Microbial Toxins

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VOLUME I

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Preface

The first major attempt to compile and critically evaluate most of the knowledge that had been accumulated concerning all or a large portion of the toxins elaborated by microorganisms within a single volume was made by Professor W. E. van Heyningen in 1951 and resulted in the publication of "Bacterial Toxins." During the past two decades, however, research on microbial toxins has expanded at a rapid rate, and much new and exciting information has been obtained. Not only has the number of known isolatable toxins increased but we now have more profound insights into the structure, mode of action, and role played in disease by a number of toxin molecules that have been discussed in the literature since the early part of this century. This, of course, is not to imply that the bulk of the problems has been solved. On the contrary, much remains to be learned about the major aspects of microbial toxin research. Nevertheless, the stage is now set to reexamine our previous concepts about these microbial toxic products in the light of our current findings and to clear pathways for the future. Consequently, the purpose of this multivolume treatise is to fill the need for a comprehensive treatment of microbial toxins with an emphasis on contemporary work. Although stress must be placed on recent achievements, primary consideration will be given to covering all aspects so that new material can be synthesized from and integrated with previous investigations. Stimulating critical evaluations of current views and suggestions for productive lines of research have been encouraged. These volumes are intended for professional scientists, including graduate students, in microbiology, biochemistry, immunology, and related fields.

The toxins to be included in this treatise can be placed into four primary categories: (1) bacterial protein toxins, (2) bacterial lipopolysaccharide endotoxins, (3) algal toxins, and (4) fungal toxins. The aim has been to discuss each, wherever feasible, in terms of a number of general topics applicable to the group as a whole as well as by giving a detailed account of each individual toxin. The present plan calls for six volumes. The first one covers some of the general problems and approaches in the area of bacterial protein toxins, whereas Volumes II and III are concerned with considerations of specific toxins. Since it is commonly accepted that all of the lipopolysaccharide endotoxins produced by gram-negative bacteria are quite similar in structure, function, and mode of biosynthesis, no attempt will be made to cover each toxin separately.

Consequently, Volume IV will refer primarily to the biochemistry of endotoxins as a whole as related to their structure and biosynthesis and Volume V will deal with their physiology, pharmacology, and immunology. The algal and fungal toxins will be covered in Volume VI.

The editors are convinced that these volumes should be as inclusive as possible but they are aware that in this respect they have not been completely successful. This is due in part at least to the above outlined, perhaps arbitrary, classification of the toxins. Some bacterial toxins, for example, which are not proteins or lipopolysaccharides have not been included (e.g., *Pseudomonas tabaci, Rhizobium japonicum,* and the nucleotide exotoxin of *Bacillus thuringiensis*). For any omissions, typographical errors, inconsistencies in the references, etc., the editors take full responsibility.

This undertaking has been made much easier by the excellent cooperation of all of the contributors and by the advice and practical assistance rendered by the staff of Academic Press. Particular thanks and gratitude go to Miss Loretta Battista who has been the focal point of all of the complexities involved with the administration of this effort and without whose help this treatise could not have been readily achieved. We hope that this work will aid in stimulating and accelerating future research in the overall area of microbial toxins.

November, 1969

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Contents of Future Volumes

Introductory Remarks

Roux and Yersin (1) more than 80 years ago demonstrated that sterile filtrates from cultures of the diphtheria bacillus contained a poison or toxin that upon injection into guinea pigs, rabbits, and pigeons was capable of mimicking the symptoms and type of death produced by infection with living organisms. In their initial experiment, no less than 35 ml of culture filtrate injected intraperitoneally was required in order to kill a guinea pig. Nevertheless, in their first paper they noted that low doses of the filtrate caused a type of paralysis in guinea pigs and rabbits that was reminiscent of post-diphtheritic paralysis in man and that injection of a concentrated preparation in an amount sufficient to kill 100 guinea pigs produced no ill effects in a 10-g mouse. Roux and Yersin even demonstrated that the urine of children taken shortly before death from diphtheria may contain sufficient toxin to kill guinea pigs with symptoms similar to those produced by culture filtrates. Thus, for the first time, the mechanism of pathogenicity of a microorganism for man became clarified and could be explained in terms of a soluble toxic substance released by the bacteria. The earlier prediction of Loeffler (2) was thus verified. In his experiments establishing the etiology of the disease, Loeffler had observed that, although in fatal cases of diphtheria, characteristic lesions could be found widely distributed in organs throughout the body, these lesions were invariably found to be sterile and the diphtheria bacillus itself could only be isolated from the primary local lesion ("false membrane") in the throat.

The search for other toxin-producing bacteria began without delay and tetanus toxin was discovered in 1890 independently by Knut Faber (3) and by Briegel and Fränkel (4). The latter authors injected culture filtrates from many bacteria into animals and obtained some evidence for extracellular toxins produced by cholera vibrios and staphylococci as well as by the tetanus bacillus. Proof of the vital importance of microbial toxins as disease-producing agents was finally furnished by von Behring and Kitasato (5) who, in 1890, first prepared diphtheria and tetanus antitoxins and demonstrated their ability to specifically protect against the lethal action of the homologous toxin.

But any hopes that a specific antitoxic immunity could be developed for protection against each infectious disease of bacterial etiology or that a characteristic toxin would be found which could explain the mecha-

nism of microbial pathogenicity in each case were short-lived. Many invasive bacteria, such as the pneumococcus, are capable of causing fatal diseases in man and in animals. Yet, even though physicians refer to patients critically ill with pneumococcal lobar pneumonia as being in a highly "toxic" condition, the pneumococcus has not been shown to produce a toxin either *in vivo* or *in vitro*. In other bacterial or mycotic diseases the characteristic toxic symptoms may be attributed to development of hypersensitivity to microbial products which in normal persons or animals may show little or no primary toxicity. The role of hypersensitivity as compared with primary toxicity may be particularly hard to assess in the case of endotoxins of gram-negative bacteria. Finally, many human and animal pathogens have been shown to produce toxins and extracellular enzymes whose role in the actual disease process is far less clear than is the case with tetanus and diphtheria.

Since the discovery of diphtheria toxin, isolation and partial characterization of many toxins has been accomplished and the list of crystalline protein toxins is becoming quite long so that the present treatise is expected to run to six volumes. Despite the fact that our knowledge has expanded sufficiently to make such a treatise possible, I suspect that most of our contributors would agree that we still know very little about how most bacterial toxins cause damage to host cells; nor in many cases do we know their role in bacterial metabolism. However, it does not seem farfetched to suppose that the extracellular lecithinases produced by *Clostridium welchii* and phospholipases of other bacterial species or the extracellular nucleases, proteinases, hyaluronidases, carbohydrases, etc. released by pathogenic species such as hemolytic streptococci and staphylocci are used by these organisms to obtain assimilable nutrients. Indeed, the production of extracellular enzymes that break down large molecules to small assimilable units was obviously an important step in the evolution of parasitism by certain microorganisms.* It is far less clear to what extent such extracellular enzymes are actually responsible for the symptoms of disease. In a few instances, such as tetanus, botulism, diphtheria, and scarlet fever, the role of toxins in producing lesions or symptoms has been established without question. But only in the case of diphtheria toxin has the mechanism been established beyond reasonable doubt at the cellular level in molecular terms.

*Extracellular proteins are rarely elaborated by those species of gram-negative bacteria normally found in the intestinal tract of man and animals. Presumably in this case assimilable nutrients become available as a result of breakdown of large molecules by host enzymes of the digestive tract.

"Quelle est la nature du poison diphtérique? Est-ce un alcaloïde ou une diastase?" Roux and Yersin (1) asked these questions in 1888 and suggested that because of its heat lability, the toxin resembled an enzyme ("diastase") more than an alkaloid. But 80 years intervened before it was finally proved independently in two laboratories (6,7) that diphtheria toxin is, in fact, a highly active enzyme of a novel and unique type. A few molecules of this toxin fixed at the periphery of susceptible mammalian cells (perhaps no more than one molecule per cell is required) suffice to inhibit cell protein synthesis within a few hours or less (8). The mechanism of this inhibition has now been elucidated. It has been shown that diphtheria toxin catalyzes the splitting of NAD (nicotinamide adenine dinucleotide) with release of free nicotinamide and transfer of its ADP-ribose moiety to a point at or near the active site of transferase II(6-11). This enzyme is a translocase (12) which in the presence of GTP and Mg^{2+} brings about the translocation of the growing peptide chain on the ribosome so as to be in position to accept the next amino acid from aminoacyl-tRNA. Transferase II appears to be the unique acceptor for the toxin-catalyzed transfer of the ADP-ribose group. It has been shown that addition of a "saturating" dose to HeLa cell cultures (25-50 toxin molecules fixed per cell) brings about the complete conversion of all the free cellular transferase II to its inactive ADP-ribose derivative within minutes (7). Dialyzed ribosome-free extracts from intoxicated cells show no translocase activity but their transferase II activity may be restored to its normal level by addition of both the toxin and an excess of nicotinamide (7). Recently, Baseman and Gill (13) have found protein synthesis reduced in certain tissues of intoxicated guinea pigs because of the specific inactivation of transferase II. Thus, at least in the case of diphtheria toxin at the cellular level, its primary mode of action can be explained in molecular terms even though the complex series of events leading ultimately to death of animals from intoxication remains to be worked out.

Research into the biology of infectious disease has greatly declined during the past decade. This lapse of interest can be attributed, at least in part, to the fact that successful solution of the practical problems of prevention and chemotherapy of most infectious diseases has virtually eliminated them as leading causes of death in civilized countries. Yet the fundamental biological questions that ask by what mechanisms microorganisms are able to cause disease have remained largely unanswered, even though from a theoretical point of view these problems have retained all of their original fascination. A vast amount of knowledge on the production, the isolation, and the properties of microbial toxins has accumulated over the years. It is hoped that the assembly of this information within a single series of volumes and its critical analysis by a dis-

tinguished group of experts will provide a stimulating source of reference for many years to come for those interested in host-parasite interaction and in the biology of infectious disease.

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Contents