

Microbial Toxins

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VOLUME IV

BACTERIAL ENDOTOXINS

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Preface

The field of research concerning bacterial lipopolysaccharides has, during the past decade, developed rapidly and in a most exciting manner. During this period the most definitive integration of existing information was contained in a symposium on bacterial endotoxins held at Rutgers University. The proceedings, edited by M. Landy and W. Braun, were published in 1964. In the intervening seven years there has been a remarkable expansion of our knowledge concerning endotoxins which has required comprehensive analysis. Our contributors have directed themselves toward this goal. Areas of research which underwent vigorous development include the mechanism of biosynthesis of lipopolysaccharides, the utilization of bacterial mutants to study the genetics of endotoxin formation, the relation of endotoxin structures to other biological molecules and to virulence, as well as the interaction of lipopolysaccharides with the immune system. While exciting new findings have evolved, this is not to suggest that the era of endotoxin research has come to a close. Many questions remain unanswered, and in Volumes IV and V an attempt is made to disclose the areas which need added effort. Critical evaluations of these problems have been encouraged.

We have attempted to incorporate into Volume IV a general introduction to the subject as well as research concerning structure (both morphological and physical), chemistry, immunology, biosynthesis, and genetics of bacterial endotoxins. Volume V concerns itself with discussions involving the physiology, pharmacology, and pathology of the endotoxins as well as with evaluation of the roles that endotoxins may play in the development of shock, fever, and bacterial virulence. Characterization of host response to endotoxin in terms of alterations in cell metabolism as well as neutralization and detoxication are also presented in Volume V.

As was true in each of the previous volumes in this treatise, we are indebted to the staff of Academic Press for advice and practical assistance. We thank each of the contributors for their consideration, their forbearance for the many delays, and their valuable comments and criticisms. We are also grateful to Miss Loretta Battista for her aid in the administrative aspects of the project. We assume full responsibility for any errors or omissions and sincerely hope that these volumes will stimulate the development of new insights into the various aspects of bacterial endotoxin research.

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Contents of Other Volumes

Volume I: Bacterial Protein Toxins Edited by

S. J. Ajl, S. Kadis, and T. C. Montie

General Characteristics

W. E. van Heyningen

The Nomenclature of Microbial Toxins: Problems and Recommendations

Peter F. Bonventre

Intracellular versus Extracellular Toxins

Marcel Raynaud and Joseph E. Alouf

Isolation and Purification of Bacterial Toxic Proteins

Joseph E. Alouf and Marcel Raynaud

Cytolytic Toxins of Bacteria

Alan W. Bernheimer

Relationship of Lysogeny to Bacterial Toxin Production

John B. Zabriskie

Role of Toxins in Host-Parasite Relationships

J. J. Bullen

Tissue Culture and Bacterial Protein Toxins

Morris Solotorovsky and William Johnson

Pharmacology of Bacterial Protein Toxins

Helena Rašková and Karel Mašek

Relative Toxicities and Assay Systems

C. L. Oakley

Immunology of Bacterial Protein Toxins

C. L. Oakley

Relationship of Bacterial Structure and Metabolism to Toxin Production

Iwao Kato

Uptake of Bacterial Protein Toxins by Cells

I. Mesrobian, Lydia Mesrobian, and C. Bona

AUTHOR INDEX-SUBJECT INDEX

Volume IIA: Bacterial Protein Toxins Edited by

S. Kadis, T. C. Montie, and S. J. Ajl

Botulinum Toxin

Daniel A. Boroff and Bibhuti R. DasGupta

Tetanus Toxin

W. E. van Heyningen and Jane Mellanby

Type A *Clostridium perfringens* Toxin

M. V. Ispolatovskaya

***Clostridium perfringens* Toxins Types B, C, D, and E**

Andreas H. W. Hauschild

Cholera Toxins

John P. Craig

The Exotoxin of *Shigella dysenteriae*

W. E. van Heyningen

Protein Toxins from *Bordetella pertussis*

J. Munoz

***Salmonella typhimurium* and *Escherichia coli* Neurotoxins**

Lydia Mesrobian and I. Mesrobian

Toxins of *Proteus mirabilis*

Krystyna Izdebska-Szymona

***Listeria monocytogenes* Toxin**

C. P. Sword and G. Charles Kingdon

AUTHOR INDEX-SUBJECT INDEX**Volume IIB: Bacterial Protein Toxins Edited by**

S. Kadis, T. C. Montie, and S. J. Ajl

Diphtheria Toxin

A. M. Pappenheimer, Jr.

AUTHOR INDEX-SUBJECT INDEX**Volume III: Bacterial Protein Toxins Edited by**

T. C. Montie, S. Kadis, and S. J. Ajl

Nature and Synthesis of Murine Toxins of *Pasteurella pestis*

Thomas C. Montie and Samuel J. Ajl

Site and Mode of Action of Murine Toxin of *Pasteurella pestis*

Solomon Kadis and Samuel J. Ajl

Streptolysin O

Seymour P. Halbert

Streptolysin S

Isaac Ginsburg

Erythrogenic Toxins

Dennis W. Watson and Yoon Berm Kim

Staphylococcal α -Toxin

John P. Arbuthnott

The Beta- and Delta-Toxins of *Staphylococcus aureus*

Gordon M. Wiseman

Enterotoxins

Merlin S. Bergdoll

Staphylococcal Leukocidin

A. M. Woodin

Addendum—Production of Test Toxin of P-V Leukocidin

R. Elsworth and K. Sargeant

Anthrax Toxin

Ralph E. Lincoln and Donald C. Fish

***Bacillus cereus* Toxin**

Peter F. Bonventre and Charles E. Johnson

***Bacillus thuringiensis* Toxins—The Proteinaceous Crystal**

Marguerite M. Lecadet

Toxins of *Pseudomonas*

Robert J. Heckly

The Toxins of Mycoplasma

Evangelia Kaklamanis and Lewis Thomas

AUTHOR INDEX-SUBJECT INDEX**Volume V: Bacterial Endotoxins Edited by**

S. Kadis, G. Weinbaum, and S. J. Ajl

The Relationship of Lipopolysaccharide Structure to Bacterial Virulence

Robert J. Roantree

Importance of Blood Group and Forssman Antigenic Determinants in Interactions between Man and Microbes

Georg F. Springer

Chemical Modification of Lipopolysaccharide and Inactivation of Its Biological Properties

Barnet M. Sultzter

Effects of Endotoxin Lipopolysaccharides on the Complement System

Henry Gewurz, Ralph Snyderman, Stephan E. Mergenhagen, and Hyun Seung Shin

Host-Dependent Neutralization and Detoxification of Endotoxin

Robert C. Skarnes and Fred S. Rosen

Metabolic Effects of Bacterial Endotoxins

L. Joe Berry

Release of Vasoactive Agents and the Vascular Effects of Endotoxin

Lerner B. Hinshaw

Addendum—The Effects of Endotoxins in the Microcirculation

B. Urbaschek

Endotoxin and the Pathogenesis of Fever

E. S. Snell

Experimental Hemorrhagic and Endotoxin Shock

Arnold L. Nagler and Stanley M. Levenson

Effects of Lipopolysaccharide (Endotoxins) on Susceptibility to Infections

Leighton E. Cluff

Role of Hypersensitivity and Tolerance in Reactions to Endotoxin

Louis Chedid and Monique Parant

AUTHOR INDEX-SUBJECT INDEX**Volume VI: Fungal Toxins Edited by**

A. Ciegler, S. Kadis, and S. J. Ajl

Section A *Aspergillus* Toxins**Aflatoxins and Related Compounds**

E. B. Lillehoj, A. Ciegler, and R. W. Detroy

Ochratoxin and Other Dihydroisocoumarins

P. S. Steyn

Miscellaneous *Aspergillus* Toxins

Benjamin J. Wilson

Section B *Penicillium* Toxins**Yellowed Rice Toxins****a. Luteoskylin and related compounds (rugulosin etc.)***Penicillium islandicum*; *P. rugulosum*, *P. tardum*, and
P. brunneum, *P. variable*, etc.**b. Chlorine-containing peptide***P. islandicum***c. Citrinin***P. citrinum*, etc.

Mamoru Saito, Makoto Enomoto, and Takashi Tatsuno

d. Citreoviridin

P. citreo-viride, *P. ochrosalmoneum*

Kenji Uraguchi

The Rubratoxins, Toxic Metabolites of *Penicillium rubrum* Stoll

M. O. Moss

Patulins, Penicillic Acid, and Other Carcinogenic Lactones

A. Ciegler, R. W. Detroy, and E. B. Lillehoj

Cyclopiazonic Acid and Related Toxins

C. W. Holzapfel

Miscellaneous *Penicillium* Toxins

Benjamin J. Wilson

AUTHOR INDEX-SUBJECT INDEX

Volume VII: Algal and Fungal Toxins Edited by

S. Kadis, A. Ciegler, and S. J. Ajl

Section A Algal Toxins

The Dinoflagellate Poisons

Edward J. Schantz

Blue-Green and Green Algal Toxins

John H. Gentile

Toxins of Chrysophyceae

Moshe Shilo

Section B Fungal Toxins, Toxins of *Fusarium*

F-2 (Zearalenone) Estrogenic Mycotoxin from *Fusarium*

C. J. Mirocha, C. M. Christensen, and G. H. Nelson

Alimentary Toxic Aleukia

A. Z. Joffe

Toxin-Producing Fungi from Fescue Pasture

Shelly G. Yates

Spiroepoxy Trichothecenes

James R. Bamburg and Frank M. Strong

Toxins of *Fusarium nivale*

Mamoru Saito and Takashi Tatsuno

Section C

Rhizoctonia Toxin (Slaframine)

H. P. Broquist and J. J. Synder

Section D

The Toxicology of Sporidesmins and Other Epipolythiadioxopiperazines

Alan Taylor

AUTHOR INDEX-SUBJECT INDEX

Volume VIII: Fungal Toxins Edited by

S. Kadis, A. Ciegler, and S. J. Ajl

Section A

The Isolation and Identification of the Toxic Coumarins

Donald E. Richards

The Biological Action and Metabolism of the Toxic Coumarins

Lester D. Scheel

The Natural Occurrence and Uses of the Toxic Coumarins

Vernon B. Perone

Section B

Stachybotrys Toxin

Joseph Forgacs

Section C Phytotoxins

Phytopathogenic Toxins

H. H. Luke and V. E. Gracen, Jr.

Helminthosporium Toxins

H. H. Luke and V. E. Gracen, Jr.

Alternaria Toxins Related to Pathogenesis in Plants

G. E. Templeton

A Phytotoxin from *Didymella applanata* Cultures

C. A. Salemink and F. Schuring

Compounds Accumulating in Plants after Infection

Joseph Kuć

The Toxic Peptides of *Amanita* Species

Theodor Wieland and Otto Wieland

Mushroom Toxins Other than *Amanita*

Robert G. Benedict

Ergot

D. Gröger

AUTHOR INDEX-SUBJECT INDEX

Contents

| | |
|---------------------------------|------|
| LIST OF CONTRIBUTORS | ix |
| PREFACE | xi |
| CONTENTS OF OTHER VOLUMES | xiii |

1. General Characteristics

KELSEY C. MILNER, JON A. RUDBACH, AND EDGAR RIBL

| | |
|--|----|
| I. Introduction. Toward a Definition | 1 |
| II. Source in Cell | 3 |
| III. Host-Reactive Properties | 6 |
| IV. Physicochemical Properties | 36 |
| V. A Provisional Definition | 54 |
| References | 56 |

2. The Anatomy and Chemistry of Gram-Negative Cell Envelopes

JOHN H. FREER AND M. R. J. SALTON

| | |
|--|-----|
| I. Anatomy of Envelopes | 67 |
| II. Chemistry of the Gram-Negative Cell Envelope | 102 |
| References | 122 |

3. The Physical Structure of Bacterial Lipopolysaccharides

J. W. SHANDS, JR.

| | |
|---|-----|
| I. Introduction | 127 |
| II. Problems in the Study of Lipopolysaccharide Structure | 128 |
| III. The Physical Shape of Lipopolysaccharide | 130 |
| IV. Determinants of Physical Shape | 137 |
| V. Physical Structure and Biological Function | 141 |
| References | 143 |

4. Isolation and Chemical and Immunological Characterization of Bacterial Lipopolysaccharides

OTTO LÜDERITZ, OTTO WESTPHAL, ANNE MARIE STAUB, AND HIROSHI NIKAIIDO

| | |
|---|-----|
| I. Introduction | 145 |
| II. Isolation and Properties of Lipopolysaccharides | 147 |

| | |
|---|-----|
| III. Sugar Constituents and Composition of Lipopolysaccharides | 153 |
| IV. Structure of the O-Specific Chains and Chemical Basis of the Immunological Specificities of the O-Factors | 162 |
| V. Structure of Atypical Specific Chains as Found in SR and T Mutants | 189 |
| VI. The Basal Core Structure as Provided by Analysis of R Mutant Lipopolysaccharides | 192 |
| VII. General Conclusions | 217 |
| References | 224 |

5. The Chemistry of the Unique Carbohydrates of Bacterial Lipopolysaccharides

GILBERT ASHWELL AND JEAN HICKMAN

| | |
|--|-----|
| I. Introduction | 235 |
| II. Pentoses | 236 |
| III. Uronic Acids | 237 |
| IV. Hexoses | 239 |
| V. Hexosamines | 241 |
| VI. Heptoses | 251 |
| VII. 6-Deoxyhexoses | 254 |
| VIII. 3,6-Dideoxyhexoses | 257 |
| IX. KDO (3-Deoxy-D-mannooctulosonic Acid, 2-Keto-3-deoxyoctanic Acid) | 262 |
| References | 263 |

6. The Relation of Bacteriophage Attachment to Lipopolysaccharide Structure

ANNETTE M. C. RAPIN AND HERMAN M. KALCKAR

| | |
|--|-----|
| I. Introduction | 267 |
| II. Structure of the Gram-Negative Cell Wall | 271 |
| III. The Cell Wall of Enterobacteriaceae—Relationship between Antigenic Alterations and Phage Adsorption | 272 |
| IV. Early Work on the T Phages | 277 |
| V. <i>Salmonella</i> Phages | 280 |
| VI. <i>E. coli</i> Phages | 292 |
| VII. Conclusions | 302 |
| References | 303 |

7. Chemical and Biological Heterogeneity of Endotoxins

A. NOWOTNY

| | |
|--|-----|
| I. Introduction | 309 |
| II. Morphological and Chemical Heterogeneity | 310 |
| III. Biological Heterogeneity | 321 |
| IV. Attempts to Explain the Observed Heterogeneity | 325 |
| V. Summary | 326 |
| References | 327 |

8. Biosynthesis of the Core Region of Lipopolysaccharide**M. J. OSBORN AND LAWRENCE I. ROTHFIELD**

| | |
|---|-----|
| I. Introduction | 331 |
| II. Pathway of Biosynthesis of the Outer Core | 332 |
| III. Role of Glycerophosphatides in Core Glycosyl Transferase Reactions | 340 |
| IV. Biosynthesis of the Backbone Region | 347 |
| References | 349 |

9. Biosynthesis of O-Antigens**PHILLIPS W. ROBBINS AND ANDREW WRIGHT**

| | |
|--|-----|
| I. Introduction | 351 |
| II. Variations in O-Antigen Structure | 352 |
| III. The Biosynthetic Cycle | 354 |
| IV. The Antigen Carrier Lipid | 356 |
| V. The Polymerase Reaction | 358 |
| VI. Transfer of O-Antigen Chains to the Lipopolysaccharide Core—The Ligase Reaction | 361 |
| VII. Synthesis of Other O-Antigens and Polysaccharides | 362 |
| References | 366 |

**10. Genetic Aspects of Biosynthesis and Structure of *Salmonella*
Lipopolysaccharide****B. A. D. STOCKER AND P. HELENA MÄKELÄ**

| | |
|--|-----|
| I. Introduction | 369 |
| II. Methods of Genetic Analysis in <i>Salmonella</i> | 372 |
| III. The Core | 389 |
| IV. The O Side Chains | 403 |
| V. Modifications of the LPS Structure | 417 |
| VI. T Forms | 424 |
| VII. Summary and Discussion | 426 |
| References | 433 |

| | |
|--------------------|-----|
| AUTHOR INDEX | 439 |
|--------------------|-----|

| | |
|---------------------|-----|
| SUBJECT INDEX | 459 |
|---------------------|-----|

CHAPTER 1

General Characteristics

KELSEY C. MILNER, JON A. RUDBACH, AND EDGAR RIBI

| | |
|---|----|
| I. Introduction. Toward a Definition | 1 |
| II. Source in Cell | 3 |
| A. Development of Opinion | 3 |
| B. Contemporary Questions | 4 |
| C. Extraction of Endotoxin | 5 |
| III. Host-Reactive Properties | 6 |
| A. Catalogue | 6 |
| B. One or Many | 27 |
| C. Potentiation of Effect | 30 |
| D. Distribution of Injected Endotoxin | 35 |
| IV. Physicochemical Properties | 36 |
| A. Chemistry | 36 |
| B. Inactivation of Endotoxin | 41 |
| C. Physical Properties | 47 |
| V. A Provisional Definition | 54 |
| References | 56 |

I. Introduction. Toward a Definition

Endotoxin has undoubtedly been under investigation for well over a century although the word itself was not introduced for several decades. Today, paradoxically, we find the term a misnomer but still the best available name for a mysterious bacterial product that cannot yet be described completely in two or indeed any number of volumes.

Killed cells of most gram-negative bacteria are themselves poisonous, and from them may be extracted a more or less water-soluble material that always contains phosphorus, fatty acids, divalent cations, and carbohydrates (and usually other constituents), that withstands boiling and that, upon injection into animals (especially mammals), gives rise to striking and varied pathophysiological changes. The material was called endotoxin because it was thought to be contained within cells and released from them with difficulty, perhaps only upon disruption or digestion of the cells. It was further differentiated from the classic toxins (protein toxins) by an apparent failure to be neutralized by antiserum, by failure to convert to toxoid upon aging or treatment with formaldehyde, by a lower order of potency, and by its production of essentially the same responses in animals regardless of the microbial species of origin. Much further discussion proceeds, however, from the foregoing statements, and not all of these traditional ideas are correct.

The beginning of research on bacterial endotoxins is probably to be found in studies of the relationship between putrefaction and disease during the prebacteriological era. The extent to which we can identify endotoxin in these old reports should be of some assistance in formulating a definition. Although many of the earlier descriptions of the effects of injecting putrefying tissues into animals are compatible with the known host reactivity of endotoxin, there was, in most cases, the complication of injecting what we recognize as living bacteria that also produced infection. A great Danish pathologist and physiological chemist, who worked with both Virchow and Claude Bernard and whose accomplishments deserve to be better remembered, made a restrictive definition of what he called "putrid intoxication" and deliberately set out to isolate the toxic principle (*putride Gift*) (Panum, 1856). His starting materials were aqueous infusions of decomposing feces, blood, brain, and other tissues. These he filtered clear and distilled. The activity remained in the residue and could be redissolved in water but not in alcohol. Even after being boiled for 11 hours, the poison would kill a dog in a dose of 0.12 gm and produce fever in a much smaller dose. Because Panum was very sure that neither "ferments" nor living organisms could have survived in his preparations, he was led to espouse a chemical theory of putrefaction and septic disease. Thus, he enters history as one who was on the wrong side—an opponent of microbial theories of etiology. Billroth, probably a less talented observer, wrote voluminously and introduced many of the terms employed by microbiologists. He is often credited with the first demonstration of pyrogen in ordinary distilled water, but the attribution is unwarranted. [Billroth (1865) correctly noted that the slow 1°C rise and fall in temperature of only one dog, following subcutaneous inoculation with distilled water, was within the normal diurnal fluctuation for the particular animal. Other dogs inoculated intravenously with the same water or with fresh well water showed no fever.] We can be fairly sure from the data given by Panum that he was working with endotoxin. In part, that is because the elegance and precision of his work stand out from that of his contemporaries, in part because we read a great deal into his descriptions from our more informed status. We assume, for example, that his starting materials contained kinds of bacteria which he did not know existed. In order to avoid confusion today, we should know much more about a material before labeling it "endotoxin."

The concept of endotoxin as a poison, which is part of the living substance of bacteria and which escapes only upon disintegration of the cells, we owe mainly to Pfeiffer (1892; see also succeeding works in same journal). This persists as the dictionary meaning of the word. By combining this idea with their knowledge of immune lysins, Pfeiffer and col-