

# INTRODUCTION TO BIOCHEMICAL TOXICOLOGY

Edited by  
Ernest Hodgson and Frank E. Guthrie

# **Introduction to Biochemical Toxicology**

---

*Edited by*

**Ernest Hodgson and Frank E. Guthrie**

Interdepartmental Program in Toxicology  
North Carolina State University, Raleigh, North Carolina

---

**BLACKWELL SCIENTIFIC PUBLICATIONS**  
OXFORD LONDON EDINBURGH BOSTON MELBOURNE

© 1980 Elsevier North Holland, Inc.

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise without the prior permission of the copyright owner.

First published in 1980 by:

Elsevier North Holland, Inc.

52 Vanderbilt Avenue, New York, New York 10017

Distributed in Australia by:

Blackwell Scientific Book Distributors

214 Berkeley Street, Carlton, Victoria 3053

Library of Congress Cataloging in Publication Data

Main entry under title.

Introduction to biochemical toxicology.

Includes bibliographies and index.

1. Toxicology. 2. Poisons—Metabolism. 3. Poisons—Physiological effect.

I. Hodgson, Ernest, 1932- II. Guthrie, Frank Edwin, 1923- [DNLM:

1. Poisoning—Metabolism. 2. Poisons—Metabolism. QV600 I68]

RA1216.I55 615.9 80-11374

ISBN 0 632 00645 5

*Editorial Services* Barbara Conover

*Design* Edmée Froment

*Art Editor* Virginia Kudlak

*Production Manager* Joanne Jay

*Compositor* Bi-Comp, Inc.

*Printer* Halliday Lithograph

Manufactured in the United States of America

---

## Preface

---

As a result of a training program in environmental and biochemical toxicology, funded by National Institute of Environmental Health Sciences Training Grant ES-07046, a course in Biochemical Toxicology has been taught at North Carolina State University for the past several years. A disadvantage to both students and teachers has been the lack of an adequate textbook for this subject. Although several pharmacology texts contain much excellent material, they are not directed toward considerations of toxicants per se. The present book is aimed at the senior-beginning graduate student level and is largely confined to considerations of the biochemistry of toxicants, their uptake, distribution, metabolism, mode of action, and elimination.

The editors share the view that an introductory text must present fundamental information in as uncomplicated a manner as possible. For this reason, the book may seem too simple to the advanced student. To further readability, references have been deleted. However, a list of suggested readings at the end of each chapter will permit students to extend their knowledge in any of the areas covered. For a reference work with an extensive bibliography the reader is directed to *Biochemical Toxicology* by A. de Bruins (Elsevier, 1976).

The book should be easily understood by any student with adequate background in biology and chemistry, including biochemistry. It has been our experience that when a fundamental understanding of biochemistry is lacking, the student should be advised to postpone an undertaking in biochemical toxicology.

Because this is a new venture in a field not previously covered by a separate text, future editions might well be quite different from the present one. To ensure improvement as well as change the editors would welcome constructive criticism and suggestions not only on the material presented but also on the choice of material to present. The views of those using the book for instructional purposes would be of especial value.

In addition to authors reviewing each other's chapters the following colleagues were kind enough to act as reviewers during the course of preparation and their efforts are gratefully acknowledged: J. R. Bend, E. McConnell, R. M. Philpot, B. R. Smith, L. Valcovic, and A. Wilson of the National Institute of Environmental Health Sciences; F. E. Bell, S. G. Chaney, J. L. Irvin, P. G. Kaufman, H. C. Smith, and J. H. Wilson of the University of North Carolina at Chapel Hill; C. E. Anderson, E. V. Caruolo, R. C. Fites, H. R. Horton, S. C. Huber, D. Huislingh, R. G. Noggle, J. F. Roberts, P. V. Shah, and D. S. Smith of North Carolina State University; C. F. Arntzen and H. M. Hall of the U. S. Department of Agriculture; F. E. Hastings of the U. S. Forest Service; H. M. Mehendale of the University of Mississippi Medical Center; L. G. Tate of the University of South Alabama; and B. A. Pappas of Carleton University, Ottawa. The expert typing and editorial assistance of Faye Lloyd are gratefully acknowledged.

Ernest Hodgson  
Frank E. Guthrie

*Raleigh, North Carolina*

---

## Contributors

---

DAUTERMAN, WALTER C.

Department of Entomology and Toxicology Program, North Carolina State University,  
Raleigh, N. C. 27650

de SERRES, FREDERICK J.

Office of the Director, National Institute of Environmental Health Sciences, P. O. Box  
12233, Research Triangle Park, N. C. 27709

DONALDSON, WILLIAM E.

Department of Poultry Science and Toxicology Program, North Carolina State University,  
Raleigh, N. C. 27650

GRALLA, EDWARD J.

Chemical Industry Institute of Toxicology, P. O. Box 12137, Research Triangle Park, N. C.  
27709

GROSCH, DANIEL S.

Department of Genetics and Toxicology Program, North Carolina State University,  
Raleigh, N. C. 27650

GUTHRIE, FRANK E.

Department of Entomology and Toxicology Program, North Carolina State University,  
Raleigh, N. C. 27650

HODGSON, ERNEST

Department of Entomology and Toxicology Program, North Carolina State University,  
Raleigh, N. C. 27650

HOLBROOK, DAVID J., JR.

Department of Biochemistry and Nutrition, School of Medicine, University of North  
Carolina, Chapel Hill, N. C. 27514

**KULKARNI, ARUN P.**

Department of Entomology and Toxicology Program, North Carolina State University,  
Raleigh, N. C. 27650

**MAILMAN, RICHARD B.**

Department of Psychiatry and Biological Sciences Research Center, University of North  
Carolina School of Medicine, Chapel Hill, N. C. 27514

**MAIN, A. RUSSELL**

Department of Biochemistry and Toxicology Program, North Carolina State University,  
Raleigh, N. C. 27650

**MATTHEWS, H. B.**

Pharmacokinetics Branch, National Institute of Environmental Health Sciences, P. O. Box  
12233, Research Triangle Park, N. C. 27709

**MORELAND, DONALD E.**

U. S. Department of Agriculture, Departments of Crop Science and Botany and  
Toxicology Program, North Carolina State University, Raleigh, N. C. 27650

**TUEY, DANIEL B.**

Biometry Branch, National Institute of Environmental Health Sciences, P. O. Box 12233,  
Research Triangle Park, N. C. 27709

## Abbreviations

These abbreviations are used throughout the book. Abbreviations used in a single chapter are not included but are defined on initial use.

ACTH	adrenocorticotrophic hormone
AChE	acetylcholinesterase
ATP	adenosine triphosphate
AMP	adenosine monophosphate
BuCh	butyrylcholine
BuChE	butyrylcholinesterase
cAMP	cyclic AMP
cGMP	cyclic GMP
ChE	cholinesterase
CoA	coenzyme A
CoQ	coenzyme Q
DAO	diamine oxidase
DNP	2,4-dinitrophenol
DPIP	2,6-dichlorophenolindophenol
ED <sub>50</sub>	median effective dose
EF-2	elongation factor 2
EPA	Environmental Protection Agency
FAD	flavin adenine dinucleotide
FDA	Food and Drug Administration
FFA	free fatty acids
FMN	flavin mononucleotide
FP	flavoprotein
GABA	$\gamma$ -aminobutyric acid
GMP	guanosine monophosphate
GSH	reduced glutathione
GTP	guanosine triphosphate
LD <sub>50</sub>	lethal dose for 50% of population
MAO	monoamine oxidase
PAM	pyridine aldoxime methiodide
PCB	polychlorinated biphenyl
Pi	inorganic phosphate
PMS	phenazine methosulfate
TCDD	2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin
UDP	uridine diphosphate
UDPGA	uridine diphosphate glucuronic acid
UMP	uridine monophosphate
UTP	uridine triphosphate
VLDLP	very low density lipoprotein



---

# Contents

---

Preface	xvii
Contributors	xix
Abbreviations	xxi
<b>Chapter 1. Biochemical Toxicology: Definition and Scope</b>	<b>1</b>
Ernest Hodgson and Frank E. Guthrie	
1.1. Introduction	1
1.2. Relationship of Toxicology to Other Sciences	2
1.3. Scope of Toxicology	3
1.4. Biochemical Toxicology	4
1.4.1. General Description	4
1.4.2. Portals of Entry	4
1.4.3. Distribution	5
1.4.4. Metabolism	5
1.4.5. Sites of Action	7
1.4.6. Excretion	8
1.5. Conclusions	9
Suggested Reading	9
<b>Chapter 2. Absorption and Distribution</b>	<b>10</b>
Frank E. Guthrie	
2.1. Absorption	10
2.1.1. Membranes	10
2.1.1a. Ionization	14
2.1.1b. Partition Coefficients	15
2.1.2. Mechanisms of Transport	16
2.1.2a. Passive Transport	16
2.1.2b. Filtration	16
2.1.2c. Special Transport	16
2.1.2d. Endocytosis	17
2.1.3. Rate of Penetration	17

2.1.4. Route of Penetration in Mammals	19
2.1.4a. Skin Penetration	19
2.1.4b. Gastrointestinal Penetration	21
2.1.4c. Respiratory Penetration	23
2.1.5. Absorption in Some Nonmammalian Systems	25
2.1.5a. Gills	25
2.1.5b. Invertebrates	25
2.1.5c. Plants	26
2.2. Distribution	29
2.2.1. Distribution by Body Fluids	29
2.2.2. Ligand-Protein Interactions	33
2.2.3. Types of Binding	34
2.2.3a. Covalent Binding	34
2.2.3b. Noncovalent Binding	34
2.2.4. Experimental Treatment of Interactions	35
2.2.4a. Method of Study	35
2.2.4b. Factors Involved	35
2.2.4c. Analysis of Data	36
2.2.4d. Competitive Binding	37
2.2.4e. Other Factors Affecting Distribution	38
Suggested Reading	38

### Chapter 3. Toxicokinetics 40

Daniel B. Tuey

3.1. Introduction	40
3.2. Basic Concepts	41
3.2.1. Exponential Growth and Decay	41
3.2.2. Curve Fitting	43
3.2.3. Pharmacokinetic Models	44
3.3. Toxicokinetic Analysis	45
3.3.1. Toxicokinetic Analysis Models	45
3.3.2. One-Compartment Model	45
3.3.2a. First-Order Elimination	45
3.3.2b. First-Order Absorption	48
3.3.2c. Constant Input	49
3.3.3. Two-Compartment Model	50
3.3.4. Chronic Ingestion	52
3.4. Toxicokinetic Synthesis	54
3.4.1. Physiological Compartmental Models	54
3.4.2. Mass Balance and Flow-Limited Transport	55
3.4.3. Polychlorinated Biphenyls: An Example	56
3.4.4. Hexabromobiphenyl: An Example	59
Suggested Reading	65

### Chapter 4. Metabolism of Toxicants: Phase I Reactions 67

Ernest Hodgson and Walter C. Dauterman

4.1. Introduction	67
4.2. Microsomal Mixed-Function Oxidations	67
4.2.1. Microsomes and Mixed-Function Oxidations: General Background	67
4.2.2. Constituent Enzymes of the Mixed-Function Oxidase System and the Cytochrome P-450 Reaction Mechanism	69
4.2.3. Distribution of Cytochrome P-450	72

4.2.4. Multiplicity of Cytochrome P-450 and Purification and Reconstitution of Mixed-Function Oxidase Systems	73
4.2.5. Microsomal Mixed-Function Oxidase Reactions	76
4.2.5a. Epoxidation and Aromatic Hydroxylation	76
4.2.5b. Aliphatic Hydroxylations	78
4.2.5c. Dealkylation: O-, N-, and S-Dealkylation	78
4.2.5d. N-Oxidation	80
4.2.5e. Oxidative Deamination	81
4.2.5f. S-Oxidation	82
4.2.5g. P-Oxidation	82
4.2.5h. Desulfuration and Ester Cleavage	82
4.3. Other Microsomal Oxidations	83
4.4. Nonmicrosomal Oxidations	84
4.4.1. Alcohol Dehydrogenases	84
4.4.2. Aldehyde Dehydrogenase	84
4.4.3. Amine Oxidases	85
4.4.3a. Monoamine Oxidases	85
4.4.3b. Diamine Oxidases	85
4.5. Reduction Reactions	86
4.5.1. Nitro Reduction	86
4.5.2. Azo Reduction	86
4.5.3. Reduction of Pentavalent Arsenic to Trivalent Arsenic	87
4.5.4. Reduction of Disulfides	87
4.5.5. Ketone and Aldehyde Reduction	87
4.5.6. Sulfoxide and N-Oxide Reduction	87
4.5.7. Reduction of Double Bonds	88
4.6. Hydrolysis	88
4.7. Epoxide Hydration	89
4.8. DDT-Dehydrochlorinase	89
Suggested Reading	90

## Chapter 5. Metabolism of Toxicants: Phase II Reactions 92

Walter C. Dauterman

5.1. Introduction	92
5.2. Glycoside Conjugation	93
5.2.1. Glucuronides	93
5.2.1a. O-Glucuronides	93
5.2.1b. N-Glucuronides	94
5.2.1c. S-Glucuronides	95
5.3. Sulfate Conjugation	95
5.4. Methyltransferases	97
5.4.1. N-Methylation	97
5.4.2. O-Methylation	98
5.4.3. S-Methylation	99
5.4.4. Biomethylation of Elements	99
5.5. Glutathione S-Transferases	100
5.6. Acylation	102
5.6.1. Acetylation	102
5.6.2. Amino Acid Conjugation	103
5.6.3. Deacetylation	104
5.7. Phosphate Conjugation	104
Suggested Reading	104

<b>Chapter 6. Comparative Toxicology</b>	<b>106</b>
Arun P. Kulkarni and Ernest Hodgson	
6.1. Introduction	106
6.2. Variations Among Taxonomic Groups	107
6.2.1. In Vivo Toxicity	107
6.2.2. In Vivo Metabolism	108
6.2.2a. Binding to Macromolecules	109
6.2.2b. Biological Half-Life	109
6.2.2c. In Vivo Metabolite Production	110
6.2.3. In Vitro Metabolism and Biochemical Considerations	121
6.2.3a. Phase I Reactions	121
6.2.3b. Phase II Reactions	126
6.3. Selectivity	127
6.4. Genetic Differences	128
6.4.1. In Vivo Toxicity	128
6.4.2. Metabolite Production	129
6.4.3. Enzyme Differences	130
6.5. General Conclusions	132
Suggested Reading	132
 <b>Chapter 7. Physiological Factors Affecting Metabolism of Xenobiotics</b>	 <b>133</b>
Walter C. Dauterman	
7.1. Introduction	133
7.2. Age and Development	133
7.3. Sex Differences	136
7.4. Hormones	137
7.4.1. Thyroid Hormone	137
7.4.2. Adrenal Hormones	137
7.4.3. Insulin	138
7.4.4. Other Hormones	138
7.5. Pregnancy	138
7.6. Diet	139
7.7. Disease	141
Suggested Reading	142
 <b>Chapter 8. Chemical and Environmental Factors Affecting Metabolism of Xenobiotics</b>	 <b>143</b>
Ernest Hodgson	
8.1. Chemical Factors	143
8.1.1. Inhibition	144
8.1.1a. Types of Inhibition: Experimental Demonstration	144
8.1.1b. Synergism and Potentiation	150
8.1.1c. Antagonism	152
8.1.2. Induction	153
8.1.2a. Specificity of Mixed-Function Oxidase Induction	153
8.1.2b. Mechanism and Genetics of Induction in Mammals	155

8.1.2.c. Effect of Induction	157
8.1.2.d. Induction of Xenobiotic-Metabolizing Enzymes Other Than Mixed-Function Oxidases	158
8.1.3. Biphasic Effects: Inhibition and Induction	158
8.2. Environmental Factors	160
8.2.1. Temperature	160
8.2.2. Ionizing Radiation	160
8.2.3. Light	160
8.2.4. Moisture	161
8.2.5. Altitude	161
8.2.6. Other Stress Factors	161
Suggested Reading	161

## **Chapter 9. Elimination of Toxicants and Their Metabolites 162**

<b>H. B. Matthews</b>	
9.1. Introduction	162
9.2. Renal Excretion	163
9.2.1. Glomerular Filtration	163
9.2.2. Tubular Reabsorption	164
9.2.3. Tubular Secretion	164
9.2.4. Factors Affecting Renal Excretion of Xenobiotics	165
9.3. Hepatic Excretion	165
9.3.1. Liver Morphology	166
9.3.2. Bile Formation and Secretion	167
9.3.3. Enterohepatic Circulation	167
9.4. Examples of Renal and Hepatic Excretion of Organic Xenobiotics	168
9.4.1. Effect of Polarity on Absorption and Excretion	168
9.4.2. Metabolic Facilitation of Excretion	169
9.4.2.a. Degradative Metabolism	169
9.4.2.b. Oxidative Metabolism	170
9.4.3. Excretion of Poorly Metabolized Xenobiotics	170
9.4.4. Effect of Molecular Weight on Excretion	170
9.5. Xenobiotic Elimination by Lungs	171
9.5.1. Factors Affecting Xenobiotic Expiration	172
9.5.2. Examples of Volatile Xenobiotics Expired by the Lungs	172
9.5.3. Alveolobronchiolar Transport Mechanisms	172
9.6. Elimination of Toxic Inorganic Xenobiotics	173
9.6.1. Fluorine and Strontium	173
9.6.2. Cadmium	173
9.6.3. Mercury	174
9.6.4. Selenium and Beryllium	174
9.6.5. Lead	174
9.7. Minor Routes of Toxic Xenobiotic Elimination	175
9.7.1. Sex-Linked Routes of Elimination	175
9.7.1.a. Milk	175
9.7.1.b. Eggs	176
9.7.1.c. Fetus	177
9.7.2. Alimentary Elimination	177
9.7.3. Obscure Routes of Xenobiotic Elimination	179
Suggested Reading	179

<b>Chapter 10. Toxicant-Receptor Interactions: Fundamental Principles</b>	<b>180</b>
A. Russell Main	
10.1. Introduction	180
10.2. Receptors	181
10.2.1. Origins of the Receptor Concept	181
10.2.2. Identification of Real Receptors	182
10.2.3. Nature of Receptors	183
10.3. Messengers	183
10.3.1. Messengers: Neurotransmitters and Hormones	183
10.3.2. Structural Classification of Messengers	185
10.4. Enzymes as Receptors	186
10.5. Theory of Toxicant-Receptor Interactions	186
10.6. Receptors and Toxicants	192
Suggested Reading	192
<b>Chapter 11. Cholinesterase Inhibitors</b>	<b>193</b>
A. Russell Main	
11.1. Introduction	193
11.2. Substrate Specificity and Classification of Cholinesterases	194
11.3. Acylated Intermediate and Active Site of Cholinesterases	198
11.3.1. Acylated Intermediate	198
11.3.2. Active Site of Cholinesterases	200
11.3.3. Explanation of Inhibition at High Substrate Concentrations	204
11.4. Reversible Inhibitors	205
11.5. Irreversible Inhibitors of Cholinesterases	207
11.5.1. Kinetics of Irreversible Inhibition	207
11.5.2. $pI_{50}$	207
11.5.3. Bimolecular Rate Constant $k_1$	208
11.5.4. Affinity ( $K_d$ ) and Acylation ( $k_2$ ) Constants	212
11.5.5. Carbamylated Active Site and Decarbamylation	214
11.5.6. Inhibition in the Presence of Substrates and Reversible Inhibitors	216
11.6. Spontaneous Regeneration, Aging, and Reactivators	217
11.6.1. Spontaneous Regeneration	217
11.6.2. Aging or Dealkylation	219
11.6.3. Reactivators	220
11.6.4. Structures of Organophosphate and Carbamate Inhibitors of Cholinesterases	223
Suggested Reading	223
<b>Chapter 12. Biochemical Toxicology of the Central Nervous System</b>	<b>224</b>
Richard B. Mailman	
12.1. Introduction	224
12.2. Blood-Brain Barrier	225
12.3. Toxicant Metabolism in the Central Nervous System	227
12.4. Biochemical Sites of Action of Toxicants	227
12.4.1. Nerve Cell and Synaptic Transmission	227
12.4.2. Specific Biochemical Sites of Action	230

12.4.2a. Changes in Transmitter (Modulator) Levels	230
12.4.2b. Receptor Interactions	230
12.4.2c. Cyclic Nucleotide Biochemistry	231
12.4.2d. Ion Balance and Flow	231
12.4.2e. Intermediary Metabolism and Miscellaneous Sites of Action	232
12.5. Specific Mechanisms and Examples	233
12.5.1. Primary and Secondary Actions	233
12.5.1a. Depletion of Neurotransmitters	233
12.5.2. Natural Toxins	235
12.5.2a. Plant Products	235
12.5.2b. Microbial and Fungal Toxins	237
12.5.2c. Animal Products	238
12.5.3. Pharmaceutical Agents	238
12.5.3a. Major Tranquilizers	238
12.5.3b. Ethanol	239
12.5.3c. Anesthetics	239
12.5.4. Heavy Metals	240
12.5.5. Considerations in Long-Term or Chronic Toxicant Exposure	241
12.5.5a. Types of Changes Occurring After Toxicant Exposure	241
12.5.5b. Developmental Factors in Nervous System Toxicology	242
12.6. Conclusions	243
Suggested Reading	243

## **Chapter 13. Effects of Toxicants on Oxidative and Photophosphorylation** 245

**Donald E. Moreland**

13.1. Oxidative Phosphorylation and Respiration	245
13.1.1. Introduction	245
13.1.2. Electron Transport and Phosphorylation	247
13.1.3. Mechanism of Electron Transfer	248
13.1.4. ATP Generation	249
13.1.4a. Chemiosmotic Hypothesis	249
13.1.4b. Chemical Hypothesis	249
13.1.4c. Conformational Coupling Hypothesis	249
13.1.5. Methods of Study	250
13.1.6. Differences Between Plant and Animal Mitochondria	251
13.1.7. Classification of Inhibitors	252
13.1.7a. Electron Transport Inhibitors	252
13.1.7b. Uncouplers	252
13.1.7c. Energy Transfer Inhibitors	253
13.1.7d. Multiple Types of Inhibition	253
13.2. Photophosphorylation and Photosynthesis	255
13.2.1. Introduction	255
13.2.2. Photoinduced Electron Transport	255
13.2.3. Classification of Inhibitors	257
13.2.3a. Electron Transport Inhibitors	258
13.2.3b. Uncouplers	258
13.2.3c. Energy Transfer Inhibitors	258
13.2.3d. Inhibitory Uncouplers	259
13.2.3e. Electron Acceptors	259
13.2.4. Studies with Intact Plants	259
Suggested Reading	260

<b>Chapter 14. Effects of Toxicants on Nucleic Acid and Protein Metabolism</b>	<b>261</b>
David J. Holbrook, Jr.	
14.1. Introduction	261
14.1.1. Precursor Incorporation into Macromolecules	261
14.1.2. Correlation of Precursor Incorporation and Rate of Macromolecular Synthesis	263
14.1.3. Cellular Heterogeneity Within Tissues	264
14.1.4. Interrelationships in the Synthesis of Macromolecules	264
14.2. DNA Synthesis	265
14.2.1. Cell Cycle and DNA Synthetic Phase	265
14.2.2. DNA Replication	265
14.2.3. DNA Polymerases	265
14.2.4. Precursors Incorporated into DNA	267
14.2.5. Liver Regeneration and Partial Hepatectomy as a Model System	267
14.3. Modification of DNA Metabolism by Toxicants	268
14.3.1. Toxicants and Thymidine Incorporation into DNA	268
14.3.2. Toxicants and Cellular Heterogeneity in Liver	269
14.4. RNA Synthesis	270
14.4.1. Synthesis of Multiple Forms of RNA	270
14.4.1a. Ribosomal RNA	270
14.4.1b. Messenger RNA	270
14.4.1c. Transfer RNA	271
14.4.2. Mammalian RNA Polymerases	271
14.4.3. Precursor Incorporation into RNA	272
14.5. Modification of RNA Metabolism by Toxicants	274
14.5.1. Effects of Toxicants on Precursor Incorporation into Total Cellular, Nuclear, and Cytoplasmic RNAs	274
14.5.2. Effects of Toxicants on RNA Polymerases	275
14.6. Protein Synthesis	278
14.6.1. Sequential Steps in Protein Synthesis	278
14.6.2. Synthesis of Proteins on Free and Membrane-Bound Polyribosomes	279
14.7. Modification of Protein Metabolism by Toxicants	280
14.8. Summary	282
Suggested Reading	283
 <b>Chapter 15. Genetic Poisons</b>	 <b>285</b>
Daniel S. Grosch	
15.1. Introduction	285
15.2. Cytostatic Agents	286
15.2.1. Specific Spindle Poisons	286
15.2.2. Nonspecific Agents	289
15.2.3. Cytochalasins	290
15.3. Chromosome Damage	290
15.3.1. Methods of Detection	291
15.3.2. Effective Agents	291
15.3.3. Mode of Action	293
15.4. Gene Mutation	294
15.4.1. Modification of the Genetic Code	294



15.4.2. Methods of Detection	295
15.4.2a. Higher Organisms	295
15.4.2b. Microbial Tests Modified by Mammalian Influences	296
15.4.2c. Tests with Cultured Mammalian Cells	298
15.4.3. Molecular Events	298
15.4.4. Types of Chemical Mutagens	299
15.4.4a. Destructive	299
15.4.4b. Additive	300
15.4.4c. Substitutive	304
15.4.5. Comparative and Quantitative Results	304
15.5. Matters for Human Concern	306
15.6. Concluding Remarks	307
Suggested Reading	307

## **Chapter 16. Chemical Carcinogenesis** **310**

David J. Holbrook, Jr.

16.1. Introduction	310
16.2. Mechanism of Chemical Carcinogenesis and Role of Somatic Mutation	311
16.2.1. Initiation and Promotion	311
16.2.2. Proposed Mechanisms of Chemical Carcinogenesis	311
16.3. Chemical Nature and Reactivity	313
16.3.1. Electrophilic Reactive Species	313
16.3.2. Intrinsically Reactive Carcinogens	313
16.3.3. Organic Carcinogens that Undergo Metabolic Activation	313
16.4. Covalent Reaction of Organic Carcinogens with DNA and Other Macromolecules	316
16.4.1. Intact Cellular Systems	316
16.4.2. Reactions In Vitro	316
16.4.2a. Isolated Microsomes	316
16.4.2b. Isolated Nuclei	317
16.4.3. Metabolism of Xenobiotics in Relation to Covalent Reactions and Carcinogenesis	317
16.4.4. Molecular Sites of DNA and RNA Susceptible to Covalent Reactions	319
16.5. Metal Carcinogenesis	320
16.5.1. Metallic Cation Interactions	320
16.5.2. Metallic Cation-Induced Miscoding of Viral DNA Polymerases	321
16.6. DNA Repair	322
16.6.1. DNA Repair Processes in Mammalian Cells	322
16.6.1a. DNA Excision Repair	322
16.6.1b. Postreplication Repair	323
16.6.1c. Removal of Alkylated Bases	323
16.6.2. Persistence of DNA Damage in Relation to Carcinogenesis	324
16.6.3. Assay of Chemical Carcinogens in Mammalian Systems	325
16.6.3a. DNA Damage Measured by Centrifugation on Sucrose Gradients	326
16.6.3b. Unscheduled DNA Synthesis	327
Suggested Reading.	327