Proc R Coll Physicians Edinb 1997; **27**: 153-161

BOTULISM: POISON AND PROBE*

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Lord Lister had associations, not only with Edinburgh, but with my medical school, King's College Hospital, London. He was head-hunted from Edinburgh for a very specific purpose: to strengthen the consultants' hand against the Matron who was denying medical staff entry to the wards at certain times. The senior surgeon at the time was Sir William Ferguson, renowned for his lion-tooth forceps for removing sequestrum from osteomyelitis of the mandible. If the two firms met in the corridor - one with lapels up, the other down - after a brief exchange Sir William would mutter 'Whoof, carbolic', and sail on by.

The disinfectants that Lister used were capable of sterilising the surface of the body but were of little protection against micro-organisms which thrive anaerobically in the intestinal tract and beneath the skin after wounding. James Lovelock, in '*Gaia - A New Look at Life on Earth*' suggests that when life first began it was anaerobic.[1] When the atmosphere subsequently became 'poisoned' with oxygen, anaerobic organisms sought refuge in the soil and in the intestines of the new aerobic creatures. However from time to time they return to reek their vengeance on the second generation of living organisms. Most dangerous among these soil-based organisms which are widely established in the environment are the Gram-positive bacilli of the *Clostridium* species (Table 1); they produce heat-resistant spores which can germinate into vegetative organisms in a non-acidic ambient and produce exotoxins (Table 2).

TABLE 1

Clostridia	
perfringens	A = food poisoning B = necrotising haemorrhagic enteritis
welchii	gas gangrene
tetanis	tetanus *tetanus neonatorum*
botulinum	wound botulism intestinal infection intoxication

The exotoxin of *Clostridium botulinum* has been purified and the various intoxications and infections caused by it will be reviewed. This toxin has also therapeutic uses and in future has potential as a vehicle for introducing therapeutic agents into the nervous system.

* Based upon a Lister Fellowship Lecture delivered at the Symposium on *Infection and Neurological Disease* held in the Postgraduate Medical Centre, Sharoe Green Hospital, Preston, on 22 November 1996

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TABLE 2

Clostridial Toxins

Toxins	Optimum growth temp.	*State of Activation*	At risk
A	38 to 40 °C	fully synthesised dichains	humans & animals
B	38 to 40 °C	nearly fully synthesised	humans & animals
E	33 to 35 °C	single chains activated by trypsin	humans & animals (mainly fish products)
C 1 & 2	40 to 42 °C	partly activated by trypsin	animals & birds
D	40 to 42 °C	partly activated by trypsin	animals & birds

Botulinum toxins

Toxins from *Clostridium botulinum* are responsible for several hundred deaths of wild, domesticated and exotic species of birds and animals each year and are also an occasional source of mortality and morbidity to humans. Most intoxications are food-borne, but toxico-infective and wound botulism also occur. Some winter and spring outbreaks, particularly involving ducks on the Norfolk Broads, originally described as water-borne have since been realised not to be due to persistence of toxins in the frozen water but to changes in the water level affecting invertebrates harbouring Clostridia.[2]

The first intimation of an epidemic among animals and birds is frequently the occurrence of several deaths for no apparent reason. Some of the risk factors involved in such outbreaks are also of interest, for example the development of pica due to calcium or phosphorus deficiency causing the animals to diversify their food intake, contamination and fermentation of fodder, and ingestion of infected fish or meat scraps. Pollution can thus endanger the environment adding to the overall risk.

Passive immunisation can protect cattle, horses, mink and exotic animals and is also available to humans whose work entails serious risks of exposure, however few animals in their natural surroundings ever develop antibodies to botulinum toxin.

Considerable variation between species in their response to these toxins is also known. Predators who eat carrion are least affected. In NW England in an outbreak of botulism resulting from consumption of a carton of hazelnut yoghurt shared among two children and a cat, the cat was not affected. In a zoo outbreak, clostridia-infected carcasses did not affect coatis or jaguars, and only caused mild ataxia and no respiratory paralysis in lions.[4] Cattle and sheep are less susceptible than horses[5] and a dose of toxin which may kill a horse may not necessarily kill a mouse.

Human Botulism

Human botulism is an infrequent, sporadic disease and its diagnosis requires a high level of suspicion.[6] It may result in some sudden unexpected infant deaths.[3] In 1988 the diagnosis of type B botulism in two sisters in Montreal led to the identification of a further 36 previously unrecognised victims of the same outbreak; all of whom had eaten garlic bread prepared from chopped garlic in soybean oil in a restaurant in Vancouver, British Columbia, six weeks earlier.[7] Misdiagnoses are frequent and include myasthenia gravis, psychiatric disorders and stroke;[8] the co-existence of patients with a similar range of diagnoses in the same hospital can add further to the confusion.[9] The diagnosis can even be more difficult to reach as the more severely affected patients have the shortest incubation period, and because of the severity of their illness do not exhibit the classical pattern of electro-diagnostic abnormalities.[10,11,12] An early diagnosis is more likely once clustering of cases has

occurred when a small group of people, usually a family unit or dining companions, can be identified. Several outbreaks have been associated with wakes, as at Ellezelles,[13] the guests having unwittingly drunk[14] or eaten[15] the same food that caused the initial death which was being celebrated.

Mortality from adult botulism has fallen precipitously from its previous almost invariably fatal outcome due to the skills available in Intensive Care Units in treating the signs associated with this exotoxaemia such as oropharyngeal paralyses, hypoventilation and respiratory paralysis; parenteral feeding is used for patients with gastric dilatation, intestinal ileus or prolonged swallowing difficulties.

Establishing the diagnosis by serology or identification of the toxin in stools or food can be difficult and may be delayed. Early treatment with the appropriate antitoxin, particularly with types A and E botulism, should not be delayed if there is enough clinical evidence at presentation to presume a diagnosis, and this can considerably reduce the mortality. Botulinum toxin rapidly fixes to nerve endings and once endocytosis occurs, its effects cannot be reversed. At a later stage in the disease the risk of an anaphylactic reaction to horse serum probably outweighs any therapeutic advantage of antitoxin administration.

Toxico-infective botulism is seen predominantly in children whose intestinal flora has not stabilised. Spores enter the large bowel when they germinate to produce toxin. In adults toxin production is commoner in patients with prolonged achlorhydria, gastrointestinal surgery and 'blind loops'. Some babies who die acutely and unexpectedly may be due to colonisation by these entero-toxigenic bacteria. (Table 3)

TABLE 3
Enterotoxigenic bacteria in Sudden Infant Deaths

	119 SIDs %	Controls %
Cl. perfringens	45.4	19.8
Cl. difficile	27.7	14.8
Cl. botulinum	5.0 (toxin in 1 child)	NIL
(Staph. aureus	27.3	NIL)

The high incidence of toxico-infective infant botulism in California is linked to spores found in 10 per cent of US honey specimens which introduce clostridia to the gut and alter its pH and flora, after weaning.[16] Taussig and Amon[17] are aiming to treat 100 children in California with human botulinum immune globulin (BIG), and preliminary results have already confirmed its value. Endemic botulism is known to occur among families who make home preserves at altitude, as in the Rocky mountains near Denver,[18] with the home production of soft cheeses, as in Italy,[19] or where raw or inadequately salted fish-products are eaten.

Wound botulism in the Western World has been associated with trauma, compound fractures and drug addiction.[20] Relatively few outbreaks of botulism were documented in the UK (Table 4) and of most interest are the Birmingham outbreak[20] and the larger, NW England and Wales outbreak.[9,21]

The incubation period is variable ranging from 6 hours to several days (average 12-36 hours) (Table 5). Where the presentation is oculo-bulbar there will be double vision, droopy eyelids, a nasal or slurred speech, and difficulty in swallowing, in which case the diagnosis is unequivocal but as the disease progresses rapidly, the severity of the ensuing respiratory difficulties often reflects that of the ocular symptoms.[22] Characteristically a descending paralysis ensues with loss of deep tendon

reflexes in proportion to the degree of weakness; the motor weakness is always bilateral but not necessarily symmetrical. Upper motor neurone signs and cranial involvement e.g. transient deafness, can occur but mental functions and sensory perception are usually well preserved. About 40 per cent of patients may develop more protean autonomic nervous system involvement; these may precede the muscle weakness or even dominate the clinical picture. A third of the patients develop cramps or abdominal distension e.g. a police officer was unable to put on his trousers due to the rapid expansion of his stomach. Others develop dry, red or watery eyes, blurred vision, difficulty in focusing, dizziness, headache and hypotension. The mouth may be dry, red, hot and injected, with coating of the tongue and fauces, and the saliva becomes thick, dry and stringy. Such patients were often treated initially for pharyngitis with antibiotics.

TABLE 4

Outbreaks of Botulism in the U.K

Year	No of cases	No of deaths	Food involved	*C.botulisum* type
1922	8	8	duck paste	A
1932	2	1	rabbit and pigeon broth	A
1934	1	0	jugged hare	?
1935	5	4	vegetarian nut brown	A
1935	2	1	minced meat pie	B
1947	5	1	macaroni cheese	?
1955	2	0	imported pickled fish	A
1978	4	2	imported canned salmon	E
1987	1	0	kosher airline meal	A
1989	27	1	hazelnut yoghurt	B

TABLE 5

Incubation Times

Food-borne Intoxication - Botulism.

Fast -	under 36 hours	Early - Orofacial involvement
Slow -	up to 12 days	Delayed - Respiratory involvement

Clinical lessons from botulism

Because of the rarity of this serious condition its clinical recognition does not come easily, the increasing sophistication of food preparation and packaging, and the criminal adulteration of foods in supermarkets and elsewhere must heighten our vigilance; only then can unnecessary deaths be avoided such as the one in the outbreak in NW England.

Some patients continue to experience symptoms for some time after the disease has been treated and which are apparently unrelated to the initial severity of the acute illness or the treatment received. Studies of these patients should theoretically have given us some insight into parallel conditions such as the chronic fatigue syndrome; however in these follow-up studies the findings were essentially of negative value.[23,24,25]

In conjunction with the Centre for Applied Microbiology and Research at Porton Down, newer bio-assay techniques are being developed to detect antibody formation in the survivors of an outbreak.

Botulinum toxin as treatment

The neurological effects of botulinum toxin has suggested its use in neuromuscular conditions associated with spasticity. Botulinum toxin A (Dysport, Botox) has been used in the treatment of focal dystonias, writer's cramp and spasticity (Table 6). Blepharospasm and hemifacial tics are helped by toxin injections of approximately 120 i.u. divided between three or four different sites, above and below the eyes; in blepharospasm a further smaller pretarsal injection into the upper lip is also required. Cervical dystonia (spasmodic torticollis) can be helped by injecting 400 - 500 i.u. of Dysport into two or more muscles, repeated every six weeks or six months thus weakening the turning movement and correcting the faulty neck posture. The treatment of other forms of dystonia and local spasticity are developing rapidly but to date are still experimental. A dystonia clinic has been based at the Royal Preston Hospital for six years with results favourably comparable with those presented from elsewhere.[26]

Single fibre EMGs of muscles at some distance from the site of injection suggested that some of the toxin diffuses to other tissues of the body;[27] more work is required to identify toxin localisation and to explain such side-effects of this therapy as difficulty in swallowing. In those patients who are particularly resistant to the use of botulium toxin, it is now possible to check for antibodies and to use other purified types of botulinum toxins which do not have cross-immunity.[28]

The therapeutic use of botulinum toxin has raised questions as to the timing of response to it and its mechanism of action; the same questions can also be asked of the sequence of events in botulism. Is the toxin response followed by a separate CNS adjustment? For example the early effect of squint correction is an overcorrection, followed some weeks afterwards by the eye adopting a new central position. With blepharospasm the muscles are weakened and the blink rate reduced; in treated spasmodic torticollis (cervical dystonia) changes are identified in the action of untreated muscles (Table 6).

TABLE 6

Therapeutic I.M. Injections of Toxin

	Early Effect	Late Adjustment
Squint Correction	overcorrection (not dependent on retention of sight)	central position possible
Blepharospasm	after 5-8 days	reduced blink rate
Hemifacialspasm	weakened muscles	
Spasmodic torticollis	7-12 days	changes in untreated muscles
Cervical Dystonia		

(Case Report[37] Systemic botulism ocurred in a patient 21 days after a repeat injection for cervical dystonia.)

Purification and action of the toxin

The toxin used therapeutically is produced by anaerobic culture and purified in crystalline form as a high molecular weight protein of 900,000 kDa. It is distributed as a stable, freeze-dried product composed of two molecules of neurotoxin (150 kDa) bound to a haemagglutinin or non-toxic complex.[29,30] The neurotoxin consists of two disulfide-linked polypeptide chains. The heavy chain (100 kDa) is involved in neuro-specific binding and cell penetration; the light chain is responsible for the blockade of neuro-transmitter release.

The first stage on administration is receptor-mediated endocytosis i.e. passage of the toxin from the extracellular space into the fluid part of the cytoplasm (the cytosol) with a latent period up to 5 hours where, by limited proteolysis, the heavy chain can be divided into two fragments of approximately equal size. (Fig.1) The C-terminal binds to membranes, whereas the N-terminal forms transient pores in the membrane lipids which enable the toxin to pass into the cytoplasm and cross the endosome membrane by a pH-dependent translocation mechanism.[31,32] The heavy chain

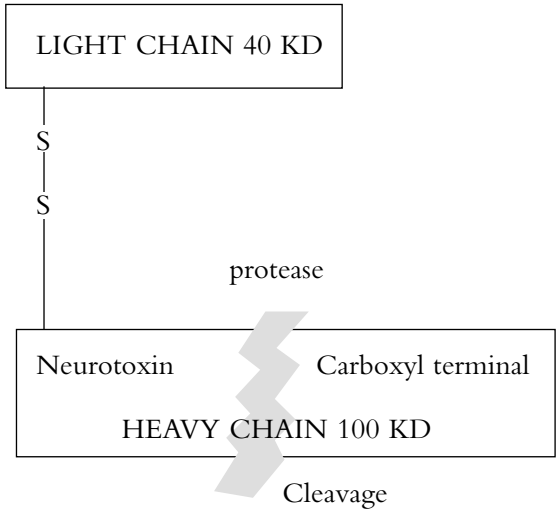

FIGURE 1
Entry of Clostridia toxin into cells by endocytosis

probably has three roles: the specific binding of the neurotoxin to motor neurones, the delivery of neurotoxin to the cytosol of the pre-synaptic nerve terminal, and as with tetanus, axonal retrograde transport and trans-synaptic delivery to the cytosol of spinal inhibitory neurones. (Fig.2) Limited retrograde transport occasionally leads to upper motor neurone manifestations and can also occur in botulism.

The light chains are zinc-endopeptidases which specifically target an insoluble core of three synaptic proteins, collectively termed SNARE proteins, developed at an early stage of evolution, and are responsible for vesicle docking and neurotransmitter release. Similar substrate proteins are found elsewhere in the body e.g. in chromatin and other non-neuronal cells, where they mediate a number of vesicle-dependent functions. Whether clostridial toxins also impair the secretory activities of these cells is unclear. The proteins, VAMP (synaptic vesicle protein synaptobrevin), and the synaptic membrane proteins syntaxin and SNAP 25

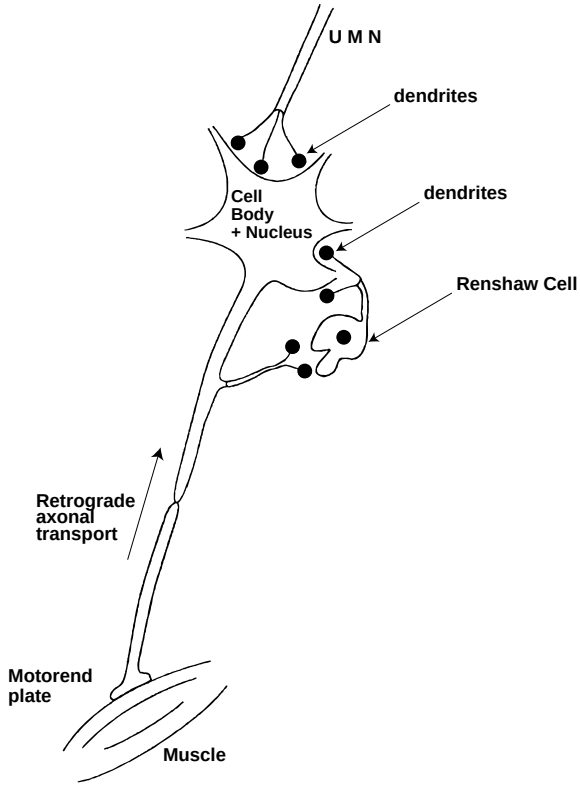

FIGURE 2
Tetanus. Toxin En-do-cytosis across synapse after retrograde transport to enter UMN and especially Renshaw cells.

(synaptosomal associated protein) - are intercoiled in alpha-helices and interact. Clostridial toxins attack the SNARE proteins, cleaving single or double peptide bonds.[34,35] The different botulinum toxin strains vary in their sites of action.

Vehicle for therapy

The excitement engendered by the detailed examination of the mechanism of action of the clostridial toxins and the physiological processes they reveal, does not stop there. A potential exists for the future manipulation of clostridial toxoids as vehicles for introducing substances into the peripheral nerves, navigation across synapses and retrograde transport within the central nervous system. Examples include the inhibition of free radicals, and the delivery of genetic material or enzymes, such as superoxidase dismutase proteins, to anterior horn cells. Experiments by an Anglo-American group working at Imperial College and the University of Maryland involving superoxide dismutase bound to tetanus toxin have already been presented at an international conference.[36]

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The snowcapped Cairngorm Mountains from Nethy Bridge, Highland
(Photograph by David H. A. Boyd)