BOTULISM

The Organism, Its Toxins, The Disease

LOUIS DS. SMITH, Ph.D.

Almost every aspect of the bacteria that cause botulism and the characteristics of the disease itself are covered in this volume. Individual chapters deal with the organisms and their occurrence in nature, the bacteriocins and bacteriophages that affect their growth and toxin production, the heat and radiation resistance of their spores, the properties and action of the toxins they produce, and the susceptibility of different species to their various toxins. The incidence and characteristics of botulism in man, domestic animals and wild birds are described and methods of control, where available, are given.

American Lecture Series®

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By

LOUIS DS. SMITH, Ph.D.

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FOREWORD

THE GENESIS OF THIS SERIES, *The American Lecture Series in Clinical Microbiology*, stems from the concerted efforts of the Editor and the Publisher to provide a forum from which well-qualified and distinguished authors may present, either as a book or monograph, their views on any aspect of clinical microbiology. Our definition of clinical microbiology is conceived to encompass the broadest aspects of medical microbiology not only as it is applied to the clinical laboratory but equally to the research laboratory and to theoretical considerations. In the clinical microbiology laboratory we are concerned with differences in morphology, biochemical behavior and antigenic patterns as a means of microbial identification. In the research laboratory or when we employ microorganisms as a model in theoretical biology, our interest is often focused not so much on the above differences but rather on the similarities between microorganisms. However, it must be appreciated that even though there are many similarities between cells, there are important differences between major types of cells which set very definite limits on the cellular behavior. Unless this is understood it is impossible to discern common denominators.

We are also concerned with the relationships between microorganisms and disease—any microorganism and any disease. Implicit in these relations is the role of the host which forms the third arm of the triangle: microorganism, disease and host. In this series we plan to explore each of these; singly where possible for factual information and in combination for an understanding of the myriad of interrelationships that exists. This necessitates the application of basic principles of biology and may, at times, require the emergence of new theoretical concept which will create new principles or modify existing ones. Above all, our aim is to present well-documented books which will be informative, instructive and useful, creating a sense of satisfaction to both the reader and the author.

Closely intertwined with the above *raison d'etre* is our desire to produce a series which will be read not only for the pleasure of knowledge but which will also enhance the reader's professional skill and extend his technical ability. *The American Lecture Series in Clinical Microbiology* is dedicated to biologists—be they physicians, scientists or teachers—in the hope that this series will foster better appreciation of mutual problems and help close the gap between theoretical and applied microbiology.

While it is clearly within the scope of this Series to include a monograph devoted solely to one disease and its etiologic agent, one might legitimately ask, "Is there a need for a treatise on botulism and, if so, who should write it?"

Breaking this question into two parts, the reply to the first part is a definite "Yes." *Clostridium botulinum* is a very ubiquitous and complex organism and its distribution and habitat in nature are variable, ranging from soil, sea and fresh water sediment, and possibly as obligate parasites or at least normal flora of some land and sea animals and birds.

The United States Public Health Service, through its Center for Disease Control (CDC) in Atlanta, maintains a continual surveillance program for botulism outbreaks. The CDC reports that the number of confirmed outbreaks in the United States increased from four in 1972 to ten in 1973, and to twenty-one in 1974. In 1975, fourteen confirmed outbreaks were reported. In each of the above years, there were two or more deaths attributable to botulinal toxin. While the case fatality rate has shown a gradual decline (from 23% from 1970-73 to 11% in 1975), and the total number of persons involved each year is not great, botulism is nonetheless a most serious disease which can be effectively treated if properly and promptly diagnosed. Control measures can also be initiated to protect the public health. Recent information has demonstrated that botulism is not entirely a foodborne disease; wound infections have been found to be a portal of entry of *Clostridium botulinum* into the host, where, after suitable growth conditions are established, the toxin is elaborated and the typical signs and symptoms of botulism prevail. With this background, it should be apparent that improved diagnosis, control, and therapy of botulism will come from a more complete understanding of the

organism and its interrelationships with man and their mutual environment.

The second part of the question is answered with brevity and simplicity. There is only one person who can and now has written a monograph on botulism. Professor Louis DS. Smith has devoted his professional career to the study of anaerobic bacteria, with emphasis on the clostridia. He is a recognzed and respected international authority and is regarded by many as being without peer in this area. This book will be a welcome addition to the libraries of physicians, microbiologists, epidemiologists, public health officials, and the food industry.

ALBERT BALOWS, Ph.D.
Editor

PREFACE

In this book, I have endeavored to bring together some of the information on *Clostridium botulinum*, its toxins, its spores, and the disease that it produces in animals and in man. The groups of organisms that are lumped together under this species name are uniform neither in their cultural nor serological charcteristics, nor have they identical terrestrial, marine, or animal habitats. The susceptibility of different species of animals, apparently including man, to the various types of botulinum toxin also differs greatly, no matter the route of administration of the toxin. Within the last decade, however, there has been a considerable advance in knowledge of the organisms themselves, their geographical distribution and the toxins that they produce, and it seemed that the time was opportune for collecting information concerning them.

The division of *C. botulinum* into four cultural groups that do not entirely coincide with the toxin types suggested some years ago seems to be holding up well in other characteristics such as heat resistance of spores, ability to grow at low temperatures, susceptibility to bacteriophage, and serological characteristics. This division, consequently, has been emphasized here.

Information on the occurrence of *C. botulinum* in nature and on the incidence of botulism in domestic animals and in man is given in some detail. The information on human botulism in the United States has largely been taken from the Handbook, *Botulism in The United States, 1899-1973*, of the Center for Disease Control, a publication that should be in the hands of everyone concerned with this disease in man.

An effort has been made to provide an introduction to the literature concerning *C. botulinum* and botulism. A definitive literature survey seemed out of the question, for it would be unduly long. Indeed, when one searches the literature on this subject, one is inclined to accept the quip that botulism is the only

disease on which there are more papers than cases. This is not true, of course, for there are about 500 human cases a year in Europe and the United States and hundreds of thousands of cases annually in animals and birds. The references which follow each chapter represent only a small part of the literature and have been limited to those papers with extensive bibliographies or those containing information that the reader might want in more detail than can be given here. This restriction necessitated the omission of many worthwhile papers, and I can only echo a plea voiced by Hilda Heller in 1922, "The anaerobist working on bibliography is surely a creature to be pitied and is not to be blamed for failing to follow the work of others conscientiously and thoroughly." Those interested in a more extensive bibliography will find useful that by L. S. McClung published in *Botulism*, the proceedings of a United States Public Health Service symposium, which gives selected references to 1964. A complete listing is in *The Anaerobic Bacteria, A Subject Bibliography* by E. McCoy and L. S. McClung, of which an up-to-date supplement is in preparation.

I take pleasure in thanking the many who helped me; to all who sent me reprints, sometimes their last copy; to the several librarians who obtained copies of specific papers for me, ranging from those of somewhat obscure modern foreign literature to the edicts of an emperor of Byzantium; to Mary Harvey and Donya Broyles who converted into clean manuscript much interlining and overscribbling; and especially to Albert Balows for his encouragement and support.

LOUIS DS. SMITH

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BOTULISM

The Organism, Its Toxins, The Disease

CHAPTER 1

INTRODUCTION AND HISTORY

BOTULISM IS A CURIOUS DISEASE. In man, it results from eating improperly preserved food. Not just spoiled food, not food that is being decomposed by the molds and bacteria that so uniformly contaminate us and all our surroundings, but food that we have tried to preserve by some inadequate means. This is always some means that kills or inhibits the usual spoilage bacteria and thus leaves the way open for *Clostridium botulinum* to grow without competition.

So long as man has tried to preserve food in some parts of the world he has had to do it in the presence of *C. botulinum* and every once in a while he must have set up conditions that were just right for this organism. We can only guess at the foods that brought this paralytic disease to our ancestors, but we can probably guess fairly accurately. We can think of ham in a barrel of brine too weak to be entirely bacteriostatic in Western Europe; of poorly dried herring from the Baltic; of trout netted in the lakes of Scandinavia, only lightly salted, and tightly packed to ferment in willow baskets; of the sturgeon roe piled in a heap on an old horsehide and not yet cured because the salt man had not yet come up from Crimea; of a lightly boiled and lightly smoked liver sausage swinging from the rafters of a hut in Austria. We can think of strips of whale flipper tenderizing and slowly rotting in a barrel of seal oil on the Labrador coast; of the damp pemmican packed in the spare moccasins of a Nez Perce warrior on a long raid to the Shoshone country in a rainy spring; of the little fishes, gutted, beheaded, and betailed, soaking in water until the

chopped chrysanthemum leaves, the carrots, the lettuce, and the malted rice are added, all being capped by a mat of woven rice straw weighed down with well washed stones. Indeed, if your ancestors came from the northern half of the northern hemisphere, some of them probably died of botulism.

Whatever food was responsible, in whatever age, we can be sure that the disease was the same. Nausea and vomiting often but not always, double vision, inability to focus, thickness of speech and inability to swallow, and generalized weakness would follow one or two days after eating the peccant food. The increasing paralysis would be due to the action of the botulinum toxin in preventing the passage of stimuli from the motor nerves to the muscles. As the disease progressed, more and more muscles would fail to respond to their specific stimuli until the muscles needed for breathing—the diaphragm and the muscles between the ribs—or the cardiac muscle of the heart would falter and fail in their essential contractions.

There is little in history before the 19th century concerning botulism. The suggestion that it was the sudden disease that killed the leaders and captains of Sennacherib's army in 701 B.C. and thus saved Jerusalem (2 Chronicles 32:21) has little, if anything to support it. Some of the ancient dietary laws and taboos may have had their origins in the recognition of some circumstance connected with other food poisoning outbreaks, but any clear-cut relationship between such taboos and botulism is difficult to find. One that has been suggested, however, is the 10th century edict of Emperor Leo VI of Byzantium (886-911) in which he forbade the manufacture of blood sausage, although Leo's interests were neither broad nor much concerned with the health of his people. True, he was known as "The Wise" and "The Philosopher" but only, as Gibbon acidly points out, because he "was less ignorant than the greater part of his contemporaries in church and state."

Whatever that situation may have been, it seems clear from his edict that Leo objected to blood sausage entirely on theological grounds and, moreover, he felt strongly about it. The penalty laid down in the edict for anyone preparing blood sausage

was to have "all his property confiscated, and after having been severely scourged, and disgracefully shaved, . . . be exiled for life" (Leo VI). Leo also made certain that civic authorities were vigilant in this matter, for the edict also provided that the chief magistrate of any city in which blood sausage was made would be fined ten pounds of gold (about $20,000 dollars at today's prices).

If Leo's edict had been obeyed down through the ages, some of the "sausage poisoning" outbreaks would not have taken place in Germany centuries later, even the well-recorded outbreak in Wildbad, Wurttemburg in 1793 that really started serious study of botulism.* Thirteen people were involved in this outbreak; seven of them recovered. Two opinions were advanced as to the cause of the six deaths—the official opinion of the chief medical officer of Wurttemburg was that this outbreak was due to belladonna poisoning; a more widely accepted view was that the illness of thirteen people and the death of six were caused by eating *Blunzen*, or *Schweinsmagen*, a type of blood sausage which was locally popular. *Blunzen* were made by washing out pigs' stomachs, filling them with blood and other ingredients, tying up the orifices, boiling them briefly, and preserving them by exposure to wood smoke. So prepared, they would be stored at room temperature for weeks.

The number of reported cases of sausage poisoning, *Wurstvergiftung*, increased rapidly during the next few decades and prompted a study of this disease by Justinius Kerner (1786-1862). A study of the sausage poisoning outbreaks convinced him that this was a special kind of poisoning, quite unlike any other. He had studied medicine and literature at the University of Tubingen and, after a few years of private practice, was appointed district medical officer in Wurttemburg, a position he held for the rest of his life. Kerner was far better known as a poet than as a physician; he was one of the more inspired poets of the Swabian school. His house was the Mecca of literary pilgrims, from minor versifiers to Gustav IV of Sweden, who arrived on foot with a knapsack on his back. In spite of the time that Kerner gave to literature, he remained active as a medical officer. He

*For an excellent review of the early history of botulism, see Dickson, 1918.

collected data on 230 cases of botulism and published several monographs on this disease (Kerner 1820, 1822). Kerner's poetry has largely disappeared from all but literary history, with the possible exception of his drinking song *Wohlauf, noch getrunken,* but his thorough and thoughtful examination of sausage poisoning brought to the attention of the German medical profession this uncommon, but unfortunately not rare disease.

Kerner was in a good position to scan the field of sausage poisoning. Germany, particularly southern Germany, was the home of certain types of sausage with which this disease was often associated. Besides *Blunzen,* some of these were *Schlackwurst,* made from pork and veal scraps and calf blood; *Leberwurst,* liver sausage; *Blutwurst,* blood sausage; *Presskopf,* made from the ground liver, tongues, and meat from the heads of pigs. In northern Germany, where sausage poisoning was rare, sausages were made almost entirely from the muscular portions which, when properly prepared, were much less apt to become vehicles of botulinum toxin.

Kerner not only accumulated all the information that he could on outbreaks of sausage poisoning, but he also experimented widely and became so well known for his work on botulism that it is sometimes known as "Kerner's disease." He noted that sausage did not become toxic if air pockets were left in the casings although they might spoil otherwise, that large sausages packed in casing made from stomachs or large intestines were more apt to become poisonous than sausages in small casings, probably the first hints that some anaerobic form of life was basically responsible. Moreover, as others pointed out, the only sausages that became poisonous were those that had been boiled in the process of manufacture, or in the case of liver sausage, those which had been smoked. Today, it is obvious that these last findings pointed to some heat-resistant microorganism.

Outbreaks of botulism that were associated with eating preserved fish occurred in Scandinavia and especially in Russia. Smoked herring, smoked salmon, lightly salted trout, sturgeon, and even crabs were involved and the similarity of the symptoms of *Fischvergiftung,* fish poisoning, to those of sausage poisoning

was recognized. The disease became known as *botulism,* from the Latin for sausage, *botulus,* although some medical workers whose background in the classics was stronger in Greek than Latin called the disease *allantiasis,* from the Greek word, *allantox,* meaning sausage.

The nature and the origin of the poisonous substance in the sausages and the fish were the subject of much work and even more conjecture for many years. Among the substances suggested as being responsible for the toxicity were prussic acid, pyroligneous acid from the wood smoke, choline, copper, lead, creosote, various fatty acids, vegetable alkaloids, various molds, *Trichina spirallis,* neuridine, and most fantastic of all, *aqua toffana. Aqua toffana* was supposed by the Romans to be a toxic substance secreted in the saliva of slaves tortured to death in the arena, and at least one German investigator suggested that since the method of killing hogs in Wurttemburg was slow, a substance similar to *aqua toffana* had been produced in the saliva of the hogs and that the flesh had become contaminated with it.

Many years after Kerner's work, the investigation in 1896 of an outbreak in the small Belgian village of Ellezelles, province of Hainaut, furnished the basic information on which much of our modern knowledge of botulism depends. Here, a music club performed at the funeral of one of its members. After the ceremonies, the amateur musicians gathered at the home of one of their number where they had refreshments, including a portion of raw, salted ham. During the next two days, twenty-three of the thirty-four members became ill with increasing neuromuscular paralysis; three of them died and ten very nearly died. This tragedy came almost at once to the attention of Emile Pierre Marie van Ermengem (1851-1922), professor of bacteriology at the medical school of the University of Ghent. Van Ermengem was born and spent his early years in Louvain, where he studied medicine. After he received his medical degree, he studied in London, Edinburgh, Paris, Vienna, and in Berlin with Koch, then the world's greatest name in bacteriology, with the possible exception of Pasteur. Van Ermengem was not only an astute and intelligent man, but he was also probably better trained to in-

vestigate an outbreak such as this than anyone else in the world.

He found that everyone who had come down with botulism had eaten ham. Those who had eaten smaller amounts had survived and had milder symptoms. The ham, which had come from a healthy pig, had been cured in brine for about four months, but in accordance with local custom it had not been smoked, nor had it been cooked before it was eaten. The odor had been noticed to be slightly off, somewhat like that of mildly rancid butter, but not unpleasant and definitely not putrid. Another ham was in the same barrel, but it had not been covered with brine and had become too spoiled to eat. The remains of both hams were available and when Van Ermengem examined them in the laboratory, he found that extracts of the ham that had been served to the patients were toxic to laboratory animals, producing a paralytic disease that much resembled botulism in man. From this ham and from the spleen of one of the men who had died, he isolated a spore-forming, obligately anaerobic bacterium. He used gelatin to solidify his medium—agar was not then used—and incubated the culture containing 2 percent glucose for eight days under flowing hydrogen. Because he considered that the Ellezelles patients were suffering from the same disease as sausage poisoning, he suggested that the bacterium be called *Bacillus botulinus*.

Van Ermengem found this organism to grow readily in the laboratory, provided that the temperature was between 25 and 30°C; that the salt concentration was not above five percent; that the medium in which it was grown contained an appreciable concentration of protein or protein breakdown products; and that it was cultivated in the absence of air. The toxin that his strain of *Bacillus botulinus* produced was not heat resistant, being inactivated by five minutes boiling, by thirty minutes at 80°C, or by one hour at 70°C. It was resistant to treatment with alcohol, proteolytic enzymes, mild acid, and to the action of other organisms. Mice, monkeys, guinea pigs, and rabbits were killed by small amounts of toxin when it was given by mouth or when it was injected. Cats, chickens, dogs, pigeons, and rats were resistant to toxin given by mouth, although cats and pigeons were quite