

## *Clostridium botulinum*

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### History

- About 900's (roughly): It was realized that certain foods caused typical poisoning. Emperor Leo VI of Byzantium forbade the manufacture of blood sausages.
- 1793: An outbreak caused by blood sausages was described in Wildbad, Germany
- 1820: Justinus Kerner collected data on 230 cases of typical poisoning in Germany and the disease became known as "Kerner's Disease."
- 1897: van Ermengem in Belgium isolated an anaerobic bacterium from cured raw ham that had caused "Kerner's Disease" in 23 people and killed 3. Extract from ham and a culture of the organism killed a number of different experimental animals with the same signs as the disease in humans. Dogs and cats proved to be fairly resistant.

van Ermengem called the organism *Bacillus botulinus* after botulus (Latin word for sausage). It was later named *Clostridium botulinum*, and the one van Ermengem isolated was later designated type B. The name of the disease was changed from "Kerner's Disease" to Botulism.

- 1904: Landman in Germany investigated botulism caused by canned, white beans. The signs and symptoms were typical for botulism but the antitoxin Landman produced did not crossreact with van Ermengem's strain. Landman had discovered *C. botulinum* type A.
- 1918–1922: 297 cases, 185 deaths in USA, mainly in California
- 1922: Bengston in the US and Seddon in Australia isolated *C. botulinum* type C that caused paralysis in chickens and cattle, respectively.
- 1929: Gunnison and Meyer in the USA designated Bengston's strain C alpha and Seddon's C beta; the first produced toxins C<sub>1</sub> and C<sub>2</sub> the second only C<sub>2</sub>.
- 1929: Robinson in South Africa isolated *C. botulinum* type D from cattle that died from paralysis (lamziekte).
- 1936: Gunnison characterized strains isolated from smoked fish that caused botulism in USA and Russia (formerly Union of Soviet Socialist Republics; USSR) as *C. botulinum* type E.
- 1951: Wound botulism was described for the first time.
- 1960: Moller and Scheibel in Denmark isolated *C. botulinum* type F from liver paste that caused human botulism.

- 1970: Gimenez and Ciccarelli in Argentina isolated *C. botulinum* type G from soil; no reported cases of poisoning with this type in man or animals.
- 1976: Infant botulism was recognized.
- 1985: Hall et al. found that a strain of *C. barati* produced type F botulinal toxin.
- 1986: Aureli et al. and McCroskey et al. isolated strains of *C. butyricum* that produced type E botulinal toxin.
- 1973-1996: CDC documented 724 cases of verified foodborne botulism in American adults; mainly associated with home-canned vegetables.

### Categories of human botulism

There are **four** categories of human botulism and these are:

#### 1. **Foodborne botulism**

This type of food poisoning is caused by the ingestion of foods containing the potent neurotoxin. The neurotoxin is formed in the food during growth of the organism (i.e., *C. botulinum*).

#### 2. **Infant botulism**

This type of poisoning affects infants under the age of 12 months. It was first recognized in 1976, and it is caused by the ingestion of *C. botulinum* spores which colonize the intestinal tracts of infants, germinate, multiply, and produce neurotoxin that travels through the bloodstream to the central nervous system and causes flaccid paralysis.

Infant botulism has been reported in 41 states nationwide. The incidence is 1 case per 100,000 of live births, and the case fatality rate is below 4%.

In California the incidence from 1985 to 1995 was 7.1 cases per 100,000 of live births. The estimated medical cost per case was \$85,000 for a total cost of \$31 million.

Honey is one vehicle that has been associated with infant botulism by a number of laboratory and epidemiological studies. Honey is now thought to account for no more than 5% of cases. Most California cases may come from spores on wind-blown dust.

#### 3. **Wound botulism**

This illness results from the pathogen itself infecting a wound, and foods are not the vehicle of transmission. The microorganism produces the neurotoxin which is transmitted to other parts of the body via the blood. This form of *C. botulinum* illness is rare.

#### 4. **Unclassified**

This type of botulism resembles infant botulism, however, it affects adults. *C. botulinum* colonizes the intestinal tract of adults and produces the toxin in vivo. This has been thought to occur after antibiotic treatment depleted the indigenous intestinal flora. It appears the few reported cases have not been well validated.

### *C. botulinum* in the US

Botulism is not a very common disease, but it is much feared.

#### **Recorded botulism cases in the US, 1973–1996 (Shapiro et al., 1998)**

<b>Botulism Type</b>	<b>Range/year</b>	<b>Total (all years)</b>
<b>Foodborne</b>	8–86	724
<b>Infant</b>	0–99	1444
<b>Wound</b>	0–25	103
<b>Unclassified or unknown</b>	Not available	39

#### **Classification of *C. botulinum***

There are seven types of *C. botulinum* (A, B, C, D, E, F, and G). Type C has two antigenic subtypes — C<sub>1</sub> and C<sub>2</sub>.

The classification of the seven types is based on the serological specificity of the neurotoxin produced.

Types A, B, E, and, very rarely, F are associated with human botulism (foodborne, wound and infant types).

Types C and D affect animals.

Type G has not been linked to illness up to this date.

In addition to the above classification, *C. botulinum* strains are divided into four groups (i.e., I, II, III, and IV) based on physiological differences (e.g., growth temperature, pH, water activity, and sodium chloride concentration), and the ability to metabolize certain substrates.

Group I: strains are proteolytic and produce neurotoxins type A, B, and F.

Group II: strains are nonproteolytic and produce neurotoxins type B, E, and F.

Group III: strains are variably nonproteolytic or proteolytic, and produce neurotoxins type C and D.

Group IV: strains are proteolytic and produce neurotoxin type G.

Strains from groups I and II are the most commonly involved in human illness.

**Distribution of serotypes in human botulism in the US**

Types	Cases (%)	Deaths (%)
A	38	52
B	38	12
E	9.7	10
F	0	0
Unknown	13	0

**Characteristics of *C. botulinum***

- All *C. botulinum* are gram positive spore-forming anaerobic rods that produce a potent neurotoxin.
- *C. botulinum* spores are usually oval in shape and subterminal. They swell the cell leading to a typical tennis-racket shape.
- pH values for growth  
Types A and proteolytic B, pH 4.6–8.5  
Minimum pH for E pH 6.2 at 5°C, pH 5.4 at 30°C
- Limiting water activity  
Type A 0.95  
Type B 0.94  
Type E 0.97
- Limiting salt concentration for growth  
10.7–12% NaCl, nonproteolytic most sensitive
- Growth temperature  
Type A and proteolytic B 10–50°C  
E and nonproteolytic B and F 3.3–45°C  
Spores are highly resistant to freezing
- Redox potential  
Optimum growth occurs at Eh of -350 mV  
Growth can occur in the Eh range of +30 to +250 mV  
0–100 mV, E is the least anaerobic
- Heat resistance  
Decimal reduction time (D value; 90% kill)  
121.1°C (250°F)/ 0.20–0.21 min for the most resistant (A and proteolytic B)  
121.1°C (250°F) / 3 min to achieve 10<sup>12</sup> fold reduction (standard for low acid canned foods)  
0.3–0.6 min causes 10<sup>6</sup> fold reduction and is standard for canned, cured meats
- Radiation resistance  
47–54 kGy causes 10<sup>12</sup> fold reduction of type A spores  
10–11 kGy causes 10<sup>12</sup> fold reduction of type B spores  
7–9 kGy causes 10<sup>12</sup> fold reduction of type E spores

12 kGy causes  $10^{12}$  fold reduction of type F spores  
 48 kGy is the accepted dose for sterilization of food spores

### **Foods implicated in botulism**

Any food that can support the growth of this pathogen or allow the germination of its spores and eventually toxin production can be associated with this illness.

For example, low acid foods (pH>4.6) can support the growth of this microorganism, and thus might pose a potential problem.

Home Canned or Preserved Low Acid Vegetables

- ✓ asparagus, tomatoes, beans, mushrooms, carrots
- ✓ peppers, corn, baked potato, chopped beets
- ✓ garlic in soybean oil

North American Indian Specialties

- ✓ fish and fish eggs
- ✓ seal flippers

Other foods that have been implicated with botulism include luncheon meats, ham, sausage, smoked and salted fish, and lobster.

If conditions are anaerobic, *C. botulinum* growth and toxigenesis are not inhibited by resident (spoilage) microflora in raw foods — in contrast to *S. aureus*.

Growth  $\approx$  toxigenesis in *C. botulinum* — in contrast to *S. aureus*.

### **Toxin production in selected foods**

Type of Food	Toxin Produced	Toxin Not Produced
Hamburger sandwich*	7 days, 25°C	
Hamburger sandwich**	4 days, 25°C	
Sausage sandwich*		7 days, 25°C
Sausage sandwich**	21 days, 25°C	
Baked potato	3-7 days, 22–30°C	

\* In the presence of oxygen

\*\*Absence of oxygen

### **Nature of the illness**

Onset of illness is about 18–36 hr after ingestion of the food containing the neurotoxin. Symptoms of intoxication vary from a mild to severe illness.

Early symptoms include nausea and vomiting which is followed by neurological signs such as blurred or double vision, difficulty in speaking or swallowing, fatigue, lack of muscle coordination and difficulties in breathing. Other symptoms include gastrointestinal problems such as cramps or abdominal pain, diarrhea, or constipation.

### Toxigenic dose

It is estimated that a few nanograms of *C. botulinum* neurotoxin can cause illness. The neurotoxin produced by *C. botulinum* probably the most toxic compounds made by a biological system. About 1 oz. (28.35 g) of this toxin can kill 200 million people. Fortunately, the incidence of the illness is low.

### *C. botulinum* toxins

*C. botulinum* produces eight toxins (A, B, C<sub>1</sub>, C<sub>2</sub>, D, E, F and G). All of the toxins are neurotoxins except C<sub>2</sub>.

Some strains produce pairs of toxins, and these strains are designated subtypes in which a capital letter identifies the type of toxin in greater amount and the following, lower case letter identifies the type of toxin produced in lesser amount.

For example, one strain that was isolated by Gimenez & Ciccarelli is subtype A<sub>F</sub>, and another strain isolated from a case of infant botulism was classified as subtype B<sub>A</sub>.

### Principal types of *C. botulinum* and toxins

Type	Toxins Produced							
	A	B	C <sub>1</sub>	C <sub>2</sub>	D	E	F	G
A	+	-	-	-	-	-	-	-
B	-	+	-	-	-	-	-	-
C	-	-	+	+	(+)	-	-	-
D	-	-	(+)	(+)	+	-	-	-
E	-	-	-	-	-	+	0	-
F	-	-	-	-	-	0	+	-
G	-	-	-	-	-	-	-	+

( ): that toxin produced in small amounts

0 : minor antigenic similarity between types E and F

### **Mechanism and characteristics of toxin**

As mentioned earlier, the botulinal neurotoxin is the most toxic compound produced by a biological system.

The LD<sub>50</sub> in mice is <0.1ng/kg.

The neurotoxin binds in a few minutes to glycoprotein or glycolipid of neurons and prevents the presynaptic release of acetylcholine, which is needed for the transmission of a nerve impulse across the synapse.

It is speculated that the neurotoxin functions as a zinc-protease that cleaves a protein associated with the vesicles responsible for the usage of acetylcholine.

The botulinal neurotoxin is fairly closely related to tetanus toxin, and the difference seems to be that they have different binding sites.

The neurotoxin, 150 kDa, is part of a large complex progenitor toxin (two additional peptides in the type A complex, fewer in others); the toxin itself is called derivative toxin. The progenitor toxin is environmentally more stable than the derivative.

The derivative toxin consists of a heavy chain (100 kDa) and a light (50 kDa) chain connected by a S-S bridge. The derivative toxin is synthesized as a single 150 kDa unit which is then proteolytically clipped to form the active toxin.

It is believed that the amino terminal of the heavy chain forms a channel in the neuronal membrane through which the light chain enters.

### **Summary of toxin mechanism**

Neurotoxin → binds to neurons → internalized → prevents release of acetyl choline

Other possible scenarios, depending on type of toxin:

Toxin → binds to many cells → internalized → ADP ribosylate → disrupts cytoskeleton, increases vascular permeability, like enterotoxins

Toxin → ADP ribosylate, other functions unknown

### **Prevention**

The minimum requirements for most commercial food preservation methods are the assurance of destruction or inhibition of *C. botulinum* (pH<4.6).

Keep foods out of the temperature danger zone (4.4–60°C or 40–140°F). Foods that require heating or cooling should undergo that process rapidly.

Botulinum toxin is destroyed by heating at 80°C for 30 min or boiling or a few minutes. If all foods were boiled immediately before consumption there would be no foodborne botulism.

### **Detection of organism and toxin**

Enrichment culture is done in cooked liver or cooked meat medium, these media are anaerobic when freshly boiled; sometimes 1–2% soluble starch is added to absorb toxic compounds.

Incubation at 25–30°C for up to one week. Plating can be done on blood agar or egg yolk agar, and anaerobic incubation of the microorganism is required.

Toxin is detected and typed by mouse inoculation. A fairly sensitive method for detection of toxin by ELISA has been developed.

For techniques see: Compendium of Methods for the Microbiological Examination of Foods.

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