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.

GEORGE WEINBAUM
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CHAPTER 1

General Characteristics

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

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-