



Drug Bioavailability

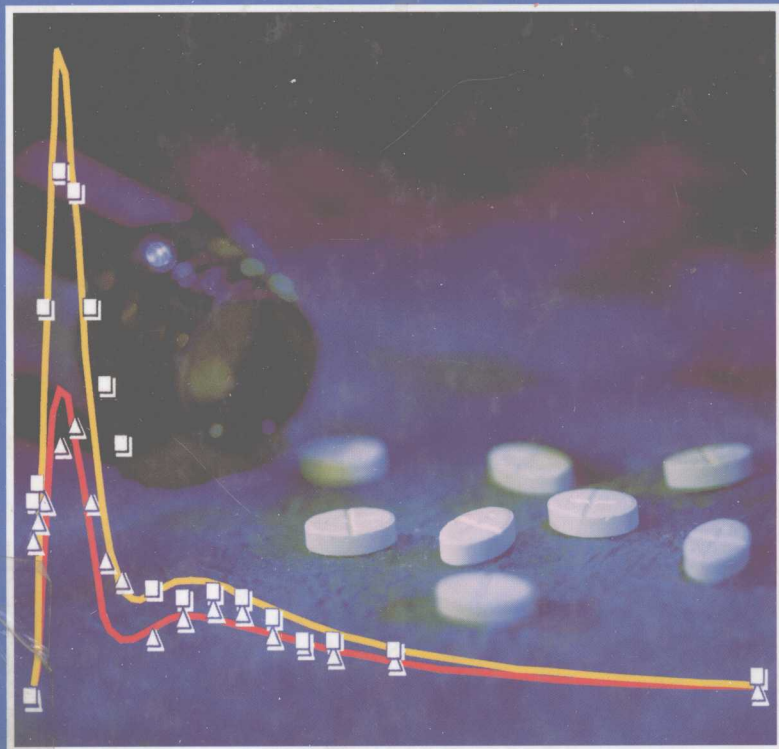
Estimation of Solubility, Permeability, Absorption
and Bioavailability

Edited by
H. van de Waterbeemd, H. Lennernäs and P. Artursson

**Methods
and Principles
in Medicinal
Chemistry**

Volume 18

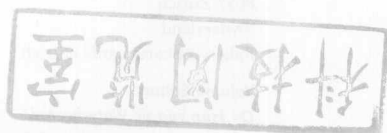
Edited by
R. Mannhold,
H. Kubinyi,
G. Folkers



Drug Bioavailability

Estimation of Solubility, Permeability, Absorption and Bioavailability

Edited by Han van de Waterbeemd, Hans Lennernäs and Per Artursson



 **WILEY-VCH**

Series Editors

Prof. Dr. Raimund Mannhold

Biomedical Research Center
Molecular Drug Research Group
Heinrich-Heine-Universität
Universitätsstraße 1
40225 Düsseldorf
Germany
raimund.mannhold@uni-duesseldorf.de

Prof. Dr. Hugo Kubinyi

BASF AG Ludwigshafen
c/o Donnersbergstraße 9
67256 Weisenheim am Sand
Germany
kubinyi@t-online.de

Prof. Dr. Gerd Folkers

Department of Applied Biosciences
ETH Zürich
Winterthurerstr. 190
8057 Zürich
Switzerland
folkers@pharma.anbi.ethz.ch

Volume Editors

Dr. Han van de Waterbeemd

Pfizer Global Research and Development
Department of Drug Metabolism, IPC 351
Sandwich, Kent CT13 9NJ
UK
han_waterbeemd@sandwich.pfizer.com

Prof. Dr. Hans Lennernaäs

Biopharmaceutics Group
Department of Pharmacy
Uppsala University
S-751 23 Uppsala
Sweden
hans.lennernaas@biof.uu.se

Prof. Dr. Per Artursson

Division of Pharmaceutics
Department of Pharmacy
Uppsala University
S-751 23 Uppsala
Sweden
per.artursson@galenik.uu.se

■ This book was carefully produced.

Nevertheless, authors, editors and publisher do not warrant the information contained therein to be free of errors. Readers are advised to keep in mind that statements, data, illustrations, procedural details or other items may inadvertently be inaccurate.

Library of Congress Card No.: applied for

British Library Cataloguing-in-Publication Data: A catalogue record for this book is available from the British Library
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>

© 2003 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

All rights reserved (including those of translation into other languages). No part of this book may be reproduced in any form – by photoprinting, microfilm, or any other means – nor transmitted or translated into a machine language without written permission from the publishers. Registered names, trademarks, etc. used in this book, even when not specifically marked as such, are not to be considered unprotected by law.

Printed in the Federal Republic of Germany.

Printed on acid-free paper.

Composition Asco Typesetters, Hong Kong

Printing betz-druck gmbh, Darmstadt

Bookbinding Litges & Dopf Buchbinderei GmbH, Heppenheim

ISBN 3-527-30438-X

Drug Bioavailability

Estimation of Solubility,
Permeability, Absorption
and Bioavailability

Edited by H. van de

*Waterbeemd, H. Lennernäs
and P. Artursson*

Methods and Principles in Medicinal Chemistry

Edited by

R. Mannhold

H. Kubinyi

G. Folkers

Editorial Board

H.-D. Höltje, H. Timmerman, J. Vacca,

H. van de Waterbeemd, T. Wieland

Preface

The processes involved in drug discovery have changed considerably in the past decade. Today we have access to the full human as well as several bacterial genomes offering a rich source of molecular targets to treat diseases. Methods in biology have moved to ultra-high-throughput screening (uHTS) of such precedented and unprecedented targets. Chemistry adapted to this progress by developing methods such as combinatorial and parallel synthesis allowing the rapid synthesis of hundreds to hundreds of thousands molecules in reasonable quantities, purities and timelines.

Historical data on the fate of potential drugs in development indicate that major reasons for attrition include toxicity, efficacy and pharmacokinetics/drug metabolism. Therefore, in today's drug discovery the evaluation of absorption, distribution, metabolism and elimination (ADME) of drug candidates is performed early in the process. In the last 10 years drug metabolism and physicochemical *in vitro* screening methods have increasingly been introduced. In recent years these methods more and more became medium to high throughput in order to cope with increasing numbers of compounds to evaluate after HTS.

Although HTS seems to be a very efficient approach, it must be stressed that there is also a high cost associated with it. Interest is thus shifting to prediction and simulation of molecular properties, which might hopefully lead to overall more efficient processes.

The next vague of tools will be around computational or *in silico* ADME approaches. These will allow to include ADME into the design of combinatorial libraries, the evaluation of virtual libraries, as well as in selecting the most promising compounds to go through a battery of *in vitro* screens, possibly even replacing some of these experimental screens. Several of these computational tools are currently under development as will be discussed in this volume.

For reasons of convenience for the patient and compliance to the therapy, most drugs are administered orally. To keep the dose at the lowest possible level, high oral absorption and high bioavailability are prime properties to optimise in a new drug. Drug bioavailability is the outcome of a complex chain of events, and is among others influenced by the drug's solubility, permeability through the gastrointestinal wall, and its first pass gut wall and liver metabolism. Excluding liver metabolism, all other factors are characterized by the term oral absorption. Per-

meability through the gut wall can be favoured or hindered through the effect of various transporter proteins such as P-glycoprotein. Our increased knowledge and understanding of all of these processes involved in permeability, oral absorption and bioavailability will make predictive tools more robust.

This volume gives an overview of the current status and an outlook to future more reliable predictive approaches. It is subdivided in five sections dealing with studies of membrane permeability and oral absorption, drug dissolution and solubility, the role of transporters and metabolism in oral absorption, computational approaches to drug absorption and bioavailability, and finally with certain drug development issues.

The series editors would like to thank Han van de Waterbeemd, Hans Lennernäs, and Per Artursson for their enthusiasm to put together this book and to work with such a fine selection of authors. We also express our gratitude to Frank Weinreich and Gudrun Walter of Wiley-VCH for their valuable contributions to this project.

March 2003

Raimund Mannhold, Düsseldorf
Hugo Kubinyi, Weisenheim am Sand
Gerd Folkers, Zürich

Foreword

This book aims at bringing together the strategies and tools currently available to investigate and make predictions about oral absorption and bioavailability of drugs in humans. Ideally, such predictive models can be used in drug discovery from the design of compounds and libraries throughout lead optimisation to clinical candidate selection. This book also aims to discuss more complex *in vivo* aspects of oral drug delivery.

The volume is divided into five sections. Part one looks at the experimental study of membrane permeability and oral absorption. In Part two, problems of measuring and prediction solubility, as one of the key determinants in the absorption process, will be discussed in detail. In the next part, progress in the science around transporter proteins and gut wall metabolism and their effect on the overall absorption process is presented. Part four looks at the *in silico* approaches and models to predict permeability, absorption and bioavailability. In the last part of the book, a number of drug development issues will be highlighted, which could have an important impact of the overall delivery strategies for oral pharmaceutical products.

In summary, progress in predicting oral absorption is based on a much better understanding of the transport processes across the intestinal epithelium along the gastrointestinal tract. The identification of the key physicochemical properties, and in addition the identification of key transporter proteins and metabolising enzymes in the gut wall has led to the development of new *in vitro* and *in vivo* screens that allow reasonably accurate estimates of oral absorption in man to be made. Predicting bioavailability is more challenging, but very promising progress has been made in recent years, both via the combination of several *in vitro* measures, as well as the development of predictive *in silico* tools. In many cases, the validity and the accuracy of the applied methods have been investigated to some extent, but more mechanistic research is needed in order to improve the performance of the various methods used in this field of drug development.

We are very grateful to the many contributors to this book. Their insightful chapters are the body of this book. Some were prepared to stand in at the last minute, and still delivered within the deadline, which is always a relief to the editors. Finally we thank Frank Weinreich for his continuous encouragement and light pressure to get the chapters in press on time.

December 2002,
Sandwich
Uppsala
Uppsala

Han van de Waterbeemd
Hans Lennernäs
Per Artursson

List of Authors

Dr. Bertil Abrahamsson
AstraZeneca R&D
Department of Preformulation and
Biopharmaceutics
431 83 Mölndal
Sweden

Dr. Balaji Agoram
USC School of Pharmacy
Department of Pharmaceutical Sciences
1985 Zonal Ave. PSC 700
Los Angeles, CA 90089-9121
USA

Prof. Dr. Gordon Amidon
University of Michigan
College of Pharmacy
Ann Arbor, MI 48109
USA
glamidon@umich.edu

Prof. Dr. Per Artursson
Department of Pharmacy
Biomedical Centre
Uppsala University
751 23 Uppsala
Sweden
per.artursson@galenik.uu.se

Dr. Alex Avdeef
PION Inc
5 Constitution Way
Woburn, MA 01801
USA
aavdeef@pion-inc.com

Kevin Beaumont
Pfizer Global Research and Development
Department of Drug Metabolism
IPC 331
Sandwich, Kent CT13 9NJ
UK
kevin_beaumont@sandwich.pfizer.com

Dr. Christel A. S. Bergström
Division of Pharmaceutics
Department of Pharmacy
Biomedical Centre, Box 580
Uppsala University
751 23 Uppsala
Sweden
christel.bergstrom@farmaci.uu.se

Prof. Dr. Michael B. Bolger
USC School of Pharmacy
Department of Pharmaceutical Sciences
1985 Zonal Ave. PSC 700
Los Angeles, CA 90089-9121
USA
bolger@usc.edu

Dr. Emanuele Carosati
Laboratory for Chemometrics and
Chemoinformatics
Chemistry Department
University of Perugia
Via Elce di Sotto 10
06123 Perugia
Italy

Dr. John Comer
Sirius Analytical Instruments Ltd.
Riverside
Forest Row Business Park
Forest Row, East Sussex RH18 5HE
UK
john.comer@sirius-analytical.com

Dr. Charles L. Crespi
Gentest™ a BD Biosciences Company
6 Henshaw Street
Woburn, MA 01801
USA
charles_crespi@BD.com

Prof. Dr. Gabriele Cruciani
Laboratory for Chemometrics

University of Perugia
Via Elce di Sotto 10
06123 Perugia
Italy
gabri@chemiome.chm.unipg.it

Dr. Chau M. Du
Pion Inc.
5 Constitution Way
Woburn, MA 01801
USA

Dr. Anne Engelbrecht Thomsen
Royal Danish School of Pharmacy
Department of Pharmaceutics
2 Universitetsparken
2100 Copenhagen
Denmark
antt@dfh.dk

Dr. Holger Fischer
University of Basel, Biocenter
Klingelbergstrasse 70
CH-4056 Basel
Switzerland

Dr. Robert Fraczekiewicz
USC School of Pharmacy
Department of Pharmaceutical Sciences
1985 Zonal Ave. PSC 700
Los Angeles, CA 90089-9121
USA

Prof. Dr. Sven Frøkjær
Royal Danish School of Pharmacy
Department of Pharmaceutics
2 Universitetsparken
2100 Copenhagen
Denmark
sf@dfh.dk

Dr. Gladys E. Granero
University of Michigan
College of Pharmacy
Ann Arbor, MI 48109
USA

Dr. Markus Haeberlein
AstraZeneca R&D Södertälje
151 85 Södertälje
Sweden

Dr. Barry Jones
Pfizer Global Research and Development
Department of Drug Metabolism
IPC 664
Sandwich, Kent CT13 9NJ

UK
barry_jones@sandwich.pfizer.com

Dr. Johan Karlsson
AstraZeneca R&D
DMPK and Bioanalytical Chemistry
431 83 Mölndal
Sweden

Dr. Christopher P. Landowski
University of Michigan
College of Pharmacy
Ann Arbor, MI 48109
USA

Dr. Ewa Landwojtowicz
University of Basel, Biocenter
Klingelbergstrasse 70
CH-4056 Basel
Switzerland

Prof. Dr. Hans Lennernäs
Biopharmaceutics Group
Department of Pharmacy
Uppsala University
751 23 Uppsala
Sweden
hans.lennernas@biof.uu.se

Dr. Xiaochun Li Blatter
University of Basel, Biocenter
Klingelbergstrasse 70
CH-4056 Basel
Switzerland

Dr. Chris Lipinski
Pfizer Global Research and Development
Eastern Point Road
Groton, CT 06340
USA
christopher_a_lipinski@groton.pfizer.com

Dr. Chris Logan
AstraZeneca R&D Charnwood
Physical & Metabolic Science
Bakewell Road
Loughborough, Leics LE11 5RH
UK
chris.logan@astrazeneca.com

Dr. Arun Mandagere
Pfizer Global Research and Development
Ann Arbor Laboratories
Strategic Resources/Lead Discovery
2800 Plymouth Road
Ann Arbor, MI 48106
USA
arun.mandagere@pfizer.com

Prof. Dr. Raimund Mannhold
Department of Laser Medicine
Molecular Drug Research Group
Heinrich-Heine-Universität
Universitätsstr. 1
40225 Düsseldorf
Germany

Dr. James W. McFarland
Reckon.dat Consulting
217 Blood Street
Lyme, CT 06371-3509
USA
reckon.dat@attglobal.net

Dr. M. Meniconi
Laboratory for Chemometrics and
Chemoinformatics
Chemistry Department
University of Perugia
Via Elce di Sotto 10
06123 Perugia
Italy

Dr. Ulf Norinder
AstraZeneca R&D
Discovery – Medicinal Chemistry
S-151 85 Södertälje
Sweden
ulf.norinder@astrazeneca.com

Dr. Niclas Petri
Biopharmaceutics Group
Department of Pharmacy
Uppsala University
S-751 23 Uppsala
Sweden

Dr. Chandrasekharan Ramachandran
University of Michigan
College of Pharmacy
Ann Arbor, MI 48109
USA

PD Dr. Anna Seelig
University of Basel, Biocenter
Department of Biophysical Chemistry
Klingelbergstrasse 70
CH-4056 Basel
Switzerland
anna.seelig@unibas.ch

Dr. Ho-Chul Shin
University of Michigan
College of Pharmacy
Ann Arbor, MI 48109
USA

Dr. Boyd Steere
USC School of Pharmacy
Department of Pharmaceutical Sciences
1985 Zonal Ave. PSC 700
Los Angeles, CA 90089-9121
USA

Prof. Dr. Bente Steffansen
Royal Danish School of Pharmacy
Department of Pharmaceutics
2 Universitetsparken
2100 Copenhagen
Denmark
bds@dfh.dk

Prof. Dr. Yuichi Sugiyama
University of Tokyo
Graduate School of Pharmaceutical Sciences
7-3-1 Hongo, Bunkyo-ku
Tokyo 113-0033
Japan
sugiyama@mol.f.u-tokyo.ac.jp

Dr. Duxin Sun
University of Michigan
College of Pharmacy
Ann Arbor, MI 48109
USA

Prof. Dr. Hiroshi Suzuki
University of Tokyo
Graduate School of Pharmaceutical Sciences
7-3-1 Hongo, Bunkyo-ku
Tokyo 113-0033
Japan
hsuzuki@mol.f.u-tokyo.ac.jp

Dr. Staffan Tavelin
Division of Pharmaceutics
Department of Pharmacy
Biomedical Centre, Box 580
Uppsala University
S-751 23 Uppsala
Sweden
staffan.tavelin@farmaci.uu.se

Dr. Anna-Lena Ungell
AstraZeneca R&D
DMPK and Bioanalytical Chemistry
S-431 83 Moelndal
Sweden
anna-lena.ungell@astrazeneca.com

Dr. Han van de Waterbeemd
Pfizer Global Research and Development
PDM, Department of Drug Metabolism
IPC 351

Sandwich, Kent, CT13 9NJ
UK
han_waterbeemd@sandwich.pfizer.com

Prof. Dr. Clive G. Wilson
University of Strathclyde
Department of Pharmaceutical Sciences
27 Taylor Street
Glasgow, G4 0NR

UK
c.g.wilson@strath.ac.uk

Dr. Ismael Zamora
Lead Molecular Discovery
Fransecs Cananes 1-3, 2-1
08190 Sant Cugat del Valles
Spain
ismael.zamora@telefonica.net

Contents

Preface xvii

Foreword xix

List of Authors xxi

I Studies of Membrane Permeability and Oral Absorption 1

1 Physico-chemical Approaches to Drug Absorption 3

Han van de Waterbeemd

Abbreviations 3

Symbols 3

1.1 Introduction 4

1.2 Drug-like Properties 5

1.3 Dissolution and Solubility 6

1.3.1 Calculated Solubility 7

1.4 Ionization (pK_a) 7

1.5 Lipophilicity 8

1.5.1 Calculated log P 9

1.6 Molecular Size and Shape 9

1.6.1 Calculated Size Descriptors 9

1.7 Hydrogen Bonding 9

1.7.1 Calculated Hydrogen-Bonding Descriptors 10

1.8 Amphiphilicity 11

1.9 Permeability 11

1.9.1 Artificial Membranes 11

1.9.2 IAM, ILC, MEKC, and BMC 12

1.9.3 Liposome Partitioning 13

1.9.4 Biosensors 13

1.9.5 Ghost Erythrocytes and Diffusion Constants 13

References 14

2 High-throughput Measurement of log D and pK_a 21

John E. A. Comer

Abbreviations 21

Symbols 21

2.1	Introduction	22
2.2	Relationship between Ionization and Lipophilicity	24
2.3	Measuring log D	26
2.3.1	Shake-flask Method	26
2.3.2	pH-metric Method	27
2.3.3	Direct Chromatographic Methods	28
2.3.3.1	Chromatographic Hydrophobicity Index (CHI)	28
2.3.3.2	Microemulsion Electrokinetic Chromatography (MEEKC)	29
2.3.3.3	Chromatography in the Presence of Octanol	29
2.3.3.4	Reversed-Phase Chromatography	30
2.3.3.5	Liquid-Liquid Partition Chromatography	30
2.4	Measuring pK_a	32
2.4.1	Review of Methods	32
2.4.2	The Effect of Co-solvents on pK_a	34
2.4.3	pH-Metric Titration	34
2.4.4	Hybrid pH-Metric/UV Method	35
2.4.5	Other Methods	35
2.4.6	pH Gradient Titration	36
2.5	Some Thoughts about High-throughput Analytical Chemistry	39
	Acknowledgments	41
	References	42
3	High-throughput Measurement of Permeability Profiles	46
	<i>Alex Avdeef</i>	
	Abbreviations	46
	Symbols	46
3.1	Introduction	47
3.2	Key Historical Developments in Artificial-Membrane Permeability Measurement	47
3.3	The Ideal <i>in vitro</i> Artificial Membrane Permeability Model	52
3.3.1	Lipid Compositions in Biological Membranes	52
3.3.2	Permeability-pH Considerations	53
3.3.3	Role of Serum Proteins	54
3.3.4	Effects of Cosolvents, Bile Acids, and other Surfactants	55
3.3.5	Components of the Ideal	56
3.4	New Directions in PAMPA	56
3.4.1	Concentrated and Charged Phospholipid Membranes	56
3.4.2	Gradient-pH Permeability Equation	57
3.4.3	Permeability Measurements: High-phospholipid in Surfactant-free Solutions	58
3.4.4	Membrane Retention Measurements: High-phospholipid in Surfactant-free Solutions	59
3.4.5	Egg Lecithin and the Degree of Negative Charge	60
3.4.6	Summary: Increasing Phospholipid Content in the Absence of Sink Conditions	60

3.4.7	Effects of Surfactant on High-phospholipid Membrane Permeability and Retention	61
3.4.8	Quality and Usefulness of the UV Spectra	63
3.4.9	Iso-pH and Gradient-pH Mapping in 2% DOPC-Dodecane	65
3.4.10	Iso-pH Mapping in 20% Soy Lecithin-Dodecane, with Surfactant	68
3.4.11	Predictions of <i>in vivo</i> Human Jejunal Permeabilities using the Improved 20% Soy Lecithin with Surfactant <i>in vitro</i> PAMPA Technique	68
	Acknowledgments	69
	References	69
4	Caco-2 and Emerging Alternatives for Prediction of Intestinal Drug Transport: A General Overview	72
	<i>Per Artursson and Staffan Tavelin</i>	
	Abbreviations	72
	Symbols	72
4.1	Introduction	72
4.2	Research Opportunities with the Caco-2 Cell Model	73
4.2.1	Transport Mechanisms	73
4.2.2	Prediction of Drug Permeability <i>In Vivo</i>	74
4.3	Limitations of Caco-2 Cells in Predicting Intestinal Drug Transport	76
4.3.1	Technical Issues	76
4.3.2	Limitations Related to Transport Studies and Their Solutions	77
4.3.2.1	Active Transport	77
4.3.2.2	Passive Transport	80
4.4	Conclusion	82
	Acknowledgements	82
	References	82
5	Cell Cultures in Drug Discovery: An Industrial Perspective	90
	<i>Anna-Lena Ungell and Johan Karlsson</i>	
	Abbreviations	90
	Symbols	91
5.1	Introduction	91
5.2	Permeability Screening in Different Phases of Discovery	93
5.3	Cell Cultures for Assessment of Intestinal Permeability	94
5.3.1	Caco-2	95
5.3.2	MDCK Cells	97
5.3.3	2/4/A1 Cells	97
5.3.4	HT29	98
5.3.5	Other Cell Lines	99
5.4	Screening for Intestinal Permeability	99
5.4.1	Caco-2 Culture and Transport Experiments	99
5.4.2	Automated Caco-2 Assay	101
5.4.3	Quality Control and Standardization of Caco-2 Assay	103
5.4.4	Correlation to Fraction of Oral Dose Absorbed	104

5.4.5	Optimization of Experimental Conditions: pH	108
5.4.6	Optimizing Experimental Conditions: Solubility and BSA	109
5.5	Mechanistic Use of Cell Models	111
5.5.1	Paracellular Pathway	111
5.5.2	Transcellular Pathway	113
5.5.3	Carrier-mediated Transport	113
5.5.4	Evaluation of Metabolism During Transport	116
5.5.5	Evaluation of Toxicity	117
5.5.6	Computational Models for Prediction of Intestinal Permeability	118
5.6	Concluding Remarks	120
	References	120
6	Use of Animals for the Determination of Absorption and Bioavailability	132
	<i>Chris Logan</i>	
	Abbreviations	132
	Symbols	132
6.1	Introduction	133
6.1.1	ADME/PK in Drug Discovery	133
6.1.2	The Need for Prediction	134
6.2	Consideration of Absorption and Bioavailability	136
6.3	Choice of Animal Species	138
6.4	Methods	139
6.4.1	Radiolabels	139
6.4.2	<i>Ex vivo</i> Methods for Absorption	140
6.4.2.1	Static Method	140
6.4.2.2	Perfusion Methods	140
6.4.3	<i>In vivo</i> Methods	141
6.5	<i>In vivo</i> Methods for Determining Bioavailability	141
6.5.1	Cassette Dosing	141
6.5.2	Semi-simultaneous Dosing	142
6.5.3	Hepatic Portal Vein Cannulation	143
6.6	Inhalation	144
6.7	Relevance of Animal Models	145
6.7.1	Models for Prediction of Absorption	145
6.7.2	Models for Prediction of Volume	145
6.8	Prediction of Dose in Man	146
6.8.1	Allometry	146
6.8.2	Physiologically Based Pharmacokinetics	147
6.8.3	Prediction of Human Dose	148
6.9	Conclusion	150
	References	150
7	<i>In vivo</i> Permeability Studies in the Gastrointestinal Tract of Humans	155
	<i>Niclas Petri and Hans Lennernäs</i>	
	Abbreviations	155
	Symbols	155

7.1	Introduction	156
7.2	Pharmacokinetic Definition of Intestinal Absorption (f_a), Presystemic Metabolism (E_G and E_H) and Absolute Bioavailability (F) of Drugs Administered Orally to Humans	160
7.3	Methodological Aspects on <i>in vivo</i> Intestinal Perfusion Techniques	160
7.4	Paracellular Passive Diffusion	165
7.5	Transcellular Passive Diffusion	166
7.6	Carrier-mediated Intestinal Absorption	169
7.7	Jejunal Transport and Metabolism	172
7.8	Regional Differences in Transport and Metabolism of Drugs	179
7.9	Conclusion	180
	References	180
II	Drug Dissolution and Solubility	189
8	Gastrointestinal Dissolution and Absorption of Drugs	191
	<i>Gladys E. Granero, Chandrasekharan Ramachandran, and Gordon L. Amidon</i>	
	Abbreviations	191
	Symbols	191
8.1	General Dissolution	192
8.2	Absorption Models	197
8.3	Gastrointestinal Variables	200
8.3.1	Bile Salts	201
8.3.2	Gastric Emptying	201
8.3.2.1	Effect of Volume	202
8.3.2.2	Effect of Size and Density of the Drug Particle	203
8.3.2.3	Effect of pH	203
8.3.3	Gastrointestinal Transit	203
8.3.4	Gastrointestinal pH	204
8.4	Solubilization and Dissolution	205
8.4.1	Surfactants	206
8.4.2	Effect of Surfactants and pH on Dissolution Rate	206
8.4.3	Effect of pH	207
8.4.4	Bio-relevant Dissolution Media	207
8.4.5	Particle Size	208
8.4.6	Biopharmaceutics Classification System: Redefining BSC Solubility Class Boundary	209
	References	210
9	Aqueous Solubility in Discovery, Chemistry, and Assay Changes	215
	<i>Chris Lipinski</i>	
	Abbreviations	215
	Symbols	215
	Introduction	215
9.2	Compound Synthesis	216