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The pretherapeutic history of botulinum toxin

Frank J. Erbguth

Unintended intoxication with botulinum toxin (botulism) occurs only rarely, but its high fatality rate makes it a great concern for those in the general public and in the medical community. In the United States an average of 110 cases of botulism are reported each year. Of these, approximately 25% are food borne, 72% are infant botulism, and the rest are wound botulism. Outbreaks of food-borne botulism involving two or more persons occur most years and are usually caused by eating contaminated home-canned foods.

Botulism in ancient times

Botulinum toxin poisoning probably has afflicted humankind through the mists of time. As long as humans have preserved and stored food, some of the chosen conditions were optimal for the presence and growth of the toxin-producing pathogen *Clostridium botulinum*: for example, the storage of ham in barrels of brine, poorly dried and stored herring, trout packed to ferment in willow baskets, sturgeon roe not yet salted and piled in heaps on old horsehides, lightly smoked fish or ham in poorly heated smoking chambers, and insufficiently boiled blood sausages.

However, in ancient times there was no general knowledge about the causal relationship between the consumption of spoiled food and a subsequent fatal paralytic disease, nowadays recognized as botulism. Only some historical sources reflect a potential understanding of the life-threatening consumption of food intoxicated with botulinum toxin. Louis Smith, for example, reported in his textbook on botulism a dietary edict announced in the tenth century by Emperor Leo VI of Byzantium (886–911), in which manufacturing of blood sausages was forbidden (Smith, 1977). This edict may have its origin in the recognition of some circumstances connected with cases of food poisoning. Also, some ancient formulas suggested by shamans to Indian maharajas for the killing of personal enemies give hint to an intended lethal application of botulinum toxin: a tasteless powder extracted from blood sausages dried under anaerobic conditions should be added to the enemies' food at an invited banquet. Because the consumer's death occurred after he or she had left the murderer's place with a latency of some days, the host was probably not suspected (Erbguth, 2007).

Botulism outbreaks in Germany in the eighteenth and nineteenth centuries

Accurate descriptions of botulism emerge in the German literature from two centuries ago when the consumption of improperly preserved or stored meat and blood sausages gave rise to many deaths

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throughout the kingdom of Württemberg in Southwestern Germany. This area near the city of Stuttgart developed as the regional focus of botulinum toxin investigations in the eighteenth and nineteenth centuries. In 1793, 13 people of whom 6 died were involved in the first well-recorded outbreak of botulism in the small southwest German village of Wildbad. Based on the observed mydriasis in all affected victims, the first official medical speculation was that the outbreak was caused by an atropine (*Atropa belladonna*) intoxication. However, in the controversial scientific discussion, the term "sausage poison" was introduced by the exponents of the opinion that the fatal disease in Wildbad was caused by the consumption of "Blunzen," a popular local food from cooked pork stomach filled with blood and spices.

The number of cases with suspected sausage poisoning in Southwestern Germany increased rapidly at the end of the eighteenth century. Poverty ensuing from the devastating Napoleonic Wars (1795–1813) had led to the neglect of sanitary measures in rural food production (Grüsser, 1986). In July 1802, the Royal Government of Württemberg 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 on Stuttgart again addressed the problem of "sausage poisoning," considering it to be caused by hydrocyanic acid, known at that time as "prussic acid." However, the members of the near Medical Faculty of the University of Tübingen disputed that prussic acid could be the toxic agent in sausages, suspecting a biological poison. One of the important medical professors of the University of Tübingen, Johann Heinrich Ferdinand Autenrieth (1772–1835), asked the Government to collect the reports of general practitioners and health officers on cases of food poisoning for systematic scientific analyses. After Autenrieth had studied these reports, he issued a list of symptoms of the so-called "sausage poisoning" and added a comment, in which he blamed the housewives for the poisoning, because they did not dunk the sausages long enough in boiling water, thus trying to prevent the sausages from bursting (Grüsser, 1998). The list of symptoms was distributed by a public announcement and contained characteristic features of food-borne botulism such as gastrointestinal problems, double vision, mydriasis, and muscle paralysis.

In 1815, a health officer in the village of Herrenberg, J. G. Steinbuch (1770–1818), sent the case reports of seven intoxicated patients who had eaten liver sausage and peas to Professor Autenrieth. Three of the patients had died and the autopsies had been carried out by Steinbuch himself (Steinbuch, 1817).

Justinus Kerner's observations and publications on botulinum toxin 1817–1822

Contemporaneously with Steinbuch, the 29-year-old physician and Romantic poet Justinus Kerner (1786–1862) (Figure 1.1), then medical officer in a small village, also reported of a lethal food poisoning. Autenrieth considered the two reports from Steinbuch and Kerner as accurate and important observations and decided to publish them both in 1817 in the "*Tübinger Blätter für Naturwissenschaften und Arzneykunde*" ["*Tübinger Papers for Natural Sciences and Pharmacology*"] (Kerner, 1817; Steinbuch, 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 summarized the case histories of 76 patients and gave a complete clinical description of what we now recognize 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). Kerner compared the various recipes and ingredients of all sausages which had produced intoxication and found out that among

Figure 1.1 Justinus Kerner; photograph of 1855.

Das

Fettgift

oder

die Fettsäure

und

ihre Wirkungen auf den thierischen Organismus,

ein Beytrag

zur Untersuchung des in verdorbenen Würsten giftig wirkenden Stoffes,

von

Dr. Justinus Kerner,

Oberamtsarzt zu Weinsperg.

Stuttgart und Tübingen,
in der J. G. Cotta'schen Buchhandlung.
1822.

Figure 1.2 Title of Justinus Kerner's second monograph on sausage poisoning 1822.

the ingredients blood, liver, meat, brain, fat, salt, pepper, coriander, pimento, ginger, and bread the only common ones were fat and salt. Because salt was probably known to be "innocent," Kerner concluded that the toxic change in the sausage must take place in the fat and therefore called the suspected substance "sausage poison," "fat poison" or "fatty acid." Later Kerner speculated about the similarity of the "fat poison" to other known poisons, such as atropine, scopolamine, nicotine, and snake venom, which led him to the conclusion that the fat poison was probably a biological poison (Erbguth, 2004).

In 1822, Kerner published 155 case reports including postmortem studies of patients with botulism and developed 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"] (Kerner, 1822) (Figure 1.2). 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, and gastrointestinal and bladder paralysis. Kerner also experimented on various animals (birds, cats, rabbits, frogs, flies, locusts, snails) by feeding them with extracts from bad sausages and finally carried out high-risk experiments

on himself. After he had tasted some drops of a sausage extract he reported: ". . . some drops of the acid brought onto the tongue cause great drying out of the palate and the pharynx" (Erbguth & Naumann, 1999).

Kerner deduced from the clinical symptoms and his experimental observations that the toxin acts by interrupting the motor and autonomic nervous signal transmission (Erbguth, 1996). 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 & Naumann, 1999).

In the eighth chapter of the 1822 monograph, Kerner speculated about using the "toxic fatty acid" botulinum toxin for therapeutic purposes. He concluded that small doses would be beneficial in conditions with pathological hyperexcitability of the nervous system (Erbguth, 2004). 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." The term "sympathetic nervous system" as used during the Romantic period, encompassed nervous functions in general. "Sympathetic overactivity" then was thought to be the cause of many internal, neurological, and psychiatric diseases. Kerner favored the "Veitstanz" (St. Vitus dance – probably identical with Chorea minor) with its "overexcited nervous ganglia" to be a promising indication for the therapeutic use of the toxic fatty acid. Likewise, he considered other diseases with assumed nervous overactivity to be potential candidates for the toxin treatment: hypersecretion of body fluids, sweat or mucus; ulcers from malignant diseases; skin alterations after burning; delusions; rabies; plague; consumption from lung tuberculosis; and yellow-fever. 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" (Erbguth, 1998).

Justinus Kerner also advanced the idea of a gastric tube suggested by the Scottish physician Alexander Monro in 1811 and adapted it for the nutrition of patients with botulism; he wrote: "if dysphagia occurs, softly prepared food and fluids should be brought into the stomach by a flexible tube made from resin." He considered all characteristics of modern nasogastric tube application: the use of a guide wire with a cork at the tip and the lubrication of the tube with oil.

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 melancholic poetry. Many of his poems were set to music by the great German Romantic composer Robert Schumann (1810–56) who had to quit his piano career due to the development of a pianist's focal finger dystonia. Kerner's poem "The Wanderer in the Sawmill" was the favourite poem of the twentieth-century poet Franz Kafka (Appendix 1.1). The nickname "Sausage Kerner" was commonly used and "sausage poisoning" was known as "Kerner's disease." Further publications in the nineteenth century by various authors, for example Müller (Müller, 1869), increased the number of reported cases of "sausage poisoning," describing the fact that the food poisoning had occurred after the consumption not only of meat but also of fish. However, these reports

Figure 1.3 Emile Pierre Marie van Ermengem 1851–1922.

added nothing substantial to Kerner's early observations. The term "botulism" (from the Latin word *botulus* meaning sausage) appeared at first in Müller's reports and was subsequently used. Therefore, "botulism" 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 1895–6 by the Belgian microbiologist Emile Pierre Marie van Ermengem of the University of Ghent (Figure 1.3).

The discovery of "*Bacillus botulinus*" in Belgium

On December 14, 1895 an extraordinary outbreak of botulism occurred amongst the 4000 inhabitants of the small Belgian village of Ellezelles. The musicians of the local brass band "Fanfare Les Amis Réunis" played at the funeral of the 87-year-old Antoine Creteur and as it was the custom gathered to eat in the inn "Le Rustic" (Devriese, 1999). Thirty-four people were together and ate pickled and smoked ham. After the meal the musicians noticed symptoms such as mydriasis, diplopia, dysphagia, and dysarthria followed by increasing muscle paralysis. Three of them died and ten nearly died. A detailed examination of the ham and an autopsy were ordered and conducted by van Ermengem who had been appointed Professor of Microbiology at the University of Ghent in 1888 after he had worked in the laboratory of Robert Koch in Berlin in 1883. van Ermengem isolated the bacterium in the ham and in the corpses of the victims (Figure 1.4), grew it, used it for animal experiments, characterized its culture requirements, described its toxin, called it "*Bacillus botulinus*," and published his observations in the German microbiological journal "*Zeitschrift für Hygiene und Infektionskrankheiten*" ["*Journal of Hygiene and Infectious Diseases*"] in 1897 (an English translation was published in 1979) (van Ermengem, 1897). The pathogen was later renamed "*Clostridium botulinum*." van Ermengem was the first to correlate "sausage poisoning" with the newly discovered anaerobic microorganism and 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's milestone investigation yielded all clinical facts about botulism and botulinum toxin: (1) botulism is an intoxication, not an infection, (2) the toxin is produced in food by a bacterium, (3) the toxin is not produced if the salt concentration in the food is high, (4) after ingestion, the toxin is not inactivated by the normal digestive process, (5) the toxin is susceptible to inactivation by heat, and (6) not all species of animals are equally susceptible.

Botulinum toxin research in the early twentieth century

In 1904, when an outbreak of botulism in the city of Darmstadt, Germany was caused by canned white beans, the opinion that the only botulinogenic

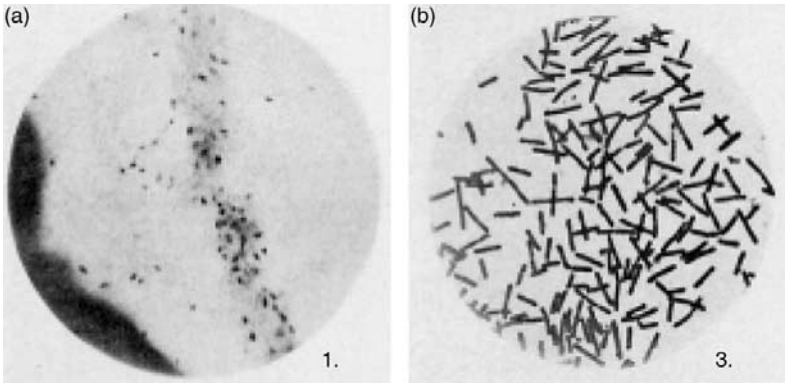

Figure 1.4 Microscopy of the histological section of the suspect ham at the Ellezelles botulism outbreak. (a) Numerous spores among the muscle fibers (Ziehl × 1000). (b) Culture (gelatine and glucose) of mature rod-shaped forms of "Bacillus botulinus" from the ham; eighth day (× 1000) (from van Ermengem, 1897).

foods were meat or fish had to be revised. The bacteria isolated from the beans by Landmann (Landmann, 1904) and from the Ellezelles ham were compared by Leuchs (Leuchs, 1910) 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 United States was caused by commercially conserved pork and beans, and dates from 1906 (Drachmann, 1971; Smith, 1977). 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 United States by Bengston and in Australia by Seddon, type D and type E were characterized some years later (type D: USA 1928 by Meyer and Gunnison; type E: Ukraine 1936 by Bier) (Kriek & 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. The essential insights into the molecular actions of botulinum toxin were gained by various scientists after 1970 (Dolly *et al.*, 1990; Schiavo *et al.*, 1992, 1993; Dong *et al.*, 2006; Mahrhold *et al.*, 2006), when its use as a therapeutic agent was pioneered by Edward J. Schantz and Alan B. Scott.

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 first in 1976 (infant botulism) and in contaminated wounds (wound botulism) in the 1950s (Merson & Dowell, 1973; Picket *et al.*, 1976; Arnon *et al.*, 1977). The number of cases of food-borne and infant botulism has changed little in recent years, but wound botulism has increased because of the use of black-tar heroin, especially in California.

Swords to ploughshares

Before the therapeutic potential of botulinum toxin was discovered around 1970, its potential use as a weapon was recognized during World War I (Lamb, 2001). The basis for its use as a toxin was investigations by Hermann Sommer and colleagues working at the Hooper Foundation, University of

California, San Francisco in the 1920s: the researchers were the first to isolate pure botulinum toxin type A as a stable acid precipitate (Snipe & Sommer, 1928; Schantz, 1994). 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 crystallization techniques at Fort Detrick was pioneered by Carl Lamanna and James Duff in 1946. The methodology was subsequently used by Edward J. Schantz to produce the first batch of toxin which was the basis for the later clinical product (Lamanna *et al.*, 1946). The entrance of botulinum toxin into the medical therapeutic armament in Europe also led from military laboratories to hospitals: 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).

APPENDIX 1.1

The Wanderer in the Sawmill (*by Justinus Kerner 1826*)

Down yonder in the sawmill
I sat in good repose
and saw the wheels go spinning
and watched the water too.

I saw the shiny saw blade,
as if I had a dream,
which carved a lengthy furrow
into a fir tree trunk.

The fir tree as if living,
in saddest melody,
through all its trembling fibers
sang out these words for me:

At just the proper hour,
o wanderer! you come,
it's you for whom this wounding
invades my heart inside.

It's you, for whom soon will be,
when wanderings cut short,
these boards in earth's deep bosom,
a box for lengthy rest.

Four boards I then saw falling,
my heart was turned to stone,
one word I would have stammered,
the blade went 'round no more.

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2

Botulinum toxin: history of clinical development

Daniel Truong, Dirk Dressler and Mark Hallett

The clinical development of botulinum toxin began in the late 1960s with the search for an alternative to surgical realignment of strabismus. At that time, surgery of the extraocular muscles was the sole treatment. However, it was unsatisfactory due to variable results, consequent high reoperation rates, and its invasive nature. In an attempt to find an alternative, Alan B. Scott, an ophthalmologist from the Smith-Kettlewell Eye Research Institute in San Francisco, CA, USA, had been investigating the effects of different compounds injected into the extraocular muscles to chemically weaken them. The drugs tested initially proved unreliable, short acting or necrotizing (Scott *et al.*, 1973). About this time, Scott became aware of Daniel Drachman, a renowned neuroscientist at Johns Hopkins University, and his work, in which he had been injecting minute amounts of botulinum toxin directly into the hind limbs of chicken to achieve local denervation (Drachman, 1972). Drachman introduced Scott to Edward Schantz (1908–2005) who was producing purified botulinum toxins for experimental use and generously making them available to the academic community. Schantz himself credits Vernon Brooks with the idea that botulinum toxin might be used for weakening muscle (Schantz, 1994). Brooks worked on the mechanism of action of botulinum toxin for his Ph.D. under the mentorship of Arnold Burgen, who suggested the project to him (Brooks, 2001). Schantz had left the US Army Chemical Corps at Fort Detrick, Maryland in 1972 to work at the Department of Microbiology and Toxicology, University of Wisconsin, Madison, WI, USA. Using acid precipitation purification techniques worked out at Fort Detrick by Lamanna and Duff, Schantz was able to make the purified botulinum toxins. In extensive animal experiments botulinum toxin produced the desired long-lasting, localized, dose-dependent muscle weakening without any systemic toxicity and without any necrotizing side effects (Scott *et al.*, 1973). Based on these results the US Food and Drug Administration (FDA) permitted Scott in 1977 to test botulinum toxin in humans under an Investigative New Drug (IND) license for the treatment of strabismus. These tests proved successful and the results of 67 injections were published in 1980 (Scott, 1980). With this publication botulinum toxin was established as a novel therapeutic agent. Before botulinum toxin could be registered as a drug the US FDA required numerous tests including tests for safety, potency, stability, sterility, and water retention in the freeze-dried product. In addition to establishing a laboratory for the tests, a sterile facility for filling and freeze-drying was set up by Scott, Schantz, and Eric Johnson, who joined the team in 1985.

By the early 1980s, Scott and colleagues had injected botulinum toxin for the treatment of strabismus, blepharospasm, hemifacial spasm, cervical dystonia, and thigh adductor spasm (Scott, 1994).

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During the 1980s, the use of botulinum toxin for therapeutic purposes increased substantially as Scott supplied investigators with various interests. In 1985, Tsui and colleagues reported the successful use of botulinum toxin for the treatment of cervical dystonia in 12 patients based on the earlier dosage data from Scott's injections (Tsui *et al.*, 1985). This was followed by a double-blind, crossover study in which botulinum toxin was found to be significantly superior to placebo at reducing the symptoms of cervical dystonia, including pain (Tsui *et al.*, 1986). Soon, botulinum toxin became the treatment of choice for cervical dystonia. The therapeutic use of botulinum toxin for the treatment of blepharospasm and hemifacial spasm proceeded along similar lines, with several groups reporting success in these indications by the mid 1980s and documenting the benefits of repeated injections after the effects waned (Frueh *et al.*, 1984; Mauriello, 1985; Scott *et al.*, 1985). Reports of the successful use of botulinum toxin in many conditions of focal muscle overactivity followed, including spasmodic dysphonia (Blitzer *et al.*, 1986), oromandibular dystonia (Jankovic & Orman, 1987), dystonias of the hand (Cohen *et al.*, 1989), and limb spasticity (Das & Park, 1989).

In December 1989, the FDA licensed the manufacturing facilities and a batch of botulinum toxin type A manufactured by Scott and Schantz in November 1979, the so-called batch 11/79. The therapeutic preparation contained 100 mouse units of toxin per vial. The FDA identified this product named Oculinum® (**ocu**l and **lin**ing-up) as an orphan drug for the treatment of strabismus, hemifacial spasm, and blepharospasm. For about 2 years, Scott's Oculinum Inc. was the licensed manufacturer with Allergan Inc., Irvine, CA, USA acting as the sole distributor. The manufacturing facilities and the license were turned over to Allergan in late 1991 and the product was later renamed Botox® (**bo**tulinum **tox**in). The name Botox was perhaps first used by Stanley Fahn, but he did not think of it as a possible trade name. A different batch of Botox was prepared in 1988 and served as the basis for European licensing. This and subsequent batches of Botox contain less neurotoxin complex protein per mouse unit, which may make them less liable to elicit antibodies than batch 11/79.

In 2000 NeuroBloc®/Myobloc® was registered with the US FDA by Elan Pharmaceuticals, South San Francisco, CA, USA with the indication of cervical dystonia. Myobloc is the trade name in the USA and NeuroBloc is the trade name used elsewhere. It was eventually sold to Solstice Neurosciences Inc., Malvern, PA, USA. Botox was also approved for cervical dystonia in 2000.

In Europe botulinum toxin was first produced for therapeutic purposes at the Defence Science and Technology Laboratory in Porton Down, Salisbury Plain, Wilts., UK. When the product was commercialized the manufacturing operations were renamed several times to Centre of Applied Microbiology and Research (CAMR), Porton Products, Public Health Laboratory Service (PHLS), and Speywood Pharmaceuticals. In 1994 Speywood Pharmaceuticals was acquired by Ipsen, Paris, France. The UK botulinum toxin product was first registered in 1991 as Dysport® (**Dys**tonia **Port**on Products). It is now distributed worldwide by Ipsen Ltd., Slough, Berks., UK. A US registration for cervical dystonia as well as a cosmetic registration under the name Reloxin is in preparation. The UK product was first used in the UK to treat strabismus and blepharospasm not long after Scott's initial reports (Elston, 1985; Elston *et al.*, 1985). C. David Marsden's movement disorders group at the National Hospital of Neurology and Neurosurgery, Queen Square, London, UK, pioneered its use in neurology (Stell *et al.*, 1988). Soon afterwards, Dirk Dressler, a student of Marsden, introduced this product to continental European neurology (Dressler *et al.*, 1989). More details about the continental European spread of the botulinum toxin therapy are described elsewhere (Homann *et al.*, 2002).

Recently, another botulinum toxin drug named Xeomin® has been marketed by Merz Pharmaceuticals from Frankfurt/M, Germany. It is a botulinum toxin type A preparation with high specific biological activity, and, as a consequence, a reduced protein load (Dressler & Benecke, 2006). Structurally, it is