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The Ecology and Natural History of *Clostridium botulinum*

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Ecology is the interaction of organisms with their environments and with other organisms. The most well-known interaction of *C. botulinum* is that it produces a toxin that is lethal for man and other animals. I've been asked today to speculate with you about the possibility of human outbreaks of botulism from types D and C, two types that may not have been involved in human botulism.

C. botulinum types A, B, C, D, E, and F, have one thing in common — the type of action of their neurotoxins. In almost all other respects, the types of *C. botulinum* fall into three distinct groups: group 1) type A and proteolytic strains of types B and F; group 2) type E and non-proteolytic strains of types B and F; and group 3) types C and D.[4] Types known to cause botulism in humans — types A, B, more recently E and also F — have received the most extensive study. Much less is known about types C and D, which are usually associated with outbreaks in animals and birds.

There is no really well-substantiated case of human botulism caused by type C or D. *C. botulinum* type C has been isolated from suspect food in 2 investigations of human botulism and type D from suspect food in one investigation. The foods either were not tested for toxicity or were not toxic. Demonstration of vegetative cells or spores of *C. botulinum* in food is not, in itself, evidence that the food was toxic. In another report concerning type C in humans,[14] *C. botulinum* type Cα was isolated from gastric contents of a patient who showed symptoms of botulism. However, type C antitoxin, as well as antitoxin to types A, B, D, and E, failed to neutralize the heat-labile toxic substance in the gastric contents or in enrichment cultures of the gastric contents. Therefore, since some heat-labile toxin other than that of *C. botulinum* types A, B, C, D, or E was present, this case cannot be attributed to *C. botulinum* type C or to any of the other types tested.

Let us now consider the factors necessary for an outbreak of botulism and some of the information we have on these specific points, particularly about types C and D:

FACTORS NECESSARY FOR AN OUTBREAK OF BOTULISM

1. Presence of viable organisms
2. Environment that will support growth *and* toxin production
3. Ingestion of toxin
4. Absorption of toxin
5. Susceptibility of host

Presence of viable organisms: C. botulinum has been isolated from all parts of the world (Europe, Asia, Africa, North and South America, and Australia); from sea sediment collected at depths of 1070 fathoms, from heights of 11,000 feet, and at altitudes in between. Isolations of types C and D from almost every continent have been reported. Therefore, viable organisms of *C. botulinum* (usually spores) may be on almost any food that is exposed to soil, dust in the air, or any kind of personal handling. For this reason, the canning industry subjects most canned goods, after sealing, to a temperature that will kill the spores, if present. *Environment that will support growth and toxin production:* When heating is not practical for preservation of some

foods because the heated product is unacceptable to the consumer, steps are taken to control growth and toxin production by drying the product, lowering the temperature, increasing the acidity, adding sugar, or adding other chemicals.[11] These control measures, however, are interdependent to some extent and the limits of absolute reliability are difficult to determine. Moreover, most of the work relating chemically-controlled environments to growth and toxin production of *C. botulinum* has been done with types A, B, and E. Results from these studies cannot be assumed to apply to types C and D, except in the most general sense, because C and D are quite different from the other types in their biochemical and metabolic properties. The limited information we have indicates that, for type C cultures, the optimum pH for germination of spores is 6.2 - 7.3 and maximum toxicity occurs at a controlled pH of 5.7.[13] Effects of NaCl, sugars, and other chemicals on germination and growth is largely unknown, except from indirect evidence; for instance, outbreaks of western duck disease do not occur in waters of high salinity (7% or greater), but may occur in marshlands adjacent to such areas, where the salinity is lower — also where the concentration of organic matter is higher. In investigating naturally occurring outbreaks, however, one must always remember that the macroenvironment may be quite different from the microenvironment in which the organism grows and produces toxin.

The *C. botulinum* toxin, a protein, is produced inside the cell and "escapes" (diffuses) through the cell wall of older cells or is released upon autolysis of the cell. Thus it does not appear outside the cell until several hours after the end of the log phase of growth. In some groups (particularly the non-proteolytic strains of E, B, and F) a non-toxic precursor (prototoxin or protoxin) is produced first; the toxin is produced from this non-toxic precursor by the action of proteolytic enzymes. Trypsin and chymotrypsin have been used in the laboratory to convert the prototoxin to active toxin. In proteolytic strains (A, B, F), specialized proteolytic enzymes produced by the organism activate the prototoxin. Activation of prototoxin apparently does not play a role in the toxicity of types C and D, although there are conflicting reports on this point. Iida[5] reports no activation of culture fluids of C or D by trypsin; Savin[18] reports a very slight (2-4 fold) increase in toxicity of type C after treatment with various proteases (including trypsin).

About 35 years ago, South African workers showed that type C strains could be divided into two groups, Cα and Cβ, on the basis of differences in the serological characteristics of their toxins, and that there was some cross reaction between the toxins and antitoxins of types C and D. This work was confirmed by Bulatova *et al.*,[1] who reported the distribution of the antigenic fractions in these types as shown in Table 1.

Ingestion of toxin: Prevention of ingestion of toxic food has been used in one type of botulism in animals — lamsiekte in cattle in South Africa. The disease occurs in areas where the range is deficient in phosphates. Phosphorus deficient cattle eat carrion and gnaw bones. These may contain preformed toxin from growth of *C. botulinum* type D in the carcasses. The incidence of the disease can be reduced by feeding phosphate. Vaccination, however, is necessary in most areas where type D is a problem.

TABLE 1. *Relative amounts of Cα, Cβ, and D antigenic fractions in toxins of Cα, Cβ, and D. (from Bulatova* et al., *1967).*

Fraction	Cα toxin	Cβ toxin	D toxin
1 (Cα)	100	20	tr
2 (Cβ)	40	100	10-20
3 (D)	tr	10	100

In man, botulism is usually controlled by preventing the growth of the organisms and toxin production in food or by denaturing the toxin. Because the toxin of *C. botulinum* is a protein, it is denatured (detoxified) by heating; any food that is heated to an equivalent of boiling for 10 minutes would not be toxic. Consequently, the biggest problem occurs with food that is not well heated before eating. In addition to heating food before eating it, we have depended on man's general aversion to putrified food. Botulism in man has often been associated with foods that most of us, because of our culture, sense of smell, etc., would consider undesirable — foods that are slightly tainted or spoiled. The growth of strains, including C and D, that are not highly proteolytic would be less apt to produce an objectionable taste or odor in the food, and thus a product containing toxin might be acceptable for consumption.

Absorption of toxin and susceptibility of host: After ingestion, the toxin is exposed to the gastric and intestinal proteolytic enzymes. These enzymes activate toxin precursor. They probably are not very important (unfortunately) in denaturing the toxin.

The site of action of the toxin of *C. botulinum* is the motor end plates of peripheral nerves, where the toxin prevents transmission of impulses from nerve to muscle. Toxin is absorbed primarily from the small intestine, appears in the lymph draining the small intestine, passes to the blood, and is carried to the receptor sites.[12,3,15] Much of the ingested toxin is not absorbed, which has led to the theory that only small toxic units (10,000 - 20,000 molecular weight) are absorbed. Some preliminary data[15] on the molecular weight of the toxin in the blood indicates that this is not the case, but that the size of the toxic molecule in the blood has a molecular weight greater than 100,000.

The work of Jensen and Gritman[6] involving laboratory studies of botulism in mallards indicates that there is some interaction between types C and E administered *per os* to mallards. When the two were administered together, toxin appeared in the blood stream more rapidly than when either toxin was administered alone, which would indicate that the mixture of toxins had some effect on the rate of absorption. We do not know if type E toxin also had some effect on the *amount* of toxin absorbed, although I suspect it did because the MLD (minimum lethal dose) of type C for mallards was about 7 times less when mixed with type E than when administered alone. Two facts indicate that this is not merely an additive effect: 1) type E is not toxic for mallards and 2) the MLD of toxin in the lethal C+E mixture was less than the lethal amount (measured in mouse MLD) of type C toxin alone. A third point of interest in this work is that type E toxin remained in the blood stream of the mallards 12-24 times as long when C toxin was also present than when E was administered alone. E toxin disappeared in 2-4 hr when only type E was fed; C was present up to 57 hr when only type C was fed; both were present after 48 hr (longest time tested) when both were fed. We do not know the fate of type E toxin in mallards; whatever it is, this process (elimination, destruction) apparently is inhibited by the presence of type C toxin. Above all, this work indicates the possible complexity of the situation we are trying to analyze. What effect would other substances have on absorption, elimination or degradation, or substrate attachment?[7]

Many of the carrion-eating species are resistant to botulinum toxin. The unusual resistance of vultures was noted by Kalmbach in 1939.[7] The reasons for their resistance are now under investigation by Pates and colleagues. They have found[16,17] a substance (in all probability antibody) in vulture serum that neutralizes type C toxin. No natural antibody to type A toxin was detected, but the intracardial lethal dose of type A for vultures was high (1-2 million mouse LD_{50}), and vultures exposed to sub-lethal doses of type A toxin developed antibodies to the toxin.[16] It should not be assumed, however, that natural antibody is important in the resistance, or varying degree of susceptibility, of other animal species to botulinum toxin. In most cases where normal serum has been examined, no antibody has been found.

There has been little work on the susceptibility of different species to different kinds of botulinum toxins. The one most pertinent to our consideration today is that of Gunnison and Meyer, reported in 1930.[2] The relative susceptibility of different species to toxins B, C, and D is given in Table 2. The MLD in monkeys was not determined because of the expense of toxin titrations in monkeys. However, from these results we can see that, of the toxins tested, the rhesus monkey is most resistant to D and Cα administered *per os* (higher toxin levels were not tested), the mouse most resistant to Cβ (subcutaneous and *per os*), and the rabbit most resistant to Cα and D (subcutaneously) and to D *(per os)*.

The results with the toxin administered subcutaneously are the better index of the susceptibility of the animal to toxin in the blood. The results of the toxin administered *per os* indicate the probability that the animal could be affected by the consumption of toxic food. Comparison of the subcutaneous and *per os* lethal doses indicates the proportion of toxin absorbed.

Size of the animal may affect the *per os* MLD, the larger animals requiring less toxin administered *per os* than the smaller animal, presumably because the intestinal tract is longer in the larger animal and there is more time and area for toxin absorption. The MLD of toxin, administered *per os*, per gram of body weight is shown in Table 3. On a body weight basis, the relative amount of toxin for an MLD *per os* in the different species is:

type B: guinea pigs (1)
< rabbits (2)
< monkeys (7)
< mice (20)

type Cα: guinea pigs (1)
= rabbits (1)
< mice (2)
< (monkeys (19+)

type Cβ: rabbits (1)
< guinea pigs (1.75)
< monkeys (7.4)
< mice (21)

type C: guinea pigs (1)
< rabbits (1.5)
< mice (2)
< monkeys (28+)

TABLE 2. *Relative amount of each toxin type per lethal dose for different animal species (adapted from Gunnison and Meyer, 1930).*

	guinea pig[1]		mouse[2]		rabbit[3]		monkey[4]	
	S-Q*	P-O*	S-Q	P-O	S-Q	P-O	S-Q	P-O
B	1	2	0.2	2	20	100		≤ 100
Cα	1	200	0.1	20	5	2,000	≤ 500	> 30,000
Cβ	1	350	0.35	210	2.5	2,000		≤ 9,450
D	1	1,000	0.5	100	5	15,000	≤10,000	>200,000

1. 300 - 400 gm
2. 17 - 20 gm
3. 2000 - 4000 gm
4. 1850 - 3772 gm

* S-Q = subcutaneous; P-O = *per os*

TABLE 3. *Number of subcutaneous lethal doses/oral lethal dose in each animal species (compiled from Gunnison and Meyer, 1930)*

	guinea pigs	mice	rabbits
B	2	10	5
Cα	200	20	400
Cβ	350	600	800
D	1000	200	3000

Lamanna and Hart[7] reported that the amount of botulinum toxin required (probably administered intraperitoneally) for a fatal dose is the same in large and small mice. This is reasonable and to be expected if there are the same number of receptor sites in each individual of the species. This is a simple concept, but the situation was complicated by similar experiments in the rat. In the rat there *did* appear to be some correlation between body size and the intraperitoneal lethal dose, the LD_{50} was larger for the larger rats. As Lamanna and Hart indicate, this could happen if elimination of the toxin or detoxification of the toxin was more efficient in the larger rat than in the smaller rat, if the number of receptor sites per nerve cell increases with the mass of the nerve cell, if the amount of acetylcholine released at the point where the toxin acts to prevent release is greater in the larger rat, or if the receptivity for toxin at the nerve endings decreases with age. There is no information now that would enable one to select one of these as being the most probable. The results further emphasize that we must not assume that all species react similarly to one type of botulinum toxin, much less to different types of toxin.

Now, I was asked to discuss the probability that we will be faced with outbreaks of human botulism caused by types C or D. Thirty-six years ago Kalmbach and Gunderson[8] expressed my present feelings very well: "This study has revealed no instance of human beings contracting botulism through eating the flesh of bird victims of duck sickness. In considering the susceptibility of man to type C botulism this fact is indeed significant. Although one would have to presuppose an adequate toxin-producing period in the flesh eaten and the absence of heat great enough to destroy toxin or the organism itself, many prefer their game rare and a bit "high", a preference that might easily lead to serious consequences were type C botulinum toxic to man. . . . Further circumstantial evidence of the relative if not absolute immunity of man to oral doses of the toxin of type C, and possibly the closely associated type D, comes from South Africa, where in areas known to be infected with lamsiekte, the natives often may be found feasting on meat that is none too fresh."

We will not know with certainty whether man is susceptible to types C or D until we have a well-confirmed outbreak or until we have definite information about the absorption of toxin from the human intestinal tract, elimination and detoxification of circulating toxin by man, sensitivity (or presence) of receptor sites in man — information that is difficult to obtain in a "non-experimental" animal. If we cannot generalize from mice to rats, can we expect to generalize from monkeys to man? Intuitively, I feel that we are not apt to see human botulism from types C and D because man is not susceptible to the toxin. If he is not susceptible only because the toxin is not absorbed from the intestinal tract, then we could suspect that other factors might alter the permeability of the wall so that the toxin could pass through. If he is not susceptible because the receptor sites are not compatible with the molecular configuration of the toxin, then we can expect no problem from C and D. His lack of susceptibility, if true, may be from other factors, or combinations of factors and circumstances. At this stage, we do not even know whether we are trying to simplify what God has made complicated or complicate what God has made simple.

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