ON THE DISCOVERY OF CLOSTRIDIUM BOTULINUM [1]

Pieter P. Devriese

Abstract

A description is given of a food intoxication in 1895 at Ellezelles, a village in Belgium.

As a result 3 persons died within a few days and others became seriously ill. A thorough investigation by E. van Ermengem led to the discovery of *Clostridium botulinum* and botulinum toxin. About 75 years later a subtype of the toxin proved to be highly effective in the treatment of dystonias and is now widely used.

Introduction

Up to the end of the last century the causes of food poisoning were still largely unclear. In 1895 there was an extraordinary case of food poisoning in the Belgian village of Ellezelles. A number of musicians who had been playing at a funeral died after sharing a meal; others became seriously ill.

E. Van Ermengem, professor of microbiology at the State University of Ghent, was called in to investigate. By examining the food and the victims Van Ermengem was able to isolate the anaerobic bacterium Bacillus botulinus (from the Latin botulus, meaning sausage) - later called Clostridium botulinum - and he carried out toxicological studies describing the effects of this unknown toxin (Van Ermengem, 1897, 1979). Then, around 1970, the medical world became interested in the medicinal potential of the toxin. The subtype A proved to be highly

[1] This paper is an abbreviated version of a publication in *Geschiedenis der Geneeskunde* (Journal of History of Medicine) reproduced by permission of the editor.

effective in the treatment of forms of dystonia which were difficult to treat by other means.

Van Ermengem could never have suspected that his discovery might have a therapeutic as well as a preventive application.

The food poisoning in Ellezelles, December 14, 1895

Ellezelles (Elzele) is a small town about 4 miles east of the town of Renaix (Ronse). It lies on the dividing line between the valleys of the river Scheldt and the river Dender. This area has been inhabited since the Neolithic age (ca. 4500 to 2500 B.C.). Agriculture is the main economic activity, with the cultivation of flax as a supplementary source of income. In 1801 the parish had 4,008 inhabitants, in 1977 5,074.

The musicians of a local brass band, the Fanfare Royale Les Amis Réunis (founded in 1864 and still active today) played at the funeral of Antoine Creteur, who had died at the age of 87, and afterwards, as was the custom, they ate together (34 people in all).

Figure 1. Ellezelles in 1997. The inn where the meal took place.

Extraordinary symptoms followed. The musicians were troubled, to varying degrees, by visual disturbances (ptosis, mydriasis, diplopia and difficulties in focussing), a general weakness in the muscles, dryness and inflammation of the mucous membranes of the nose, mouth and throat, difficulty swallowing and breathing, speech disorders (dysartria), coughing, voice trouble with complete aphonia in some cases, urine retention and constipation. In some cases the visual problems continued for 6 to 8 months.

Three young men died within 5 to 7 days. The case histories of these unfortunate people were recorded in detail by Drs. André and Noville:

Jules Hautru, agricultural labourer, age 19.
On December 14 ate ham at the meal after the funeral.
December 15: digestive trouble after the usual midday meal. Ate nothing else for the rest of the day.
December 16: coffee for breakfast, ate nothing further. At 10 a.m. copious vomiting, greenish.
Visual problems: diplopia (double vision), pupils dilated, trouble focussing, edema and paralysis of the eyelids.
Difficulty swallowing: painful red throat, right tonsil greatly swollen. General weakness in the muscles, dullness and drowsiness.
Tongue blackened and thick, no appetite, little thirst. No bowel movement until December 19. Urine not examined. No fever, pulse weak, not faster than 80 per min. Breathing shallow, 30 per min.
December 17 and 18: no change.
December 19 died in coma in the evening. (Noville)

Angel Deltenre, agricultural labourer, age 15.
December 14: around midday ate about 150 gr. ham with fat. Noticed no ill effects, went home and slept well that night.
December 15: friends came to his house to play music. All ate rabbit. He felt less well, found blowing his instrument very tiring.
December 16: had an attack of dry coughing, like croup. Said he felt ill and had a sore throat. Also complained of problems with his eyes.

December 17 and 18: same symptoms, more trouble swallowing: bringing back fluids through the nose. His parents thought he had a throat infection.
December 19: In the morning I was called in . No bowel movement and no vomiting since eating the ham. No pain. Pupils strongly dilated, partial paralysis of the eyelids. Breathing free, temperature normal, pulse 80 per min. Considerable weakness in the muscles, consciousness not affected. Purgative administered by enema had no effect.
Called out again in the evening; pulse 100 per min., temperature 37.5°C. Very rapid breathing. Patient could swallow almost nothing: everything coming back through the nose. He was now agitated; could not sit or lie comfortably. Lost consciousness briefly. Died 2 hours later, fully conscious. (André).

Firmin Creteur, saddler, age 21
He ate 2 portions of meat, about 200 grams. Had no breakfast and worked until he went to the funeral service. Back home about 3 o'clock, complaining of nausea but ate as usual in the evening. Complained that the ham was bad.
December 15: colic in the morning. Went back to bed around midday after drinking a cup of broth. In the evening ate a little meat which he brought up again during the night. Slight diarrhea (black and sticky). The vomit also contained a dark fluid.
December 16: some visual problems but still worked. Ate almost nothing. Bowels open in the evening.
December 17: no longer able to work. Ate a couple of eggs. Rumours going round about the ham and I was called in. Pupils dilated, also hoarseness and trouble swallowing. Laxatives and enema had no effect.
December 18: same symptoms. Slept poorly, restless. Mouth and throat very dry, swallowing increasingly difficult. Periods of breathlessness. Respiration normal, even so, pulse 80 per min., temperature 37° C. A red rash, no bowel movement.
December 19: the same.
December 20: symptoms worsening, dark red urine. Pulse 90 per min., temperature 38°C, tongue and lips sooty in colour. Blowing the nose and coughing produce bits of swollen mucous membrane.
December 21: the same. Bowels open after enema.

December 22: feared for his life in the morning; very rapid breathing for half an hour; slight delirium. Then better for several hours. In the evening breathing more rapid again, pulse 100 per min., regular. Died in the night in coma.. (André).

In all more than 10 people were in a dangerous condition for a time. The doctors André and Noville believed the symptoms were due to eating spoiled meat. The coroner ascribed the symptoms to a 'ptomaine', a toxic substance found in decaying flesh.

All the musicians who became ill had eaten raw ham; those who had not eaten the ham were not ill. Other people who ate the ham later, before it became clear how serious the food poisoning was, also became ill. A judicial inquiry was set up to trace the provenance of the ham and the public prosecutor ordered a chemical analysis and an autopsy of two of the victims. Part of the suspect ham, part of a second ham from the same pig and organs from the dead victims were sent for further investigation to Van Ermengem in Ghent.

The examination by E. Van Ermengem

Fig.2. E. Van Ermengem

A highly detailed examination was carried out by the microbiologist Van Ermengem in his laboratory in Ghent.

Emile-Pierre-Marie Van Ermengem was born on August 15, 1851 in Leuven (Louvain). On September 20, 1875 he was made Doctor of Medicine at the Catholic University of Leuven. Thanks to a bursary in 1876 he was able to visit the laboratories of Claude Bernard in Paris. From 1876 to 1878 he visited clinics in London, Edinburgh and Vienna. In 1883 he worked at the laboratory of Koch in Berlin and in 1885 he made a study of vaccination against cholera in Spain.

In 1888 he was appointed Professor of Microbiology at the University of Ghent. He published the first findings of his investigation into the food poisoning in Ellezelles (December 14, 1895) in 1896. After this he was called in by the authorities in various cases (such as the plague outbreak in Glasgow) and received many distinctions both at home and abroad. He died in 1932.

The examination consisted of 4 parts: clinical, toxicological and bacteriological tests and an investigation into the effects of the toxin.

The first symptoms appeared 20 to 24 hours after ingestion. Some patients had no trouble for 38 hours. An autopsy was performed on two of the victims, (A.D. and J.H.), both young men, 15 and 19 years old respectively. Histological examination of the spleen showed numerous traces of anaerobic bacilli.

The preparation of the ham was also carefully reconstructed (Fig. 3). The pig had been slaughtered on August 2, 1895 and the part which was to be cured was salted and put to keep in a barrel within 24 hours.

There are two stages in the usual curing procedure: pickling and smoking. The first piece of meat, rubbed with salt, is placed on the bottom of the barrel, in between layers of fat. A layer of fat is then laid on top, followed by a second ham, also topped with a layer of fat. Then two liters of water are added to turn the salt into brine (the Dutch word is pekel, or pickle). The barrel remains untouched for anything from 6

weeks to 3 months. Then the hams are removed from the barrel and hung for 1 to 2 weeks to let all the moisture run off. After this the hams are smoked in the chimney over a wood fire for 4 to 5 weeks.

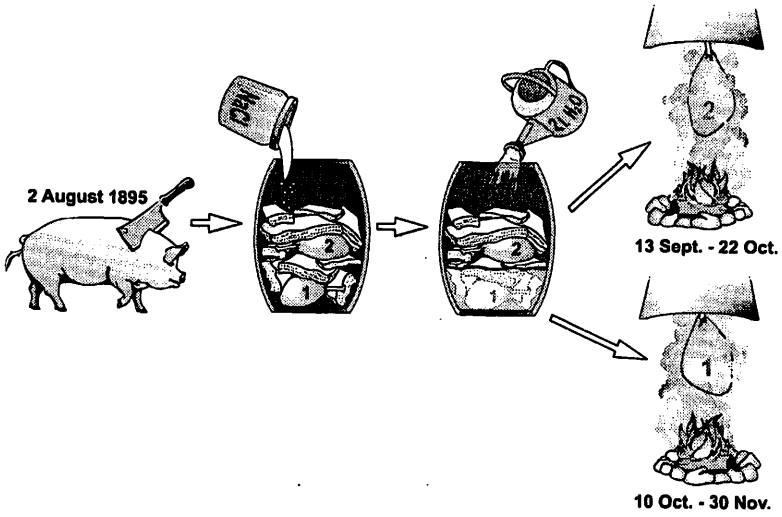

Fig. 3

The ham which caused the food poisoning, which was eaten for the first time on December 14, had been lying at the bottom of the barrel, completely immersed in the brine. The second ham, which was on top, was separated by just a layer of fat from the one beneath and was not in the brine. The bottom ham stayed in the barrel longer and was smoked from October 10 to the end of November 1895. The other was smoked from September 13 to October 22. Both hams were then hung for two weeks in the loft by the chimney (a dry place) and after that were stored in a dry, well ventilated cellar. It is interesting to note that there were a number of instances of the curing of hams failing in Ellezelles that summer.

The bottom ham, which was under the brine and not in contact with the air, showed when seen under the microscope no clear signs of decay but considerable changes resulting from an invasion of anaerobic bacteria.

Although the second ham was somewhat decayed, eating it caused no problems. And not all parts of the bottom ham were equally dangerous. It was eating the lean meat that caused problems; with the fatty meat the problems were negligible. There was also a clear correlation between the amount of meat consumed and the severity of the symptoms. The patients who died or were very ill had all eaten at least 200 grams; the others only 60 to 70 grams.

Next, experiments were carried out on a series of animals. Subcutaneous injection with tiny fragments of the ham caused comparable symptoms to those of the human victims in cats, pigeons, monkeys, guinea pigs, rabbits and mice. Administration via the digestive system provoked the same symptoms in the monkey, guinea pig and mouse. However, the ingestion of even large amounts caused no symptoms in the cat, dog and chicken.

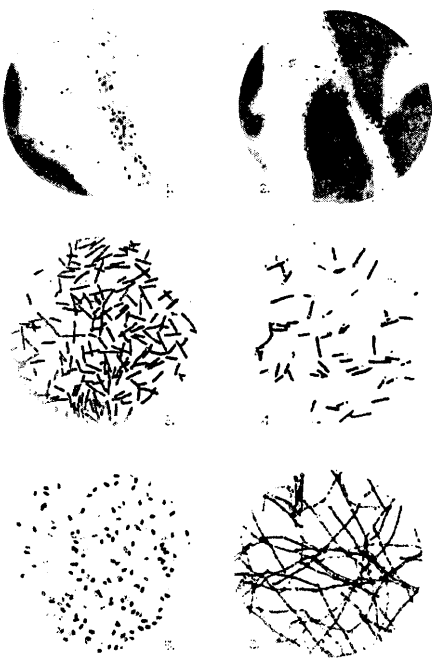

Fig. 4

The lethal dose when given by subcutaneous injection proved to be very low in the rabbit: only 0.0005 mg. The bacterium isolated from the ham and the organs of the victims is of the anaerobic type and has numerous villi. The spores produce a good deal of gas in the fermentation of sugar. In a culture medium of gelatin with glucose the bacteria form circular colonies. The bacteria give off a rancid odour, though not so unpleasant as the well-known pathogenic anaerobics. Van Ermengem named the bacterium Bacillus botulinus from the Latin botulus (sausage), because the symptoms observed were similar to those of a syndrome long known primarily in southern Germany, which occurred after eating a certain type of sausage.

Study of the events in Ellezelles and Van Ermengem's investigation indicate that the ham was probably infected with Clostridium botulinum during slaughter or preservation and that immersion in brine failed to prevent the development of the bacteria and the production of the toxin (a personal view of J. van Hoof).

Developments after 1895

After Van Ermengem's discovery of Clostridium botulinum and its toxicity, the bacterium continued to be of interest to laboratories concerned with the control of foodstuffs and military laboratories interested in the possibilities of using botulism as a biological weapon.

The toxin involved is now known as botulin toxin. Ingestion of as little as 0.1 microgram can be fatal in human beings. It prevents the release of acetylcholine at the level of the synapse (the junction of two nerve fibers), blocking the conduction of stimuli via the nerve fibers. The effect is temporary: after roughly 3 months the toxin ceases to be effective. There are 7 known sub-types of the toxin; of these, only type A is as yet being used therapeutically.

The idea of using botulinum toxin to treat squinting arose in various places in the United States during the 1970's and in Amsterdam (R.A. Crone 1984). In 1981 Scott treated the first case in this way. He

also foresaw other possible applications in humans, such as the treatment of blepharospasm and other forms of dystonia: neurological disorders typically involving the continuous involuntary contraction of muscles resulting in abnormal movements and postures

In 1981 the ophthalmologist P.V.T.M. de Jong, after a visit to Scott in San Francisco, introduced the use of the toxin at the Wilhelmina Gasthuis hospital in Amsterdam (departments of Ophthalmology and Otorhinolaryngology) to treat strabismus (squinting), blepharospasm and hemifacial spasm (spasm of the facial muscles). We were able to use the toxin provided by Scott for many years. Dr. S.H.W. Notermans, nutritional toxicologist at the National Institute of Public Health and Environment in Bilthoven, prepared and tested the toxin (Devriese, 1989; De Jong et al., 1991).

The American Food and Drug Administration agreed to a limited trial on patients in 1989. At a consensus meeting held at the National Health Institutes (Bethesda) in 1990 a list of guidelines and indications was drawn up for a broader application of botulin. Since then the therapeutic application of botulin has vastly increased: more and more countries are using it and many patients are benefiting (Blitzer et al, 1992; Jankovitz and Hallett, 1994; Moore, 1995).

Conclusion

A meticulously conducted investigation into a case of food poisoning 100 years ago has led to the development of a whole range of new therapeutic possibilities. In this way a substance which is exceedingly toxic to humans in general has proved suitable for the treatment of significant groups of patients who often feel considerably handicapped socially. As a medicine botulin toxin, produced by the Clostridium botulinum bacterium, has become essential in the treatment of many disorders which are otherwise difficult to treat medically or surgically.

Van Ermengem, who quite rightly received international acclaim for his discovery, could not have foreseen anything like this, but he

would undoubtedly have been delighted with the way things have turned out.

Acknowledgements: thanks are due to Mr. Bernard Desmaele, National General Archives, Brussels, Dr. L. Devriese, veterinarian, University of Ghent, Prof. J. Van Hoof, Department of Veterinary Inspection of Foodstuffs, University of Ghent, Mr. Jean Hustache, conductor of the Fanfare Royale Les Amis Réunis, Ellezelles, Ms. E Langendries, archivist, University of Ghent

References

BLITZER A, BRIN MF, SASAKI CT, FAHN S, HARRIS K (ed.) (1992): *Neurologic Disorders of the larynx*. NewYork: Thieme.

CRONE RA, JONG DE PTVM, NOTERMANS SHW (1984): Behandlung des Nystagmus durch Injektion von Botulines Toxin in die Augenmuskeln. *Klin Monatsbl Augenheilkd 184*: 216-217.

DEVRIESE PP (1989): Treatment of hemifacial spasm with Botulinum A toxin. In: Castro D ed., *The Facial Nerve.* Amsterdam: Kugler & Ghedini, pp. 473-477.

DEVRIESE PP (1998): Botuline, een tweesnijdend zwaard. *Geschiedenis der Geneeskunde 4:* 293-299.

VAN ERMENGEM E (1897): *Contribution à l'étude des Intoxications Alimentaires. Recherches sur des accidents à caractères botuliniques par du jambon.* Travaux du Laboratoire d'Hygiène et de Bactériologie . Université de Gand. J. Van In & Cie, Lierre/H. Engelcke, Gand. pp. 5-269. (Extrait des Archives de Pharmacodynamie, volume III).

VAN ERMENGEM E (1979): A New Anaerobic Bacillus and Its Relation to Botulism. *Reviews of Infectious Diseases. 1:* 701-719. Abridged version of 1.

JANKOVIC J AND HALLETT M (1994): *Therapy with Botulinum Toxin*. Marcel Dekker, inc. New York, Basel, Hong Kong.

JONG DE PTVM, DEVRIESE PP, NOTERMANS SHW, COZIJNSEN (1991): J. Vijftien jaar therapeutisch toepassen van botuline A toxine. In: Es JC van, Mandema E, Olthus A, Verstraete E (red.) *Het Medisch Jaar*. Houten: Bohn Stafleu Van Loghum, pp. 272-277.

MOORE P (1995). *Handbook of Botulinum Toxin Treatment*. Oxford, Blackwell Science Ltd.

SCOTT AB (1981): Botulinum toxin injection of the eye muscles to correct strabismus. *Trans Am Ophthalmol Soc 79*: 734-770.

Illustrations

Figure 1. Ellezelles in 1997 (photograph by the author). The inn 'Le Rustic' where in 1895 the meal after the funeral was eaten.

Figure 2. Photograph of E. Van Ermengem (1851 - 1932). Published with the permission of the Archives of the University of Ghent.

Figure 3. Preparation of the ham.

Figure 4.

1. Histological section of the suspect ham.
   Numerous spores among the muscle fibers. Ziehl x 1000.
2. Histological section of the suspect ham.
   Muscle tissue next to bone tissue. Ziehl x 1000.
3. Bacillus botulinus. Culture in gelatin and glucose.
   Eighth day. Mature forms. x 1000.
4. Bacillus botulinus. Same culture. Forms with spores. x 1000.
5. Bacillus botulinus. Same culture after 4 weeks. Free spores. x 1000.
6. Bacillus botulinus. Culture in bouillon and glucose at 38.5 after 48 hours. Involuted forms (x 1000). From: Van Ermengem (1897).