteine Residue within the Rat ENT2 Translocation Pore.....	330

13.5 Conclusions	332
References	333
14 Glucose Transport.....	339
14.1 Introduction	339
14.2 Specificity of Sugar Transporters	339
14.3 Net vs. Exchange Transport of Glucose.....	340
14.4 Membrane Topology of GLUTs	342
14.5 Evidence for the 12 TM Helix Model for GLUTs	342
14.5.1 Trypsinolysis	342
14.5.2 Antibody Studies.....	343
14.5.3 Scanning Mutagenesis Studies	344
14.5.4 Cysteine Scanning Mutagenesis of TM's 7, 10 and 11 Evidence for Two Hydrophilic Pores Traversing GLUT1.....	345
14.5.5 Covalent Linkage of Inhibitors.....	346
14.5.6 Conformational Change Resulting from Ligand Interactions.....	347
14.5.7 Biophysical Studies Showing α Helicity of the Transmembrane Strands	348
14.6 Structural-Functional Studies on GLUTs	349
14.6.1 Functional Differences between the Isoforms GLUT1 and GLUT4	349
14.6.2 Structure-Functional Comparisons of GLUTs 1 and 4.....	350
14.6.3 Structural Basis of Specificity Differences between GLUTs	351
14.7 Problems Relating to Glucose Transport	352
14.7.1 Problem 1: Is the Carrier a One Mobile or Two Fixed Site Transporter?	352
14.7.2 Problem 2: Can Ligands Bind to Inside and Outside Sites Simultaneously?	356
14.7.3 Problem 3: Is GLUT1 an Asymmetric Transporter?	356
14.8 ATP Interactions with Sugar Transport	358
14.8.1 ATP Effects on Human GLUT1	358
14.8.2 ATP on Glucose Transport in Avian Red Cells	360
14.8.3 Structural Basis for ATP Interaction with GLUT1	360
14.9 Drug Interactions with Glucose Transport.....	361
14.9.1 Barbiturates	361
14.9.2 Glucose Transporter Deficiency Syndrome (GTDS) in Relation to Drug Action	362
14.9.3 Steroids, Flavones and Isoflavones	362
14.9.4 The L-Type Channel Antagonists	363
14.9.5 Antipsychotic drugs.....	363
14.9.6 Actions of HIV Protease Inhibitors on GLUTs	363
14.10 Conclusions.....	364
References	365
15 Calcium Homeostasis in Normal and Abnormal Human Red Cells	373
15.1 Introduction	373
15.2 Methodological Considerations	374

15.3 Use of Incorporated Ca^{2+} Chelators.....	375
15.4 Use of Ca^{2+} Ionophores	376
15.5 Inhibition of the Ca^{2+} Pump.....	376
15.6 Calcium Homeostasis in Physiological Conditions	377
15.6.1 Total and Total Exchangeable Calcium Content of Red Cells	377
15.6.2 The Physiological $[\text{Ca}^{2+}]_i$ Level.....	378
15.6.3 Cytoplasmic Ca^{2+} Buffering.....	379
15.7 Passive Ca^{2+} Transport	380
15.8 The Ca^{2+} Pump and the Physiological Pump-Leak Turnover of Ca^{2+}	381
15.9 The $V_{\max}$ of the Ca^{2+} Pump	381
15.10 Effects of Deoxygenation and pH_i on the $V_{\max}$ of the Ca^{2+} Pump	382
15.11 Elevated Cell Ca^{2+} in Experimental Conditions	383
15.11.1 Secondary Effects of Elevated $[\text{Ca}^{2+}]_i$	383
15.11.2 Effects of Physiological Agents on the Performance of the Ca^{2+} Pump in Intact Red Cells	385
15.11.3 Population Response to Increased Ca^{2+} Influx.....	386
15.12 Elevated Cell Ca^{2+} in Pathological Conditions.....	388
15.12.1 Malaria	389
15.12.2 Sickle Cell Anaemia.....	392
15.12.3 Thalassaemia	395
References.....	396
16 Magnesium Transport.....	407
16.1 Introduction.....	407
16.2 Magnesium Homeostasis in Red Cells.....	407
16.3 Red Cell Magnesium Content.....	408
16.3.1 Distribution of Magnesium between Bound and Free Forms.....	408
16.3.2 Levels of Free Ionized Magnesium	410
16.3.3 Changes in Magnesium Content with Cell Age	410
16.4 Red Cell Membrane Magnesium Permeability	412
16.5 Is Active Magnesium Transport Needed?.....	413
16.6 Magnesium Transport Mechanisms	413
16.7 Methods for Investigating Magnesium Transport in Red Cells	414
16.8 Magnesium Transport in Human Red Cells.....	415
16.8.1 Sodium-Dependent Magnesium Transport.....	415
16.8.2 Inhibitors of Sodium-Dependent Magnesium Transport.....	416
16.8.3 Interactions between Sodium and Magnesium across the Membrane	416
16.8.4 How Many Sodium Ions are Exchanged with Each Magnesium Ion?.....	417
16.8.5 Can Magnesium Efflux Occur against an Electrochemical Gradient?.....	417
16.8.6 Role of ATP	418
16.8.7 Sodium-Independent Magnesium Efflux.....	418
16.8.8 Changes in Magnesium Transport in Disease	419
16.9 Magnesium Transport in Ferret Red Cells.....	420
16.9.1 Magnesium Efflux from Ferret Red Cells	420

16.9.2 Magnesium Uptake	422
16.9.3 Transport in Magnesium-Loaded Cells	423
16.10 Magnesium Transport in Rodent Red Cells	423
16.10.1 Rat Red Cells	424
16.10.2 Hamster and Guinea-Pig Red Cells	425
16.11 Magnesium Transport in Avian Red Cells	426
16.12 Conclusions	427
References	429
17 Trace Metal Transport	435
17.1 Introduction	435
17.2 Transport Systems	437
17.2.1 As Free Metal (Hydrated Ions)	437
17.2.2 Transport by Band 3 as an Anionic Complex	442
17.2.3 Uptake Dependent on Formation of Metal-Amino Acid Complexes	443
17.3 Conclusions	446
References	447
18 Monocarboxylate and other Organic Anion Transport	451
18.1 Introduction	451
18.2 Three Pathways for Monocarboxylate Transport across the Plasma Membrane of Red Cells	452
18.3 Non-Ionic Diffusion of Monocarboxylates	452
18.4 Band 3-Mediated Transport of Monocarboxylates	453
18.5 The Specific Monocarboxylate Transporter (MCT1)	454
18.5.1 Kinetics Properties	454
18.5.2 Substrate Specificity	456
18.5.3 Inhibitors	459
18.6 Identification, Cloning and Sequencing of the Red Cell Monocarboxylate Carrier	461
18.7 MCT1 is a Member of a Family of Proton-Linked Monocarboxylate Transporters	463
18.8 Topology of MCT1 and Other Members of the MCT Family	464
18.9 Structure Function Relationships in MCT1	465
18.10 MCT1 is Tightly Associated with the Cell Surface Glycoproteins GP70 (Embigin) and CD147 (Basigin)	466
18.11 Monocarboxylate Transport into Malaria Infected Red Cells	468
18.12 Conclusions	469
References	470
19 Water Permeability	477
19.1 Introduction	477
19.2 Water Diffusion through Lipids	477
19.3 Evidence for Discrete Permeability Pathways	478
19.4 Molecular Identification of Water Channels	479
19.5 The Aquaporin Family	480

19.6 Structure-Function Relationships.....	481
19.7 Aquaporins and Red Cells	484
19.8 Summary.....	485
References.....	485
20 Gas Transport.....	489
20.1 Introduction.....	489
20.2 Basic Principles of Gas Transport.....	489
20.2.1 Oxygen Transport: Factors Affecting Haemoglobin Function	489
20.2.2 Factors Affecting Carbon Dioxide Transport and Excretion in Blood.....	491
20.3 Control of Red Cell pH	493
20.3.1 Control of Red Cell pH in the Absence of Significant Secondarily Active Cation or Proton Transport	493
20.3.2 Effect of Na^+/H^+ Exchange on Red Cell pH	494
20.3.3 Effect of K^+-Cl^- Cotransport on Red Cell pH	497
20.4 Roles of pH Changes in Regulation of Oxygen Transport.....	498
20.5 Red Cell Volume and Haemoglobin Oxygen Affinity	499
20.6 Interactions between Membrane Transport and Regulation of Haemoglobin Oxygen Affinity by Organic Phosphates	500
20.7 Patterns of Carbon Dioxide Transport and Excretion in Vertebrates.....	500
References.....	504
21 'The Hereditary Stomatocytosis and Allied Conditions': Inherited Disorders Na^+ and K^+ Transport	511
21.1 Introduction.....	511
21.2 Normal Red Cell Cation Transport	511
21.3 History; Nosology	512
21.4 Temperature Effects.....	513
21.5 Clinical Aspects	515
21.6 Genetic Mapping.....	516
21.7 The Stomatocytic Red Cell	516
21.8 The Stomatatin Protein	517
21.9 Possible Pathogenic Mechanisms for the Stomatocytosis	518
References.....	520
22 Metabolic Disorders	525
22.1 Introduction.....	525
22.2 To Live Perilously: the Life of a Normal Red Cell.....	527
22.3 The Damaging Role of Hemichromes, Free Haem and Free Iron.....	528
22.4 Pathophysiological Consequences of Oxidative Damage.....	532
22.5 G6PD Deficiency	533
22.5.1 Structure of Normal and Mutant G6PD.....	533
22.5.2 Intracellular Regulation of G6PD in Normal and G6PD- Deficient Red Cells	534
22.5.3 Oxidant-Induced Haemolysis	535
22.5.4 The Favic Crisis	535

22.5.5 Toxic Components of Fava Beans and their Haemolytic Activity	536
22.5.6 Oxidant Stress and Calcium Homeostasis	538
22.5.7 Predominantly Extravascular Haemolysis in Oxidatively Stressed G6PD-Deficient Red Cells.....	539
22.6 Red Cell Alterations in Diabetes Mellitus	540
22.6.1 Glycation of Proteins in Diabetes.....	541
22.6.2 Functional Effects of Red Cell Protein Glycation	542
22.6.3 Increased Phagocytosis of Diabetic Red Cells: Possible Mechanisms	543
22.6.4 Role of Red Cell Alterations in Diabetes Complications	544
22.7 Concluding Remarks	544
References	545
23 Sickle Cell Disease	549
23.1 Introduction	549
23.2 Mechanisms of Sickle Red Cell Membrane Damage	549
23.2.1 Oxidation.....	549
23.2.2 Loss of Membrane.....	550
23.2.3 Binding of Hb S to the Inner Membrane	550
23.3 Red Cell Membrane Structural Abnormalities in Sickle Cell Disease...	550
23.3.1 Altered Cytoskeleton.....	550
23.3.2 Altered Membrane Lipid Composition	551
23.4 Red Cell Membrane Transport Abnormalities in Sickle Cell Disease...	551
23.4.1 K ⁺ -Cl ⁻ Cotransport	553
23.4.2 Ca ²⁺ -Activated K ⁺ Channel (Gardos Pathway)	554
23.4.3 Deoxygenation-Induced Cation Fluxes	555
23.5 Therapeutic Approaches Based on Inhibition of Sickle Cell Dehydration	556
23.5.1 Inhibition of Gardos Channel.....	556
23.5.2 Inhibition of K ⁺ -Cl ⁻ Cotransport	557
23.5.3 Inhibition of Anion Permeability	558
23.5.4 Inhibition of Deoxygenation-Induced Fluxes.....	558
References	558
24 The Membrane Physiology of the ‘Malaria-Infected’ Red Cell	569
24.1 Introduction	569
24.2 The Intraerythrocytic Phase of the Parasite Life Cycle	570
24.3 Transport Characteristics of the Parasitised Red Cell Membrane.....	572
24.4 Electrophysiological Characteristics of the Parasitised Red Cell Membrane.....	574
24.5 Roles and Consequences of the Altered Permeability of the Infected Red Cell Membrane.....	576
24.6 The Parasitophorous Vacuole and Parasite Membranes	578
24.7 Chemotherapeutic Opportunities	580
References	581

25 Hypertension	587
25.1 Introduction	587
25.2 Transporters Involved in Abnormal Monovalent Ion Handling in Experimental Models of Primary Hypertension	588
25.2.1 Na ⁺ /K ⁺ Pump	588
25.2.2 Na ⁺ -K ⁺ -2Cl ⁻ Cotransport	589
25.2.3 Na ⁺ /H ⁺ Exchange	589
25.2.4 Other Ion Transport Pathways	590
25.3 Ion Transporters in Essential Hypertension	590
25.3.1 Na ⁺ -K ⁺ -2Cl ⁻ Cotransport	591
25.3.2 Na ⁺ /Li ⁺ Countertransport vs. Na ⁺ /H ⁺ Exchange	591
25.4 Are Abnormalities of Red Cell Ion Transporters in Primary Hypertension Genetically Determined?	592
25.5 Role of Ion Transporters Expressed in Red Cells in Blood Pressure Regulation	593
25.6 Evidence for the Involvement of Ion Transport Abnormalities in the Pathogenesis of Hypertension	594
25.7 Molecular Determinants of Abnormal Ion Transport in Hypertension	595
25.8 Conclusion and Future Directions	597
References	598
26 Disorders of Band 3	603
26.1 Introduction	603
26.2 Erythroid Phenotypes with Partial Deficiency of Band 3	603
26.2.1 Autosomal Dominant Spherocytosis with Band 3 Deficiency	603
26.2.2 Autosomal Dominant Spherocytosis with Protein 4.2 Deficiency	605
26.2.3 Autosomal Recessive Spherocytosis with Protein 4.2 Deficiency	605
26.3 Erythroid Phenotypes with Complete Absence of Band 3	606
26.3.1 Severe Human Autosomal Recessive Haemolytic Anaemia	606
26.3.2 Mouse Band 3 Mutants and Knockouts	606
26.3.3 Severe Spherocytic Haemolytic Anaemia in a Steer	607
26.3.4 Phenotype of Congenital Dyserythropoietic Anaemia in Zebrafish	607
26.4 Other Erythroid Phenotypes Caused by Band 3 Mutations	608
26.4.1 Southeast Asian Ovalocytosis	608
26.4.2 Hereditary Acanthocytosis	609
26.4.3 Blood Group Antigens Carried by Band 3	609
26.5 Non-Erythroid Phenotypes Caused by Band 3 Mutations	610
26.5.1 Autosomal Dominant Distal Renal Tubular Acidosis	610
26.5.2 Autosomal Recessive Distal Renal Tubular Acidosis	612
26.6 Conclusion	613
References	613
27 Amino Acid Transport in Disease	621
27.1 Introduction	621

27.2 Measurement of Transport Kinetics in Red Cells	622
27.2.1 Transport Systems and Transporters	622
27.2.2 Role of L-Arginine Transport in Nitric Oxide Production	622
27.3 Uraemia, Pathophysiology and Treatment.....	623
27.3.1 Transport Alterations in Uraemia.....	624
27.3.2 L-Arginine Transport and Uraemia.....	624
27.3.3 Possible Mechanisms Explaining the Increased y^+ Transport Activity in Uraemia.....	627
27.4 Chronic Heart Failure	629
27.4.1 L-Arginine-NO Pathway and Heart Failure	629
27.4.2 Amino Acid Plasma Profile in Chronic Heart Failure.....	630
27.4.3 Alterations on L-Arginine Transport in Chronic Heart Failure.....	631
27.5 Possible Overlapping of Mechanisms Present in Both Chronic Heart Failure and Uraemia	632
27.6 L-Arginine Transport Alterations in Diabetes	633
27.7 L-Arginine Transport and Sepsis	634
27.8 Amino Acid Transport Alterations in Sickle Cell Disease	634
References	635
28 Transgenic Models of Red Cell Disorders.....	643
28.1 Introduction	643
28.2 Production of Genetically Modified Animals	643
28.2.1 Conventional Transgenesis.....	644
28.2.2 Gene Targeting.....	645
28.2.3 Tissue-Specific Gene Targeting	649
28.3 Transgenic Models of Red Cell Membrane Disorders.....	649
28.3.1 Hereditary Spherocytosis	650
28.3.2 Hereditary Elliptocytosis/Pyropoikilocytosis.....	653
28.4 Transgenic Models of Red Cell Transport Disorders	654
28.4.1 Gradient-Driven Systems	655
28.4.2 Stomatocytosis	656
28.5 Transgenic Models of Other Red Cell Disorders.....	656
28.5.1 Sickle Cell Disease.....	657
28.5.2 Haemoglobinopathies.....	658
28.5.3 The Haemophilias	659
28.5.4 Metabolic Disorders	660
28.6 Growth and Differentiation Defects.....	661
28.6.1 The Erythropoietin and the Erythropoietin Receptor	662
28.6.2 The Erythroid-Specific GATA Transcription Factors.....	663
28.6.3 Erythroid Kruppel-Like Factor.....	665
References	666
29 Red Cell Ageing	673
29.1 Introduction: the Properties of Mammalian Red Cells	673
29.2 Red Cell Ageing in Mammals.....	675
29.3 Properties of Human Red Cells of Different Density	677
29.4 Preparation of Red Cells of Well-Defined Cell Age.....	680

29.5 The Ageing of Red Cells Reinvestigated	683
29.6 Red Cell Storage in Blood Banks	685
References.....	687
30 Active and Passive Monovalent Ion Transport Association with Membrane Antigens in Sheep Red Cells: a Molecular Riddle.....	691
30.1 Introduction: a Well-Established Cellular Model	691
30.2 Cation Polymorphism through Pumps and “Leaks”	692
30.2.1 Na ⁺ /K ⁺ Pumps	693
30.2.2 “Leaks”	694
30.2.3 An Integrated View	701
30.3 M and L Membrane Antigen/Antibody Polymorphism	701
30.3.1 Genetic Basis	701
30.3.2 Molecular Properties of the M/L Antigens	702
30.3.3 The M/L Antibodies	705
30.4 Functional Association of Antigens with “Pumps and Leaks”	706
30.4.1 Na ⁺ /K ⁺ Pump Activation by the L _p Antibody	706
30.4.2 K ⁺ -Cl ⁻ Cotransport Inhibition by the L _i Antibody	707
30.4.3 Complement-Mediated Haemolysis by Anti-M and Anti-L	708
30.5 Clonal vs. Maturational Changes of Transporters and Antigens	709
30.5.1 Clonal Cellular Changes in Lambs	709
30.5.2 Reticulocyte Maturation in Anaemic Sheep	710
30.6 Overall Physiological Consequences and the HK/LK Dimorphism	711
30.7 Membrane Antigens and Transport in Species Other than Ruminants ..	711
References.....	712
31 Comparative Physiology of Red Cell Membrane Transport	721
31.1 Introduction.....	721
31.2 Carnivores – Dog and Cat.....	722
31.3 Herbivores – Horse and Deer.....	724
31.4 Rodents – Mouse and Rat	725
31.5 Lower Vertebrates.....	726
31.5.1 Birds	726
31.5.2 Amphiuma.....	727
31.6 Conclusion	728
References.....	729
Index.....	735

Contributing Authors

Arese, P., Prof. Dr.

Department of Genetics, Biology and Biochemistry, University of Torino,
Medical School,
Via Santena 5 bis, 10126 Torino,
Italy

Baldwin, S. A., Prof. Dr.

School of Biochemistry and Molecular Biology, University of Leeds,
Leeds, LS2 9JT,
UK

Bennekou, P., Prof. Dr.

LCMF, The August Krogh Institute, University of Copenhagen,
Universitetsparken 13, 2100 Copenhagen Ø,
Denmark

Bernhardt, I., Prof. Dr.

Arbeitsgruppe Biophysik, Naturwissenschaftlich-Technische Fakultät III,
Universität des Saarlandes,
Gebäude 6, Postfach 151150, 66041 Saarbrücken,
Germany

Bookchin, R. M., Prof. Dr.

Department of Medicine, Albert Einstein College of Medicine,
Bronx, New York 10461,
USA

Borgese, F., Dr.

Laboratoire de physiologie des membranes cellulaires, Université de Nice-
Sophia Antipolis, UMR 6078 CNRS, Bâtiment Jean Maetz,
284 chemin du Lazaret, 06230 Villefranche sur mer,
France

Brovelli, A., Prof. Dr.

Dipartimento di Biochimica "A. Castellani", Università di Pavia,
via Bassi 21, 27100 Pavia,
Italy

Browning, J., Dr.

Laboratory of Physiology, Oxford University,
Parks Road, Oxford, OX1 3PT,
UK

Brugnara, C., Prof. Dr.

Department of Laboratory Medicine, Children's Hospital Boston, Harvard Medical School,
300 Longwood Avenue, Boston, MA 02115,
USA

Brunini, T. M. C., Dr.

Departamento de Farmacologia e Psicobiologia, Instituto de Biologia,
Av. 28 de Setembro 87, CEP 20551-030, Rio de Janeiro,
Brazil

Cass, C. E., Prof. Dr.

Membrane Protein Research Group, Department of Oncology,
University of Alberta, and Cross Cancer Institute,
Edmonton, Alberta T6G 2H7,
Canada

Christophersen, P., Dr.

NeuroSearch A/S, Ballerup,
Pederstrupvej 93, 2750 Ballerup,
Denmark

Deuticke, B., Prof. Dr.

Institut für Physiologie, Universitätsklinikum der RWTH,
Pauwelsstrasse 30, 52057 Aachen,
Germany

Ellory, J. C., Prof. Dr.

Laboratory of Physiology, Oxford University,
Parks Road, Oxford OX1 3PT,
UK

Fischer, T. M., Dr.

Institut für Physiologie, Universitätsklinikum der RWTH,
Pauwelsstrasse 30, 52057 Aachen,
Germany

Flatman, P. W., Dr.

Membrane Biology Group, Division of Biomedical and Clinical Laboratory Sciences, The University of Edinburgh, Hugh Robson Building,
George Square, Edinburgh EH8 9XD, Scotland,
UK

Gibson, J. S., Dr.

Department of Clinical Veterinary Medicine, University of Cambridge,
Maddingley Road, Cambridge CB3 0ES,
UK

Grosveld, F. G., Prof. Dr.

MGC Department of Cell Biology and Genetics, Faculty of Medicine, Erasmus
University Medical Center Rotterdam,
PO Box 1738, 3000 DR, Rotterdam,
The Netherlands

Guizouarn, H., Dr.

Laboratoire de physiologie des membranes cellulaires, Université de Nice -
Sophia Antipolis, UMR 6078 CNRS, Bâtiment Jean Maetz,
284 chemin du Lazaret, 06230 Villefranche sur mer,
France

Haest, C. W. M., Dr.

Institut für Physiologie, Universitätsklinikum der RWTH,
Pauwelsstrasse 30, 52057 Aachen,
Germany

Halestrap, A. P., Prof. Dr.

Department of Biochemistry, School of Medical Sciences,
University of Bristol,
Bristol BS8 1TD,
UK

Heberle, J., Dr.

Forschungszentrum Jülich, IBI-2: Strukturbiologie,
52425 Jülich,
Germany

Horn, N. M., Dr.

School of Biological Sciences, University of Southampton,
Bassett Crescent East, Southampton SO16 7PX,
UK

Jarolim, P., Dr.

Department of Pathology, Brigham and Women's Hospital, Harvard Medical
School,
75 Francis Street, Boston MA 02115,
USA

Kirk, K., Prof. Dr.

School of Biochemistry and Molecular Biology, Faculty of Science, Australian
National University,
Canberra A.C.T. Australia 0200,
Australia

Knauf, P. A., Prof. Dr.

Department of Biochemistry and Biophysics, University of Rochester Medical Center,
Rochester, NY 14642,
USA

Lauf, P. K., Prof. Dr.

Department of Physiology and Biophysics, Wright State University School of Medicine,
Dayton, OH 45435,
USA

Lepke, S.

Max Planck Institut für Biophysik,
Heinrich-Hoffmann-Str. 7, 60528 Frankfurt am Main,
Germany

Lew, V. L., Dr.

Physiological Laboratory, University of Cambridge,
Cambridge CB2 3EG,
UK

Lytle, C., Prof. Dr.

Division of Biomedical Sciences, University of California, Riverside,
Riverside, California, 92521,
USA

Mendes Ribeiro, A. C., Dr.

Departamento de Farmacologia e Psicobiologia, Instituto de Biologia,
Av. 28 de Setembro 87, CEP 20551-030, Rio de Janeiro,
Brazil

Minetti, G., Dr.

Dipartimento di Biochimica "A. Castellani", Università di Pavia,
via Bassi 21, 27100 Pavia,
Italy

Motais, R., Prof. Dr.

Laboratoire de physiologie des membranes cellulaires, Université de Nice - Sophia Antipolis, UMR 6078 CNRS, Bâtiment Jean Maetz,
284 chemin du Lazaret, 06230 Villefranche sur mer,
France

Naftalin, R. J., Prof. Dr.

Physiology Division, King's College London, Guy's Campus,
London SE1 1UL,
UK

Nikinmaa, M., Prof. Dr.

Department of Biology, University of Turku,
20014 Turku,
Finland

Oakley, F., Dr.

School of Biological Sciences, University of Southampton,
Bassett Crescent East, Southampton SO16 7PX,
UK

Orlov, S. N., Prof. Dr.

Laboratory of Pathophysiology of Ion Transport Disorders, Research Centre,
University of Montreal Hospital, CHUM,
3850 rue St.-Urbain, Montreal, PQ, H2W 1T8,
Canada

Pal, P., M. Sc.

Department of Biochemistry and Biophysics, University of Rochester Medical
Center,
Rochester, NY 14642,
USA

Passow, H., Prof. Dr.

Max Planck Institut für Biophysik,
Heinrich-Hoffmann-Str. 7, 60528 Frankfurt am Main, and
Institut für Biochemie und Biophysik, Friedrich Schiller Universität Jena,
Philosophenweg 12, 07743 Jena,
Germany

Patrinou, G. P., Dr.

MGC Department of Cell Biology and Genetics, Faculty of Medicine, Erasmus
University Medical Center Rotterdam,
PO Box 1738, 3000 DR, Rotterdam,
The Netherlands

Sachs, J. R., Prof. Dr.

Department of Medicine, State University of New York at Stony Brook,
Stony Brook, NY, 11794-8151,
USA

Saliba, K. J., Dr.

School of Biochemistry and Molecular Biology, Faculty of Science, Australian
National University,
Canberra A.C.T. Australia 0200,
Australia

Schwarzer, E., Dr.

Department of Genetics, Biology and Biochemistry, University of Torino,
Medical School, Via Santena 5 bis, 10126 Torino,
Italy

Stewart, G. W., Prof. Dr.

Department of Medicine, Rayne Institute, University College London,
University Street, London WC1E 6JJ,
UK

Swietach, P.

Laboratory of Physiology, Oxford University,
Parks Road, Oxford OX1 3PT,
UK

Thomas, A. L., Dr.

School of Biological Sciences, University of Southampton,
Bassett Crescent East, Southampton SO16 7PX,
UK

Tiffert, T., Dr.

Physiological Laboratory, University of Cambridge,
Cambridge CB2 3EG,
UK

Weiss, E., Dipl.-Biophys.

Arbeitsgruppe Biophysik, Naturwissenschaftlich-Technische Fakultät III,
Universität des Saarlandes,
Gebäude 6, Postfach 151150, 66041 Saarbrücken,
Germany

Wilkins, R., Dr.

Laboratory of Physiology, Oxford University,
Parks Road, Oxford, OX1 3PT,
UK

Yao, S. Y. M., Dr.

Membrane Protein Research Group, Department of Physiology,
University of Alberta,
Edmonton, Alberta T6G 2H7,
Canada

Young, J. D., Prof. Dr.

Membrane Protein Research Group, Department of Physiology,
University of Alberta,
Edmonton, Alberta T6G 2H7,
Canada