J Neural Transm (2008) 115: 559–565
DOI 10.1007/s00702-007-0728-2
Printed in The Netherlands

Journal of Neural Transmission

Historical

From poison to remedy: the chequered history of botulinum toxin

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Received 12 December 2006; Accepted 17 March 2007; Published online 26 April 2007
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Summary Botulinum toxin poisoning has afflicted mankind through the mists of time. However, the first incident of food-borne botulism was documented as late as the 18th century, when the consumption of meat and blood sausages gave rise to many deaths throughout the kingdom of Württemberg in South Western Germany. The district medical officer Justinus Kerner (1786–1862), who was also a well-known German poet, published the first accurate and complete descriptions of the symptoms of food-borne botulism between 1817 and 1822 and attributed the intoxication to a biological poison. Kerner also postulated that the toxin might be used for treatment purposes. In 1895, an outbreak of botulism in the small Belgian village of Ellezelles led to the discovery of the pathogen "Clostridium botulinum" by Emile Pierre van Ermengem. Modern botulinum toxin treatment was pioneered by Alan B. Scott and Edward J. Schantz in the early 1970s, when the type-A serotype was used in medicine to correct strabismus. Other preparations of the type-A toxin were developed and manufactured in the United Kingdom, Germany, and China, whereas a therapeutic type-B toxin was prepared in the United States. To date, the toxin has been used to treat a wide variety of conditions associated with muscular hyperactivity, glandular hypersecretions and pain.

Keywords: Botulinum, poison

Introduction

Botulism is food poisoning caused by the neurotoxin-producing, rod-shaped, gram-positive bacterium Clostridium botulinum. The pathogen is found ubiquitously in soil and water. Seven serological sub-types of botulinum toxin (A, B, C_1, C_2, D–G) have been distinguished. The toxin consists of a high molecular weight protein (150,000 daltons) with three domains (L, H_N, and H_C) (Aoki, 2002). This molecular structure is a prerequisite for a four-step mechanism of blocking cholinergic neuromuscular and autonomic function entailing the inhibition of cholinergic transmission of nerve impulses at the neuromuscular and sympathetic cholinergic junction: binding to nerve cells mediated by the H_C domain (step 1) is followed by uptake into endocytic vesicles (step 2), which contain an ATPase proton pump. Acidification of the vesicular lumen alters the structure of the toxin so that H_N is incorporated into the membrane and L is translocated into the cytosol (step 3), where it catalyses the proteolysis of SNARE proteins (step 4). These proteins are involved in vesicular trafficking and fusion with target membranes within the cell. BTX-A and BTX-E cleave the protein SNAP-25, BTX-B, D, F and G cleave the vesicle-associated membrane protein VAMP/synaptobrevin, while BTX-C cleaves both SNAP-25 and syntaxin (Rosetto et al., 2002). The inhibition of acetylcholine release causes symptoms such as diplopia, bulbar weakness, dysphonia, dysarthria and dry mouth and subsequent generalised muscle weakness complicated by respiratory failure. Until the last century, botulism was thought to be caused exclusively by food that was contaminated with preformed toxin. This view has changed during the last 50 years, due to spores of C. botulinum being discovered in the intestines of babies in the 1970s (infant botulism) and in contaminated wounds (wound botulism) in the 1950s (Arnon et al., 1977; Merson and Dowell, 1973).

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Food-borne botulism in ancient times

Since primeval times, botulism has mostly occurred as a result of consuming improperly preserved food. As long as

man has tried to preserve and store food, there have always been storage conditions that have been optimal for the presence and growth of Clostridium botulinum in ham, sausages, fish, and vegetables in the various regions of the world. Some ancient dietary laws and taboos may have originated from an inkling of the life-threatening consequences of consuming poisoned food. Some lethal formulas drawn up by ancient shamans and warlocks contained the powder of blood sausages dried under anaerobic conditions, probably using botulinum toxin (Smith Louis, 1977). However, in ancient times, the connection between food consumption and subsequent death from a paralytic disease in cases of botulism was not generally recognised. Therefore, we have only few historical sources and documents on various causes of food poisoning prior to the 18th century. Some instances of poisoning with *Atropa belladonna* reported in the old medical literature were in fact probably cases of food-borne botulism, because the described combination of dilated pupils and fatal muscle paralysis without clouding of consciousness cannot be attributed to an atropine intoxication (Erbguth, 2004).

Botulism outbreaks in the 18th and 19th century

At the end of the 18th century, food poisoning manifested after consumption of meat and blood sausages caused many deaths throughout the Kingdom of Württemberg. Poverty ensuing from devastating Napoleonic Wars (1795–1813) had led to the neglect of sanitary measures in rural food production (Grüsser, 1986). Due to the combination of mydriasis and progressive muscle paralysis in the victims, some physicians suspected an atropine intoxication, others related the disease to the consumption of meat. In July 1802, the Royal Government in Stuttgart issued a public warning about the ''harmful consumption of smoked blood-sausages''. In August 1811, the medical section of the Department of Internal Affairs of the Kingdom of Württemberg again addressed the problem of ''sausage poisoning'', considering it to be caused by hydrocyanic acid, known at that time as ''prussic acid''. The members of the Medical Faculty of the University of Tübingen disputed that prussic acid could be the toxic agent in sausages, suspecting a biological poison. However, the Government started to collect the reports of general practitioners and health officers on cases of food poisoning. The analysis of these reports resulted in a public announcement of typical gastrointestinal, autonomic and neuromuscular symptoms (Erbguth and Naumann, 2000).

Justinus Kerner's systematic publications on 'sausage poisoning'

The German physician and romantic poet Justinus Kerner (1786–1862) (Fig. 1) was the intellectual instigator of modern botulinum toxin therapy. It is fascinating to see how his seminal ideas have materialised over the past 30 years (Erbguth and Naumann, 2000; Erbguth, 2004). As a medical officer, the 29-year-old Kerner reported a case of lethal food poisoning in 1817 in the ''Tübinger Blätter für Naturwissenschaften und Arzneykunde'' [''Tübinger papers for natural sciences and pharmacology''] (Kerner, 1817). Kerner again disputed that an inorganic agent such as hydrocyanic acid could be the toxic agent in the sausages, suspecting a biological poison instead. After he had observed further cases, Kerner published a first monograph in 1820 on ''sausage poisoning'' in which he summarised the case histories of 76 patients and gave a complete clinical description of what we now recognise as botulism. The monograph was entitled ''Neue Beobachtungen über die in Württemberg so häufig vorfallenden tödtlichen Vergiftungen durch den Genuß geräucherter Würste'' [''New observations on the lethal poisoning that occurs so frequently in Württemberg owing to the consumption of smoked sausages''] (Kerner, 1820). In 1822, Kerner published 155 case reports of patients with botulism including hypotheses on the ''sausage poison'' in a second monograph ''Das Fettgift oder die Fettsäure und ihre Wirkungen auf den thierischen Organismus, ein Beytrag zur Untersuchung des in verdorbenen Würsten giftig wirkenden Stoffes'' [''The fat poison or the fatty acid and its effects on the animal body system, a contribution to the examination of the substance responsible for the toxicity of bad sausages'']

Fig. 1. Justinus Kerner 1786–1862 (Oil painting by Alexander Bruckmann 1844)

(Kerner, 1822). The monograph contained an accurate description of all muscle symptoms and clinical details of the entire range of autonomic disturbances occurring in botulism, such as mydriasis, decrease of lacrimation and secretion from the salivary glands, gastrointestinal and bladder paralysis. Kerner also experimented on various animals by feeding them with extracts from bad sausages and finally carried out high-risk experiments on himself (Erbguth, 1996). Kerner deduced from the clinical symptoms and his experimental observations that the toxin acts by interrupting the motor and autonomic nervous signal transmission (Erbguth, 1998). He concluded: ''*The nerve conduction is brought by the toxin into a condition in which its influence on the chemical process of life is interrupted. The capacity of nerve conduction is interrupted by the toxin in the same way as in an electrical conductor by rust*'' (Kerner, 1820). Finally, Kerner tried in vain to produce an artificial ''sausage poison''. In summary, Kerner's hypotheses concerning ''sausage poison'' were (1) that the toxin develops in bad sausages under anaerobic conditions, (2) that the toxin acts on the motor nerves and the autonomic nervous system and (3) that the toxin is strong and lethal even in small doses (Erbguth and Naumann, 2000). In the eighth chapter of the 1822 monograph, Kerner speculated about using the toxin for therapeutic purposes. He concluded that small doses would be beneficial in conditions with pathological hyperexcitability of the nervous system. Kerner considered that various diseases in which he suspected an overexcited nervous ganglia system (e.g. motor hyperkinesias and glandular hypersecretion) would be appropriate indications for the therapeutic use of the ''toxic fatty acid'' botulinum toxin (Erbguth and Naumann, 2000). Kerner wrote: ''*The fatty acid or zoonic acid administered in such doses, that its action could be restricted to the sphere of the sympathetic nervous system only, could be of benefit in the many diseases which originate from hyperexcitation of this system*'' and ''*by analogy it can be expected that in outbreaks of sweat, perhaps also in mucous hypersecretion, the fatty acid will be of therapeutic value*.'' However, Kerner conceded self-critically that all the possible indications mentioned were only hypothetical and wrote: ''*What is said here about the fatty acid as a therapeutic drug belongs to the realm of hypothesis and may be confirmed or disproved by observations in the future*.''

Botulism research after Kerner

After his publications on food-borne botulism, Kerner was well-known to the German public and amongst his contemporaries as an expert on sausage poisoning, as well as for his poetry. He was nicknamed 'Sausage Kerner' and 'sausage poisoning' was known as 'Kerner's disease'. The famous German poet Heinrich Heine, who used to mock the German Romantic poets, characterised Justinus Kerner as the poet ''*who has visions of poisoned blood sausages . . .*'' (Erbguth, 2004). Further publications in the 19th century by various authors added nothing substantial to Kerner' early observations. The term 'botulism' (from the Latin word *botulus* meaning sausage) introduced in the second half of the 19th century refers to the poisoning due to sausages and not to the sausage-like shape of the causative bacillus discovered later (Torrens, 1998). The next and most important scientific step was the identification of Clostridium botulinum in 1895/1896 by the microbiologist Emile Pierre Marie van Ermengem.

The discovery of 'Bacillus botulinus' in Belgium

In December 1895, there was an extraordinary outbreak of botulism in the small Belgian village of Ellezelles after a funeral meal which included pickled and smoked ham. The 34 guests displayed symptoms such as mydriasis, diplopia, dysphagia, dysarthria and progressive muscle paralysis. Three of them died and 10 nearly died. The microbiologist Emile Pierre Marie van Ermengem of the University of Ghent (Fig. 2), who had worked in the laboratory of Robert Koch in Berlin in 1883, conducted the medical investigation (Devriese, 1999; Kreyden, 2002). van Ermengem was the first to correlate ''sausage poisoning'' with an anaerobic microorganism that he found in the ham eaten at the funeral meal and in the tissue of the victims. He concluded that ''*it is highly probable that the poison in the ham was produced by an anaerobic growth of specific microorganisms during the salting process*''. van Ermengem isolated the bacterium

Fig. 2. Emile Pierre van Ermengem 1851–1922

in the ham and in the corpses of the victims, grew it, used it for animal experiments, characterised its culture requirements, described its toxin, called it ''Bacillus botulinus'' and published his observations in a German microbiological journal in 1897 (an English translation was published in 1979) (van Ermengem, 1897). The pathogen was later renamed ''*Clostridium botulinum*''.

Progress in botulinum toxin research

In 1904, when an outbreak of botulism in Germany was caused by canned white beans, the opinion that the only botulinogenic foods were meat or fish had to be revised. The bacteria isolated from the beans by Landmann (1904) and from the Ellezelles ham were compared by Leuchs (1920) at the Royal Institute of Infectious Diseases in Berlin. He found that the strains differed and the toxins were serologically distinct. The two types of Bacillus botulinus did not receive their present letter designations of serological subtypes until Georgina Burke, who worked at Stanford University, designated them as types A and B (Burke, 1919). Over the next decades, increases in food canning and food-borne botulism went hand in hand (Cherington, 2004). The first documented outbreak of food-borne botulism in the USA was caused by commercially conserved pork and beans and dates from 1906 (Smith Louis, 1977; Drachman, 1971). Techniques for killing the spores during the canning process were subsequently developed. The correct pH (<4.0), the osmolarity needed to prevent clostridial growth and toxin production, and the requirements for toxin inactivation by heating were defined.

In 1922, type C was identified in the USA by Bengston and in Australia by Seddon, type D and type E were characterised some years later (type D: USA 1928 by Meyer and Gunnison; type E Ukraine 1936 by Bier) (Kriek and Odendaal, 1994; Geiges, 2002). Type-F and type-G toxins were identified in 1960 in Scandinavia by Moller and Scheibel and in 1970 in Argentina by Gimenex and Ciccarelli (Gunn, 1979; Geiges, 2002). In 1949, Burgen and his colleagues (Burgen et al., 1949) in London discovered that botulinum toxin blocked the release of acetylcholine at neuromuscular junctions. In 1992 Giampetro Schiavo and co-workers found that tetanus and botulinum toxins are metalloproteases that are specific to the SNARE proteins. During the last 15 years, additional findings concerning the molecular mechanisms accounting for the effect of botulinum toxin have been contributed by various groups of scientists (Dolly et al., 1990; Schiavo et al., 1992, 1993). In 2006, the protein receptor (SV2C) was defined for the type-A toxin (Dong et al., 2006; Mahrhold et al., 2006).

Swords to ploughshares

The potential use of botulinum toxin as a weapon was recognised during World War I (Lamb, 2001). Pure botulinum toxin type A was first isolated as a stable acid precipitate by Hermann Sommer and colleagues working at the Hooper Foundation, University of California, San Francisco in the 1920s (Schantz, 1994; Snipe and Sommer, 1928). With the outbreak of World War II, the United States government began intensive research into biological weapons, including botulinum toxin, especially in the laboratory at Camp Detrick (later named Fort Detrick) in Maryland. Development of concentration and crystallisation techniques at Fort Detrick was pioneered by Carl Lamanna and James Duff in 1946. The methodology was subsequently used by Edward J. Schantz (Fig. 3) to produce the first batch of toxin which was the basis for the later clinical product (Lamanna, 1946). After the end of World War II, Schantz worked at Fort Detrick as a civilian. In 1972, when Fort Detrick was closed, Schantz continued

Fig. 3. Edward J. Schantz (1908–2005)

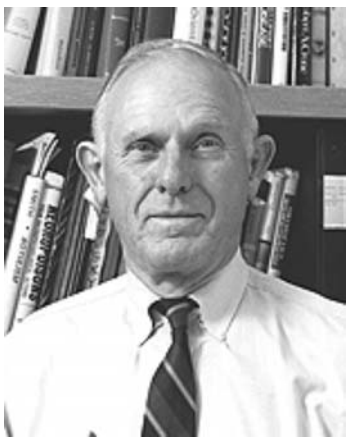

Fig. 4. Alan B. Scott

his research on the use of botulinum toxin at the University of Wisconsin (Schantz and Johnson, 1997). In 1950, Vernon Brooks, a physiologist, suggested that botulinum toxin could be used to reduce the activity of hyperactive muscles (Schantz, 1994). Around 1968, Schantz was contacted by Alan Scott (Fig. 4), an ophthalmologic surgeon at the Smith-Kettlewell Eye Research Foundation in San Francisco, who was seeking alternatives to strabismus surgery and was used to the techniques of accurately injecting ocular muscles (Scott, 2004). Since Daniel Drachmann had used small doses of Schantz's toxin to paralyse the legs of chickens, Scott regarded botulinum toxin as a potential injectable therapeutic agent for treating eye muscle hyperactivity (Drachman, 1971; Scott, 2004).

In the United Kingdom, botulinum toxin research was conducted in the Porton Down laboratories of the military section of the ''Centre for Applied Microbiology and Research'' (CAMR), which later provided British clinicians with a therapeutic formulation of the toxin (Hambleton et al., 1981).

In Germany, studies on the therapeutic efficacy of a type A toxin preparation free of non-toxic protein complexes led to an official approval in 2005.

Development of modern botulinum toxin therapy

Scott injected into the ocular muscles of monkeys various anaesthetics, alcohol, enzymes, enzyme blockers, snake neurotoxins. Eventually, motivated by Drachmann's studies, he also tried botulinum toxin. With botulinum toxin, he observed a remarkable effect. Scott recalled: *An injection of a few picograms would induce paralysis confined to the target muscles, long in duration, and with no side effects whatsoever* (Scott, 2004). The results of these animal experiments were published in 1973 (Scott et al., 1973). Human experimentation began first in healthy volunteers and strabismus patients in 1977 (Scott, 1979). Successful studies on the therapeutic value of botulinum toxin in blepharospasm and strabismus led to a first approval of the toxin batch 79-11 (prepared in November 1979) by the US Food and Drug Administration (FDA) as an orphan drug called ''Oculinum'' in 1989. The Allergan company, which had acquired the rights to distribute Oculinum, received FDA approval to change the therapeutic toxin's name from Oculinum to Botox® (Ting and Freiman, 2004).

In the UK, physicians at Moorfields Eye Hospital and the National Hospital for Nervous Diseases – particularly John Elston – repeated Dr. Scott's pioneering work, and used botulinum toxin from CAMR to treat a number of patients with periocular muscle contractions (Elston, 1985). The process of toxin production was described by Peter Hambleton and colleagues in 1981 and 1988 and a temporary approval of its use was granted (Hambleton et al., 1981; Melling et al., 1988). The toxin was then employed successfully to treat various forms of dystonia. In 1984, the new biotechnology company Porton International formed a partnership with CAMR. Association of the term ''dystonia'' to ''Porton'' led to the name Dysport®; the name of the toxin manufacturing company has been changed from Porton with ''Ipsen''. From the early 1980s, the use of type A-toxin was then extended to other forms of focal dystonia, and to other forms of contractions of smooth and skeletal muscles, as well as to conditions of glandular hypersecretion and pain.

Table 1. *Historical steps in the discovery and development of botulinum toxin*

18th century	First documented endemic outbreaks of food-borne botulism called ''sausage poisoning'' in Europe
1817–1822	Justinus Kerner and botulinum toxin: Preliminary animal experiments, systematic descriptions of its clinical effects; theoretical considerations of its possible therapeutic use
1895–1897	Emile Pierre van Ermengem: Discovery of the neurotoxin-producing pathogen Clostridium botulinum
1910	J. Leuchs: Discovery of a second serologically distinct botulinum toxin serotype (type B)
1920–1930	H. Sommer: Purification of botulinum toxin
1946	C. Lamanna and J. Duff: Techniques of toxin concentration and crystallisation
1949	A. Burgen: Description of the toxin's action on acetylcholine release at the neuromuscular junction
1970s	Description of wound and infant botulism
1941–1972	Edward Schantz: Production of a batch of toxin at Fort Detrick (USA)
1968	Contact between Alan Scott and Edward Schantz; search for therapeutic agents (e.g. botulinum toxin) to relax eye muscles
1973	Alan Scott: Publication of animal experiments with injections of botulinum toxin into eye muscles
1977–1980	Alan Scott: Treatment of strabismus patients with botulinum toxin; first publications of application in humans
1989	Approval of Alan Scott's type A toxin batch as ''Oculinum'' in the USA; later named ''Botox''
1881–1988	Development of a type A toxin preparation in the UK; later called ''Dysport''
1990s	Discovery of the molecular action of botulinum toxin (Schiavo, Montecucco, Dolly)
2000–2001	Approval of a therapeutic type B preparation in the USA and Europe (Myobloc, Neurobloc)
2005	Approval of a type A preparation in Germany (Xeomin)

The third officially approved toxin was a type B toxin formulation named Myobloc™ in the United States and Neurobloc® in Europe (Lew et al., 2000). In Germany, in 2005 another type A preparation (Xeomin® manufactured by Merz company) that is free of complex non-toxic proteins was approved for treating blepharospasm and cervical dystonia (Benecke et al., 2005; Roggenkämper et al., 2006). In China, a locally produced type A preparation is also used for clinical purposes. Other serotypes such as types C, E, and F have been under investigation for their therapeutic potential.

Use of botulinum toxin has also attracted clinical and scientific interest in many diseases, e.g. in various forms of dystonia, that had for many years been considered to be a psychosomatic (''conversion'') condition rather than an extrapyramidal movement disorder. Botulinum toxin has also been used as an experimental tool in dystonia research: by inhibiting the muscular execution of a hyperactive motor programme, sensomotor pathways of motor control could be studied using modern brain imaging or neurophysiological techniques.

References

Aoki R (2002) Physiology and pharmacology of therapeutic botulinum neurotoxins. In: Kreyden OP, Böne R, Burg G (eds) Hyperhidrosis and botulinum toxin in dermatology. Curr Probl Dermatol, vol 30. Karger, Basel, pp 107–116

Arnon SS, Midura TF, Clay SA, Wood RM, Chin J (1977) Infant botulism: epidemiological, clinical and laboratory aspects. JAMA 237: 1946–1951

Benecke R, Jost WH, Kanovsky P, Ruzicka E, Comes G, Grafe S (2005) A new botulinum toxin type A free of complexing proteins for treatment of cervical dystonia. Neurology 64: 1949–1951

Burgen A, Dickens F, Zatman L (1949) The action of botulinum toxin on the neuromuscular junction. J Physiol 109: 10–24

Burke GS (1919) The occurrence of bacillus botulinus in nature. J Bacteriol 4: 541–553

Cherington M (2004) Botulism: update and review. Semin Neurol 24: 155–163

Devriese PP (1999) On the discovery of Clostridium botulinum. J Hist Neurosci 8: 43–50

Dolly JO, Ashton AC, McInnes C, Wadsworth JD, Poulain B, Tauc L, Shone CC, Melling J (1990) Clues to the multi-phasic inhibitory action of botulinum neurotoxins on release of transmitters. J Physiol 84: 237–246

Dong M, Yeh F, Tepp WH, Dean C, Johnson EA, Janz R, Chapman ER (2006) SV2 is the protein receptor for botulinum neurotoxin A. Science 312: 592–596

Drachmann DB (1971) Botulinum toxin as a tool for research on the nervous system. In: Simpson LL (ed), Neuropoisons: their pathophysiological actions, vol. 1. Plenum Press, New York, pp 325–347

Elston JS (1985) The use of botulinum toxin A in the treatment of strabismus. Trans Ophthalmol Soc UK 104: 208–210

Erbguth F (1996) Historical note on the therapeutic use of botulinum toxin in neurological disorders. J Neurol Neurosurg Psychiatry 60: 151

Erbguth F (1998) Botulinum toxin, a historical note. Lancet 351: 1280

Erbguth F (2000) On the first systematic descriptions of botulism and botulinum toxin by Justinus Kerner (1786–1862). J Hist Neurosci 9: 218–220

Erbguth FJ (2004) Historical notes on botulism, Clostridium botulinum, botulinum toxin, and the idea of the therapeutic use of the toxin. Mov Disord 19 Suppl 8: S2–S6

Erbguth F, Naumann M (2000) Historical aspects of botulinum toxin: Justinus Kerner (1786–1862) and the ''sausage poison''. Neurology 53: 1850–1853

Geiges ML (2002) The history of botulism. In: Kreyden OP, Böne R, Burg G (eds) Hyperhidrosis and botulinum toxin in dermatology. Curr Probl Dermatol, vol 30. Karger, Basel, pp 77–93

Grüsser OJ (1986) Die ersten systematischen Beschreibungen und tierexperimentellen Untersuchungen des Botulismus. Zum 200. Geburtstag von Justinus Kerner am 18. September 1986. Sudhoffs Arch 10: 167–187.

Gunn RA (1979) Botulism: from van Ermengem to the present. A comment. Rev Infect Dis 1: 720–721

Hambleton P, Capel B, Bailey N, Tse CK, Dolly O (1981) Production, purification and toxoiding of clostridium botulinum A toxin. In: Lewis G (ed) Biomedical aspects of botulism. Academic Press, New York, pp 247–260

Kerner J (1817) Vergiftung durch verdorbene Würste. Tübinger Blätter für Naturwissenschaften und Arzneykunde 3: 1–25

Kerner J (1820) Neue Beobachtungen über die in Württemberg so häufig vorfallenden tödlichen Vergiftungen durch den Genuss geräucherter Würste. Osiander, Tübingen

Kerner J (1822) Das Fettgift oder die Fettsäure und ihre Wirkungen auf den thierischen Organismus, ein Beytrag zur Untersuchung des in verdorbenen Würsten giftig wirkenden Stoffes. Cotta, Stuttgart

Kreyden OP (2002) Botulinum toxin: from poison to pharmaceutical. In: Kreyden OP, Böne R, Burg G (eds) Hyperhidrosis and botulinum toxin in dermatology. Curr Probl Dermatol, vol 30. Karger, Basel, pp 94–100

Kriek NPJ, Odendaal MW (1994) Botulism. In: Coetzer JAW, Thomson GR, Tustin RC (eds) Infectious diseases of livestock. Oxford Press, Cape Town, pp 1354–1371

Lamanna C, Eklund HW, McElroy OE (1946) Botulinum Toxin (Type A); Including a study of shaking with chloroform as a step in the isolation procedure. J Bacteriol 52: 1–13

Lamb A (2001) Biological weapons: the facts not the fiction. Clin Med 1: 502–504

Landmann G (1904) Über die Ursache der Darmstädter Bohnenvergiftung. Hyg Rundschau 10: 449–452

Leuchs J (1920) Beiträge zur Kenntnis des Toxins und Antitoxins des Bacillus botulinus. Ztschr Hyg Infekt 65: 55–84

Lew MF, Brashear A, Factor S (2000) The safety and efficacy of botulinum toxin type B in the treatment of patients with cervical dystonia: summary of three controlled clinical trials. Neurology 55 Suppl 5: S29–S35

Mahrhold S, Rummel A, Bigalke H, Davletov B, Binz T (2006) The synaptic vesicle protein 2C mediates the uptake of botulinum neurotoxin A into phrenic nerves. FEBS Lett 580: 2011–2014

Melling J, Hambleton P, Shone CC (1988) Clostridium botulinum toxins: nature and preparation for clinical use. Eye 2: 16–23

Merson MH, Dowell J (1973) Epidemiologic, clinical and laboratory aspects of wound botulism. N Engl J Med 289: 1105–1110

Roggenkämper P, Jost WH, Bihari K, Comes G, Grafe S; for the NT 201 Blepharospasm Study Team (2006) Efficacy and safety of a new botulinum toxin type A free of complexing proteins in the treatment of blepharospasm. J Neural Transm 113: 303–312

Rossetto O, Seveso M, Caccin P, Montecucco C (2002) Botulinum neurotoxins are metalloproteases specific for SNARE proteins involved in neuroexocytosis. In: Kreyden OP, Böne R, Burg G (eds) Hyperhidro-

sis and botulinum toxin in dermatology. Curr Probl Dermatol, vol 30. Karger, Basel, pp 117–125

Schantz EJ (1994) Historical perspective. In: Jankovik J, Hallett M (eds) Therapy with botulinum toxin. Marcel Dekker, New York, p xxiii–xxvi

Schantz EJ, Johnson EA (1997) Botulinum toxin: the story of ist development for the treatment of human disease. Perspect Biol Med 40: 317–327

Schiavo G, Benfenati F, Poulain B, Rossetto O, Polverino de Laureto P, DasGupta BR, Montecucco C (1992) Tetanus and botulinum-B toxins block transmitter release by proteolytic cleavage of synaptobrevin. Nature 359: 832–835

Schiavo G, Santucci A, DasGupta BA, Mehta PP, Jontes J, Benfenati F, Wilson MC, Montecucco C (1993) Botulinum neurotoxin serotypes A and E clave SNAP-25 at distinct COOH-terminal peptide bonds. FEBS Lett 335: 99–103

Scott A (2004) Development of botulinum toxin therapy. Dermatol Clin 22: 131–133

Scott AB (1979) Botulinum toxin injection to correct strabism. Trans Am Ophthalmol Soc 79: 924–927

Scott AB, Rosenbaum A, Collins CC (1973) Pharmacologic weakening of extraocular muscles. Invest Ophthalmol 12: 924–927

Smith Louis DS (1977) Botulism. The organism, its toxins, the disease. Charles C Thomas Publ, Springfield, U.S.A.

Snipe PT, Sommer H (1928) Studies on botulinus toxin. 3. Acid preparation of botulinus toxin. J Infect Dis 43: 152–160

Stell R, Thompson PD, Marsden CD (1988) Botulinum toxin in spasmodic torticollis. J Neurol Neurosurg Psychiatry 51: 920–923

Ting PT, Freiman A (2004) The story of Clostridium botulinum: from food poisoning to Botox. Clin Med 4: 258–261

Torrens JK (1998) Clostridium botulinum was named because of association with ''sausage poisoning''. BMJ 316: 151

van Ermengem EP (1897) Über einen neuen anaeroben Bacillus und seine Beziehung zum Botulismus. Z Hyg Infektionskrankh 26: 1–56 (English version: Van Ermengem EP (1979) A new anaerobic bacillus and its relation to botulism. Rev Infect Dis 1: 701–719)