



Professor Mike Peck discusses the good, bad and ugly aspects of the *Clostridium botulinum* neurotoxins

THE BOTULINUM neurotoxins potentially impact on our lives in different ways. These may be good, bad or ugly. In recent years, extremely small quantities of these most potent of toxins have been used in a beneficial way to treat a variety of neurological conditions such as torticollis, blepharospasm and strabismus. The botulinum neurotoxins cause botulism, a severe disease of man and animals. In view of its potency, botulinum neurotoxins have also attracted attention as potentially ugly weapons of mass destruction.

From a phylogenetic point of view the taxonomic structure of *C. botulinum* leaves much to be desired. Six physiologically and phylogenetically distinct clostridia can produce botulinum neurotoxin (Table 1); four are designated *C. botulinum* and two are not! The practice has been to retain the name of *C. botulinum* to emphasise the importance of neurotoxin production. However, when some strains of *C. baratii* and *C. butyricum* were recently found to produce the neurotoxin, it was decided not to call these organisms *C. botulinum*. For each of the

six organisms, a non-neurotoxic phylogenetically equivalent organism is known (Table 1). For example, proteolytic (Group I) *C. botulinum* is similar to *C. sporogenes*, except for the ability to produce neurotoxin. The different physiology of these organisms is reflected by the circumstances in which they present a hazard. For example, proteolytic (Group I) *C. botulinum* and non-proteolytic (Group II) *C. botulinum*, and very occasionally neurotoxic *C. baratii* and *C. butyricum*, have been associated with foodborne botulism (Table 1).

Seven botulinum neurotoxins (A to G) are produced, with the location of the neurotoxin gene and toxin type dependent on the producing organism (Table 1). The neurotoxins were originally distinguished on the basis of their antigenic response. More recent research has established the amino acid sequence of the different neurotoxins, and also the crystal structure of several neurotoxins. The mode of action of the neurotoxins is now well understood. The neurotoxins act primarily at peripheral cholinergic synapses blocking release of the neurotransmitter

acetylcholine. All botulinum neurotoxins comprise a heavy chain and a light chain and are often associated with other proteins (e.g. haemagglutinin and non-toxin non-haemagglutinin).

The heavy chains are responsible for delivery of the light chains to the cytosol of the motor neuron, their site of action. The light chains possess zinc endopeptidase activity, and cleave protein components of the acetylcholine-containing synaptic vesicle docking/fusion complex. Each light chain cleaves a specific protein in this complex at a specific site. This cleavage prevents binding of acetylcholine-containing synaptic vesicles, preventing neurotransmitter release and leading to flaccid paralysis of the muscle. Flaccid paralysis of the respiratory muscles can result in death if not treated.

Foodborne botulism is an intoxication resulting from consumption of pre-formed botulinum neurotoxin. The consumption of as little as 0.1g of food in which a neurotoxin-producing clostridia has grown can result in illness (as little as 30ng of neurotoxin may be sufficient). It is a rare but severe disease. While recovery may still take months or even longer, rapid treatment with equine antitoxin and supportive therapy has led to a reduction in the fatality rate (now approximately 10% of cases). This proportion is still high for a foodborne illness. On the rare occasions when commercial foods are involved in botulism outbreaks, the medical and economic consequences can be enormous. It has been estimated that in the USA the cost per case of botulism is approximately \$30 million, compared with \$10,000-12,000 for each case of illness associated with *Listeria monocytogenes* and

Salmonella.

Proteolytic *C. botulinum* and non-proteolytic *C. botulinum* are responsible for most cases of foodborne botulism. As they differ physiologically (i.e. survive and grow under different conditions), they present a hazard in different types of foods (Table 2). Proteolytic *C. botulinum* produces spores of high heat resistance and has a minimum growth temperature of 10° to 12°C. The canning

of moderate heat resistance, but this organism can multiply and form neurotoxin at temperatures as low as 3°C. Botulism outbreaks associated non-proteolytic *C. botulinum* have occurred most frequently with processed fish, with for example outbreaks involving vacuum packed smoked fish reported in Sweden (1991 and 1994) and in Germany (1997).

The name "botulism" was given to a disease reported in

the end of the nineteenth century, Emile van Ermengem first isolated a causative organism (initially called *Bacillus botulinus*) from home made raw salted ham and the spleen of a man who later died of botulism. This outbreak, in Belgium, affected 23 musicians (three fatally). The isolated strains, now lost, were probably non-proteolytic *C. botulinum*. Over the next three decades a great number of outbreaks were identified

Table 1 Characteristics of the six phylogenetically distinct clostridia that produce botulinum neurotoxin

Organism	Non-neurotoxic equivalents	Neurotoxins produced	Location of neurotoxins gene	Associated with human botulism			Associated with animal botulism
				foodborne	infant	wound	
<i>C. botulinum</i> Group 1 (proteolytic)	<i>C. sporogenes</i>	A, B, F	Chromosome	Yes	Yes	Yes	Yes
<i>C. botulinum</i> Group 2 (non-proteolytic)	no name given	B, E, F	Chromosome	Yes	—	—	Yes
<i>C. botulinum</i> Group 3	<i>C. novyi</i>	C, D	bacteriophage	—	—	—	Yes
<i>C. botulinum</i> Group 4 (<i>C. argentinense</i>)	<i>C. subterminale</i>	G	plasmid	—	—	—	—
<i>C. baratii</i>	all typical strains	F	Chromosome	Yes	Yes	—	—
<i>C. butyricum</i>	all typical strains	E	Chromosome	Yes	Yes	—	—

Table 2 Examples of recent outbreaks of foodborne botulism

Year & Country	Food associated with outbreak	No. cases (deaths)	Toxin type: organism
1989 UK	Commercially produced hazelnut yoghurt	27 (01)	Type B: proteolytic <i>C. botulinum</i>
1991 Egypt	Commercially produced unviscerated salted fish ('faseikh')	>91 (18)	Type E: Non-proteolytic <i>C. botulinum</i>
1992 Spain	Commercially produced green beans/artichokes	04 (01)	Type B: proteolytic <i>C. botulinum</i>
1993 Italy	Commercially produced aubergine in oil	07 (00)	Type B: proteolytic <i>C. botulinum</i>
1994 USA	Restaurant potato dip ('skordalia') and aubergine dip ('meligianoslata')	30 (00)	Type A: proteolytic <i>C. botulinum</i>
1996 Italy	Commercially produced mascarpone cheese	08 (01)	Type A: proteolytic <i>C. botulinum</i>
1996 India	Crisp made of gram flour ('sevu')	34 (03)	Type E: <i>C. butyricum</i>
1997 Germany	Vacuum packed hot smoked white fish	02 (00)	Type E: Non-proteolytic <i>C. botulinum</i>
1997 Iran	Traditionally made cheese preserved in oil	27 (01)	Type A: proteolytic <i>C. botulinum</i>
1997 Argentina	Home-cured ham	06 (00)	Type E: Non-proteolytic <i>C. botulinum</i>
1998 Croatia	Ham	20 (00)	b
1998 UK	Home prepared bottled mushrooms in oil (from Italy)	02 (01)	Type B: proteolytic <i>C. botulinum</i>
1999 Morocco	Commercially produced Mortadella sausage	78 (20)	Type B: <i>C. botulinum</i> ^a
2001 USA	Fermented beaver tail and paw	03 (00)	Type E: Non-proteolytic <i>C. botulinum</i>
2001 Canada	Fermented salmon roe (two outbreaks)	04 (00)	Type E: Non-proteolytic <i>C. botulinum</i>
2001 USA	Spaghetti noodles and meat sauce	01 (00)	Type F: <i>C. baratii</i>
2001 USA	Commercially produced chilli sauce	16 (00)	Type A: proteolytic <i>C. botulinum</i>
2002 South Africa	Commercially produced tinned pilchards	02 (02)	Type A: proteolytic <i>C. botulinum</i>
2002 USA	Muktuk (from Beluga whale)	08 (00)	Type E: Non-proteolytic <i>C. botulinum</i>
2003 France	Halal sausage	04 (00)	Type B: <i>C. botulinum</i> ^a
2003 Ukraine	Home prepared canned corn	06 (00)	Type B: proteolytic <i>C. botulinum</i>
2003 South Korea	Commercially produced canned sausage	03 (00)	b
2003 Norway	Home prepared 'rakfisk'	04 (00)	Type E: Non-proteolytic <i>C. botulinum</i> ^a
2003 Germany	Home prepared dried fish	03 (00)	Type E: Non-proteolytic <i>C. botulinum</i>

^a Only toxin identified - unclear whether *C. botulinum* type B or non-proteolytic *C. botulinum* type B

^b Toxin reported as present, but type not indicated. ^c likely toxin type and organism

process for low-acid foods is designed to inactivate spores of this organism, and botulism outbreaks have occurred when the full heat treatment has not been delivered. Spores of non-proteolytic *C. botulinum* are

central Europe in the nineteenth century that was frequently associated with consumption of blood sausage; the word 'botulism' being derived from the Latin botulus meaning sausage. At

across the world. Many of these were associated with the wider use (commercial and at home) of a canning process to extend shelf life. In a seven-year period, 1918 through 1924, there were 107

botulism outbreaks in the USA involving 367 cases, of which 230 were fatal. A great number were associated with the home canning of vegetables. One particularly unfortunate outbreak occurred in Albany, Oregon, in 1924. All twelve members of the Gerber family died after consuming home-canned string beans containing type A neurotoxin. The first outbreak of botulism reported in the UK occurred at Loch Maree in August 1922, and involved consumption of wild duck-paste sandwiches containing type A neurotoxin. There were eight cases, all fatal.

Through the understanding and implementation of effective control measures, the incidence of botulism is today generally much lower than in the early part of the twentieth century. Foodborne botulism involving commercial processing is uncommon, but the consequences can be severe. Most cases are now associated with home-prepared foods, when known control measures have not been implemented. For example, in Poland from 1984-1987, there were 1301 outbreaks reported giving 1791 cases, of which 46 were fatal. In Russia from January 1998 to September 1999, there were 542 outbreaks giving 743 cases of which 62 were fatal. These high incidences were associated with an increased reliance on the home bottling/canning of foods, reflecting difficult economic conditions. The incidence in Poland has fallen considerably in recent years. Many other countries have lower, but significant rates of foodborne botulism. For example, over the past 20 years, approximately 35 cases have been reported annually in Italy, with many associated with home-prepared vegetables in oil. Approximately 25 cases have been reported annually in

France and Germany, and frequently involved home/farm prepared hams. In the USA, approximately 30 cases have been reported per year, with most cases associated with home canned vegetables or fermented marine products (e.g. fermented beaver tail and paw, muktuk (whale meat)) prepared in Alaska.



Figure 1 Infant showing "floppy head", typical symptoms of infant botulism. (Photo courtesy of Dr. Stephen Arnon, California Department of Health Services).

Approximately 10 cases have been reported annually in Spain, and involved home canned vegetables. These foods are not generally prepared at home in the UK, consequently the incidence of foodborne botulism is lower. A total of eleven outbreaks of foodborne botulism have been reported in the UK, with 58 cases, of which 19 were fatal. The largest outbreak occurred in 1989, and involved commercially produced hazelnut yoghurt. The heat treatment given to the hazelnut conserve was not sufficient to inactivate spores of proteolytic *C. botulinum*, and the conserve then supported bacterial growth and production of type B neurotoxin. This toxic mixture was then added to the natural yoghurt. The outbreak affected 27 people, of which one died. The most recent

botulism outbreak occurred in 1998, two people were affected, of which one died. The implicated food was home prepared bottled mushrooms in oil that was imported from Italy. Some examples of recent outbreaks of foodborne botulism are given in Table 2.

A recent finding has been the association of

neurotoxicogenic *C. baratii* and *C. butyricum* with foodborne botulism. A suspected outbreak involving neurotoxicogenic *C. baratii* type F, was reported in USA in January 2001, and was associated with consumption of spaghetti noodles and meat sauce by a 41 year old woman. The woman eventually recovered, although 12 weeks were spent on a life support machine. The first outbreak involving neurotoxicogenic *C. butyricum* was reported in China in 1994 and was associated with consumption of a homemade salted and fermented paste of soybeans and wax gourds (six cases, three fatal). It was subsequently established that two earlier outbreaks of type E botulism in China involving soybean dishes were also associated with neurotoxicogenic *C. butyricum*. A further

suspected outbreak was reported in India in 1996, and involved consumption of *sevu* (crisp made of gram flour) at a school cafeteria (34 cases, three fatal).

It is essential that as new technologies and approaches to food processing are introduced, measures are in place to ensure that the foodborne botulism hazard is appropriately controlled (i.e. *C. botulinum* does not become an emerging pathogen). In this respect, research has and is focused on ensuring the continued safe development of refrigerated processed foods with an extended shelflife (i.e. chilled ready meals). These foods address consumer demand in requiring minimal preparation time, are of high quality and contain few preservatives. Sales of these foods have increased tremendously over the last ten years. The foods receive a moderate heat process (typical maximum of 75° to 95°C) that is intended to minimise loss of sensory and organoleptic quality. The food is then cooled rapidly, and stored at refrigeration temperatures (<8°C). These foods are not sterile, and shelflife is dependent on a combination of the heat process, storage temperature, and perhaps also intrinsic properties of the food.

Additionally, these foods are often packed under vacuum or an anaerobic atmosphere, restricting growth of aerobic, but not anaerobic, bacteria. This minimal process favours spore-forming microorganisms that grow in the absence of oxygen at refrigeration temperatures. In particular, concern exists about the potential for growth and neurotoxin production by non-proteolytic *C. botulinum* in the absence of a competing microflora, and the associated foodborne botulism hazard.

In response to these concerns, the growth domain

has been described, and predictive models have been developed for the thermal death and growth of non-proteolytic *C. botulinum*. Several of these models are freely available through Growth Predictor (www.ifr.ac.uk/Safety/GrowthPredictor/default.html) or the Pathogen Modeling Program (www.arserrc.gov/mfs/PMP6_S tart.htm). Published (and in some cases also unpublished) original growth and death curves are compiled and also available free of charge in ComBase (www.ifr.ac.uk/combase/default.html).

A further development has been that of a process risk model for gnocchi (a minimally processed potato product). Here the techniques of quantitative risk assessment have been used to consider risk throughout the entire food chain. The product was found to be very safe with respect to the foodborne botulism hazard. This work has complemented traditional challenge testing.

Infant and wound botulism are infections. Proteolytic *C. botulinum* and neurotoxicogenic *C. baratii* and *C. butyricum* are most commonly associated with infant botulism. The first clinical cases of infant botulism were described in California in 1976, although subsequent investigations identified earlier cases. Infant botulism has now been reported in many countries, with six cases described in the UK. The most recent case was in June 2001 and involved a five month old baby. In the USA, infant botulism is the most commonly reported form of botulism, with approximately 80 – 100 cases per year. An immature intestinal flora in infants is insufficient to prevent colonisation by neurotoxicogenic clostridia, allowing ingested spores to germinate leading to cell multiplication and

neurotoxin production. Infants aged between two weeks and six months are most susceptible. The disease is characterised by extended constipation and flaccid paralysis.

A frequent observation is paralysis of the head and neck muscles, leading to difficulty in holding the head erect (Figure 1). Infant botulism is

'unsuitable for infants under 12 months'. A similar disease is very rarely reported in adults and occurs when competing bacteria in the normal intestinal flora have been suppressed (e.g. by antibiotic treatment or major surgery).

Wound botulism was first described in the USA in 1943 and was associated with




Figure 2A, Blepharospasm (focal dystonia of the orbicularis oculi; early symptoms may include, uncontrollable blinking [especially in bright light], eye irritation, photosensitivity)

rarely fatal. Equine antitoxin and antibiotics are not used to treat cases of infant botulism. Instead, treatment consists of meticulous supportive care to avoid potentially fatal complications. In the USA, human botulism immune globulin (BIG) is given to aid recovery. Two sources of spores have been identified, honey and general environmental contamination (e.g. soil, dust). It is estimated that between 10 and 100 spores are sufficient to bring about infection. Several cases of infant botulism have been linked to consumption of honey containing spores of neurotoxicogenic clostridia. In order to minimise this risk, it is recommended that jars of honey should carry an appropriate warning such as

growth and neurotoxin production by proteolytic *C. botulinum* type A in a substantial wound, following a fall from a building. Until 1982, wound botulism remained uncommon and was associated with infection following traumatic injury (e.g. cutting hand with saw, foot puncture wound). Following the description of the first case of wound botulism associated with infection following drug abuse (skin popping of heroin) in 1982, there has been an increase in the disease. Since 1995 approximately 20 – 40 cases of wound botulism have been reported annually in the USA. Prior to 2000 wound botulism had not been reported in the UK, but 18 cases were confirmed in 2000

- 2002 and all were associated with drug abuse. Strains of proteolytic *C. botulinum* type A or type B have been isolated from the wounds. Cases of wound botulism associated with drug abuse have now been reported in many countries. In the UK other clostridia (e.g. *C. novyi*, *C. tetani*, *C. histolyticum*) have also caused infection following drug abuse.

Botulism also occurs in many types of animal other than man (Table 1). Proteolytic *C. botulinum* has caused outbreaks of botulism in cattle and horses, the latter appearing particularly sensitive to neurotoxin. Outbreaks involving non-proteolytic *C. botulinum* type E have probably been associated with consumption of toxic fish. *C. botulinum* Group III is particularly associated with botulism in animals. Strains producing type C neurotoxin are associated with avian botulism and also botulism in cattle, horses and other animals, while strains producing type D neurotoxin have caused botulism in cattle and sheep. Outbreaks of botulism involving animals can be very large. For example, 5,500 beef cows died in Queensland in 1990, and 44,000 foxes died in Finland in 2002. Major outbreaks involving waterfowl can be even larger. It is estimated that 4 – 5 million waterfowl died in the western states of USA in 1952. Avian botulism is a significant cause of bird death in the UK. Avian botulism has also affected endangered species, such as the brown pelican (1996 in USA) and black-faced spoonbill (2002–3 in Taiwan).

Other aspects of botulinum neurotoxins feature in the work of Dr. Edward J. Schantz and Dr. Alan B. Scott, who pioneered the use of botulinum neurotoxin for the treatment of a variety of human diseases. 

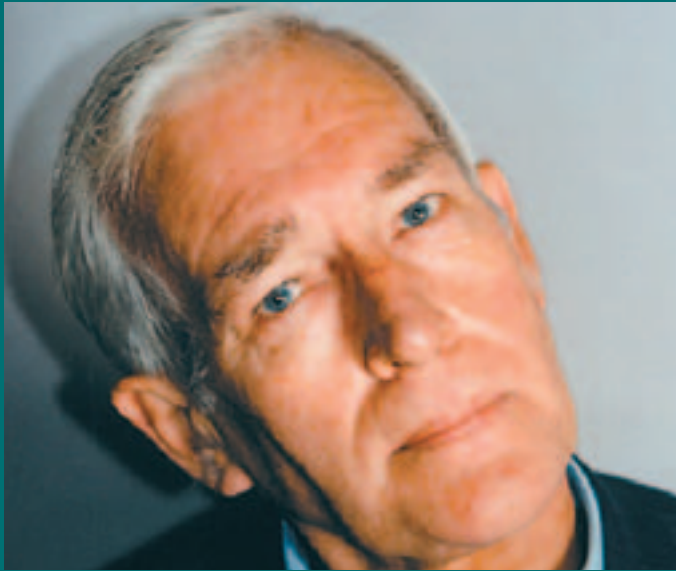


Figure 2B, Torticollis (affects the muscles of the neck, leading to inclination of the neck).

Figs 2A, 2B and 2C Photos courtesy of Ipsen

Dr. Schantz trained as a biochemist. He developed the necessary protocols, and then provided Dr. Scott with highly purified neurotoxin. Dr. Scott, an eye surgeon, explored the use of botulinum neurotoxin to block neurotransmitter release and reduce muscle activity, thereby providing an alternative to invasive surgery. Initial research (late 1960s and early 1970s) focussed on

the treatment of strabismus (hyperactive muscle activity, leading to misalignment of the eyes) in animals. Tests then moved to human volunteers, and in 1989 the US Food and Drug Administration approved the use of botulinum neurotoxin type A for the treatment of strabismus, blepharospasm (spasm of eyelid) and hemifacial spasm (spasm of face; Figs 2a - 2c).



Figure 2C, Hemifacial spasm (spasm of the face; tends to affect the left side of the face more frequently than the right, with the muscles on the side of the mouth particularly affected)

Treatment consists of the injection of a few nanograms of neurotoxin at an appropriate site.

Three preparations of botulinum neurotoxin are currently approved in various countries, Botox® and Dysport® (both preparations of type A neurotoxin), and Neurobloc® (also known as Myobloc®, a preparation of type B neurotoxin). Today a wide variety of conditions are treated with botulinum neurotoxin, including; strabismus, blepharospasm, hemifacial spasm, torticollis (inclination of the neck), excessive perspiration (excessive stimulation of the sweat glands), paediatric cerebral palsy spasticity and migraines. The botulinum neurotoxin is also much used for cosmetic purposes. A report published in October 2003 describes the successful use of botulinum neurotoxin to

treat blepharospasm in a dog.

Development and use of botulinum neurotoxin as a potential bioweapon began at least 60 years ago. Although the 1972 Biological and Toxin Weapons Convention prohibited offensive research and production of biological weapons, this did not entirely prevent further development. For example, the Soviet Union and Iraq subsequently produced botulinum neurotoxin for potential use as a bioweapon. On at least three occasions between 1990 and 1995, aerosols of botulinum neurotoxin were dispersed by the Aum Shinrikyo cult in Japan. These terror attacks apparently failed.

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Suggested reading:

- Arnon, S S; Schechter, R; Inglesby, T V; Henderson, D A *et al.* (2001). Botulinum toxin as a biological weapon. *Journal of American Medical Association* **285**, 1059-1070.
- Barker, GC; Talbot, N LC and Peck, M W (2002). Risk assessment for *Clostridium botulinum*: A network approach. *International Journal of Biodeterioration* **50**, 167-175.
- Carlin, F; Girardin, H; Peck, M W; Stringer, S C. *et al.*, (2000). Research on factors allowing a risk assessment of spore forming pathogenic bacteria in cooked chilled foods containing vegetables: a FAIR collaborative project. *International Journal of Food Microbiology* **60**, 117-135.
- Fernandez, P S; Baranyi, J and Peck, M W (2001). A predictive model of growth from spores of nonproteolytic *Clostridium botulinum* in the presence of different CO₂ concentrations as influenced by chill temperature, pH and NaCl. *Food Microbiology* **18**, 453-462.
- Johnson, E A (2000). Neurotoxic Clostridia. In 'Gram-Positive Pathogens' Eds., V A Fischetti *et al.*, pp.540-550. American Society for Microbiology, Washington DC, USA.
- Lund, B M and Peck, M W (2000). *Clostridium botulinum* in 'The Microbiological Safety and Quality of Food' Eds., B M Lund, A C Baird-Parker and G W Gould. pp.1057-1109. Aspen, Gaithersburg, USA.
- Meyer-Lindenberg, A; Wohlfarth, K M and Switzer, E N (2003). The use of botulinum toxin A for the treatment of possible essential blepharospasm in a dog. *Australian Veterinary Journal* **81**, 612-614.
- Peck, M W (1997). *Clostridium botulinum* and the safety of refrigerated processed foods of extended durability. *Trends in Food Science and Technology* **8**, 186-192.
- Schantz, E J and Johnson, E A (1997). Botulinum toxin: The story of its development for the treatment of human disease. *Perspectives in Biology and Medicine* **40**, 317-327.