Botulism in waterfowl

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Introduction

The literature on botulism in wild birds—mainly waterfowl—is extensive, and that related to the disease in man and other species vast. This review will attempt only to indicate and comment upon the more important aspects of the disease in waterfowl and gulls, paying some regard to the general context of botulism in man and other animal species.

Nature of botulism

Botulism is almost always a pure intoxication, caused by ingestion of the lethal neurotoxin produced by *Clostridium botulinum*, a bacterium whose natural habitat is soil and mud. If a suitable substance becomes contaminated with the bacterial spores, the organism will, given anaerobic conditions and a favourable temperature, multiply and produce lethal amounts of toxin. This is rapidly destroyed by boiling, but if the contaminated substance should be eaten without adequate heat treatment, some of the toxin may then be absorbed from the small intestine. Depending on the susceptibility of the species, lethal amounts of toxin may pass via the lymph and blood to receptor sites at the ends of the efferent autonomic and somatic nerves that act by the release of acetylcholine. Hence, botulism is a paralytic disease.

There are six types of *C. botulinum*, designated A-F, and a seventh, G, provisionally designated by Giménez & Ciccarelli (1970). The toxins of the various types can readily be distinguished by neutralization tests with specific antitoxins. All exert similar pharmacological effects, but their relative toxicities for various animal species often differ widely (Roberts 1959). Those of certain strains, for example types E and F (Duff *et al.* 1956; Iida 1968), are activated by trypsin. Strains of *C. botulinum* vary in respect of their somatic antigens, the heat resistance of their spores, and their capacity to produce proteolytic enzymes.

Types A, B, E and F are mainly, though not exclusively, of importance in relation to human disease; types C and D produce botulism in animals. Type D is an important cause of disease in cattle in South Africa, and type C is responsible for botulism in birds, both wild and domesticated, and in cattle and other animals, especially mink. Type E spores are found particularly in the mud of marine and other aquatic environments and human type E botulism is usually associated with eating fish in which toxin has been produced after death. The feeding of spoiled trash derived from marine fish has resulted in type E botulism in farmed trout (Huss & Eskildsen 1974). Carp, and to a lesser degree eels, were susceptible to type E toxin given by mouth, and carp were also slightly susceptible to type C toxin (Haagsma 1975).

The causal organism of botulism in waterfowl is almost invariably *C. botulinum* type C, though type E is thought to have been implicated on occasion (Fay 1966).

Some properties of *C. botulinum*

This anaerobic bacterium is a Gram-positive rod, measuring about 4–6 μm × 0·9 μm. It forms thick-walled resistant spores that are wider than the bacillus and usually subterminal. Vegetative cells are sluggishly motile and sensitive to oxygen. Colonies on agar plates are glistening and translucent with an indefinite reticular edge. Studies of types A, B (Bonventre & Kempe 1960) and C (Boroff 1955) suggest that break-down of ageing vegetative cells releases the toxin. For the production of large amounts of type C toxin Cardella *et al.* (1958) incubated cultures at 33°C for 5 days and Boroff & Reilly (1959) at 37°C for 9–10 days. Type C apparently does not grow below 10°C (Segner *et al.* 1971). Toxin can be converted to a harmless but immunogenic toxoid by formalin.

In the light of recent work, three aspects are worthy of special mention; they are (1) the relationship between the subtypes Cα and Cβ of *C. botulinum*, (2) the slight antigenic overlap between the toxins of types C and D (Dolman & Murakami 1961) and (3) the tendency (Smith 1955; McKee *et al.* 1958) for cultures of some types, including C, to lose their toxigenicity on repeated subculture. Type C was isolated by Bengston (1922), from the larvae of the blowfly *Lucilia caesar*, in the USA, and by Seddon (1922) in Australia. These isolates were representatives of two subtypes that came to be known as Cα and Cβ respectively (Gunnison & Meyer 1929), the work of Pfenninger (1924) having indicated that Cα antitoxin neutralized the toxin of both subtypes, whereas Cβ antitoxin neutralized Cβ but not Cα toxin. Botulism in waterfowl

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was usually attributable to the Cα type (Meyer 1956) although Cβ was occasionally thought to be involved (Pullar 1934; Reilly & Boroff 1967).

The composition of the toxins of types Cα, Cβ and D has been the subject of studies by Mason & Robinson (1935), Bulatova *et al.* (1967), Jansen (1971) and Eklund & Poysky (1972). Schantz & Sugiyama (1974) concluded that Cα toxin consists of a main component C1, together with components C2 and D; Cβ toxin contains the single component C2; D toxin contains a major component D, together with C1 and C2. Jansen (1971) found that the International Standard Type C Antitoxin contained antibodies against C1, C2 and D; D Antitoxin contained C1 and D antibodies. Jansen & Knoetze (1971) and Eklund & Poysky (1972) found that the toxicity of C2 in type Cβ was increased by trypsinization, and the latter authors described type C and D strains of *C. botulinum* that on subculture discontinued the production of C1 and D respectively but continued to produce C2. It is now known that the toxigenicity of types C and D depends upon a continuing association between specific bacteriophages and the clostridia, and that loss of such bacteriophage results in loss of toxin production (Inoue & Iida 1970, 1971; Eklund *et al.* 1971; Eklund *et al.* 1972). Eklund & Poysky (1974) demonstrated the interconversion of types C and D by manipulations involving specific bacteriophages, and this work was extended further (Eklund *et al.* 1974) to include interspecies conversions between *C. botulinum* (types C and D) and *Clostridium novyi (oedematiens)* type A.

Historical and geographical perspective

The disease in man became recognized by medical authorities in Europe, particularly Germany, towards the end of the 18th century. Because of its frequent association with the consumption of sausage, it became known as botulism (from the Latin *botulus*, meaning sausage). The true aetiology was discovered in Belgium in 1896 (van Ermengem 1897). Since that time, numerous outbreaks in humans have been reported in Europe, America, Japan and elsewhere. Although the disease in man is quite rare, it attracts considerable attention because of its dramatic and often fatal nature, and because the widespread distribution of the causal organism necessitates constant care in the food manufacturing industry.

From about 1910, reports appeared in the literature of mortality occurring annually—often on a massive scale—amongst waterfowl on certain lakes and mud flats in the western states of the USA. Kalmbach (1935) wrote 'Although no single outbreak has equaled (*sic*) in sheer intensity the memorable one that occurred in the marshes about Great Salt Lake in the summer of 1910, there have been years in which the mortality, even in single areas, has exceeded 100,000 birds.' The 'western duck sickness' was at first thought to be some form of chemical poisoning and toxic concentrations of alkaline salts were strongly suspected. It was not until some 20 years after the initial description that intensive and sustained research revealed the disease to be botulism (Giltner & Couch 1930; Hobmaier 1930; Kalmbach 1930; Kalmbach & Gunderson 1934) due to the organism now known as *C. botulinum* type Cα. Much of our present knowledge of botulism in waterfowl rests upon the very comprehensive American research (Kalmbach 1968) of the last half century.

Botulism in waterfowl is known also (Meyer 1956) in Canada, Argentina, Mexico, Uruguay, Australia, Germany (Lüthgen 1972), South Africa (Blaker 1967; Hay *et al.* 1973), Sweden (Nilèhn & Johannsen 1965), the Netherlands (Haagsma *et al.* 1972; Haagsma 1974), Britain (Roberts *et al.* 1972; G. R. Smith 1975) and Spain (Mountfort 1973; Anon. 1975). Recent serious outbreaks in Western Europe have attracted considerable attention but the disease has probably existed there for many years (G. R. Smith 1975).

Distribution of *C. botulinum* in nature

The pioneer soil surveys of Meyer and Dubovsky (1922a, b, c) and Dubovsky & Meyer (1922) have been followed by numerous others. The chemical and biological nature of soil or mud samples is inevitably diverse, and Meyer (1956) stressed that failure to demonstrate *C. botulinum* was no proof of the organism's absence. Nevertheless, surveys have proved of value in indicating the prevalence of various types of the organism and the strikingly regional distribution of some of them (Baird Parker 1969). Meyer (1956) suggested that the failure to demonstrate types C, D and E in early surveys may have been due to excessive preliminary heating of the samples. He considered that an exceptionally high incidence was exemplified by the figure of 30% obtained from Californian samples by Meyer & Dubovsky (1922a).

The geographical range of botulism in waterfowl demonstrates the wide distribution

of type C spores in mud. Type C was once thought (Quortrup & Sudheimer 1942; Roberts 1959) to be confined in general to areas where botulism had occurred. It is certainly true that in aquatic areas following an outbreak in waterfowl, type C can readily be demonstrated in the mud for years afterwards. However, the organism has also been identified in lakes or waterways that have no known history of the disease. In such areas Haagsma (1974) found that 30% of mud samples from a number of Dutch inland waters contained *C. botulinum* type B, C or E, but that E predominated. Smith & Moryson (1975) found that 72·5% of the lakes and waterways of London contained types B, C, D or E, but that B occurred between two and three times more frequently than C or E; D was found on one occasion only. In further unpublished studies, using constant techniques, their results varied from the uniform contamination of a large aquatic area with one or more of types B, C and E to the very low prevalence of type E in another area. In London, soil samples taken 200–300 yards from lakes positive for *C. botulinum* were usually negative. It is known (Segner *et al.* 1971) that type C organisms occur also in marine environments.

The occurrence of *C. botulinum* in soil and mud results in the not infrequent presence of the organism in the guts of birds, mammals and fish. Gunderson (1933) concluded that ducks occasionally carried types A, B and C in their livers; Dolman (1964) mentioned the isolation of types B and C from the livers of lemmings. Müller (1967) reported the presence of type Cβ in the livers of 4% of slaughtered cattle and 3% of pigs. These findings are given perspective by the knowledge that clostridial species other than *C. botulinum* sometimes occur in the internal organs and tissues of normal animals.

Epidemiology

It seems possible that the original contamination of a lake or waterway with any type of *C. botulinum* could occur through the intermediary of waterbirds that fly from one aquatic environment to another. Any bird leaving a lake on which an outbreak of avian botulism is in progress is likely to carry type C spores on its external surfaces and in its alimentary tract (Haagsma 1974). Such a bird may just have ingested a lethal dose of toxin and die after reaching a hitherto uncontaminated lake; if so, type C organisms are likely to invade the putrefying carcase from the gut and multiply profusely, thus seeding the new environment with massive numbers of organisms. Microbiological factors that might militate either for or against the establishment of various types of *C. botulinum* in a new environment have been described by Dack (1926), Quotrup & Sudheimer (1943), Grecz *et al.* (1959), Crisley & Helz (1961), Kautter *et al.* (1966), Wentz *et al.* (1967) and L. D. S. Smith (1975). Chemical and other factors may also play a part.

The factors that favour the precipitation of an outbreak of botulism in waterfowl include a prolonged spell of warm weather, enlarged areas of shallow stagnant water, alkalinity, an abundance of aquatic invertebrates, and oxygen depletion associated with large amounts of rotting vegetation or other organic matter (Kalmbach & Gunderson 1934; Coburn & Quortrup 1938; Quortrup & Holt 1941; Quortrup & Sudheimer 1942; Bell *et al.* 1955; McKee *et al.* 1958; Jensen & Allen 1960; Rosen 1971). Man-made influences such as thermal pollution of water by power stations (Haagsma *et al.* 1972) or irrigation procedures that involve the creation of shallow areas of stagnant water over stubble or other vegetation (McLean 1946) may also play a part. If type C is already present in the mud, the factors outlined above may stimulate sudden and rapid multiplication of the organism with the consequent production of lethal quantities of toxin. Whilst multiplication may occur in sludge and rotting vegetation, decaying invertebrates or waterfowl are very favourable as growth media and provide a suitable micro-environment in inhospitable surroundings (Bell *et al.* 1955). Multiplication of the organism in the carcases of birds may result in high concentrations of toxin that are then available to give rise to further deaths. In some instances the toxin may be transferred to other birds by the intermediary of dipterous fly larvae, which are not themselves susceptible but can accumulate considerable amounts by ingesting and crawling through decaying flesh. The eating of such larvae by waterfowl, pheasants and other birds may result in botulism (Lee *et al.* 1962; Rosen 1971; Smith *et al.* 1975). Occasional deaths from botulism may continue for many weeks after the peak mortality of an outbreak has subsided. Quortrup & Sudheimer (1942) were convinced that toxin was rapidly destroyed under natural conditions, but Haagsma (1974) found that it was undiminished after nine months. During an outbreak, the contamination of the environment with spores increases greatly, thus enhancing the possibility of a future outbreak.

Gulls are known to suffer from type C botulism and refuse dumps may well be the

source of toxin. Evidence regarding the possible occurrence of type E botulism in gulls and other birds on Lake Michigan is discussed by Herman (1964), Kaufmann & Fay (1964), Fay *et al.* (1965), Fay (1966), Kaufmann & Crecelius (1967) and Monheimer (1968).

Botulism in waterfowl has been described in South Africa, but reports from the tropics are rare. This may be because mortality remains unreported or undiagnosed, but mud and water temperatures in the tropics, unlike those in temperate zones, will remain comparatively high and fluctuate little.

Avian species affected

The very wide range of species affected is illustrated by numerous reports, including those of Kalmbach & Gunderson (1934), Fay *et al.* (1965), Blaker (1967) and Keymer *et al.* (1972). Certain carrion-eating species have been described as resistant to botulism (Kalmbach 1939; Holdeman 1970).

Aspects of pathogenesis

The manner in which the disease usually arises has already been outlined, but a number of points deserve special mention either because of their intrinsic interest or because they require further study.

The likelihood that a fatal dose of toxin will be ingested in any outbreak of botulism in waterfowl may depend upon the feeding habits of particular species, but in addition it is probable that different species vary in their susceptibility to type C toxin (Haagsma *et al.* 1972; Haagsma 1974).

Although it is generally accepted that botulism is most often a pure intoxication, 'wound botulism' of man (Merson & Dowell 1973) shows that multiplication of *C. botulinum* can occur in the tissues under certain circumstances. Minervin (1967) and others (see Petty 1965) believe that elaboration of toxin in the alimentary tract may play a part in certain cases of human botulism, and Roberts (1959) mentioned ruminants and birds in the same context. This is however a controversial point (Dolman 1964) and one on which direct evidence from animal experimentation is largely lacking. The administration of toxin-free type A and B spores by mouth to guinea-pigs was shown by Coleman & Meyer (1922) and Orr (1922) to produce botulism only when very large doses were used. Sugiyama *et al.* (1970) obtained no evidence of multiplication of *C. botulinum* type E in the intestinal tract of live fish. However, Bullen *et al.* (1953) and Bullen & Scarisbrick (1957) found that dietary factors influence profoundly the multiplication of *Clostridium perfringens (welchii)* in the alimentary tract of sheep and that *C. perfringens* is also not infrequently found in tissues remote from the gut. Several workers (Hobmaier 1932; Kalmbach & Gunderson 1934; Bell *et al.* 1955; Boroff & Reilly 1962; Dolman 1964; Haagsma 1974) have commented that the livers of some birds dying from botulism contain type C organisms. This matter deserves further study paying particular attention to (1) rigid aseptic precautions in technique, (2) the absolute exclusion of any possibility of invasion after death, (3) the purity of isolates, (4) the degree of invasion, and (5) the reports, already mentioned, that the livers of normal birds and animals may contain *C. botulinum* spores.

Cooch (1961, 1964) observed that the functioning of the salt gland possessed by ducks and geese that use the alkaline waters of the prairies is impaired by the action of type C toxin; a sublethal dose of toxin given by mouth to such birds became lethal if sodium chloride solution was administered at the same time.

Although *C. botulinum* types B, C and E are by no means uncommon in inland aquatic environments, the literature indicates that except for the possible occasional involvement of type E all outbreaks of botulism in waterfowl are due to type C. The reasons for this apparent paradox may well be complex, but in studies with gallinaceous birds Gross & Smith (1971) found that type C toxin was more readily absorbed through the gut wall than B or E toxin and that B toxin possessed little toxicity; type A and E toxins possessed the greatest toxicity, and D and F were non-toxic.

Experiments by Jensen & Gritman (1967) demonstrated a reinforcing effect between type C and E toxins when they were administered together to Mallard *Anas platyrhynchos*, and Jensen & Micuda (1970) found that administration of the pesticide malathion to Mallard decreased the effects of type C toxin.

Clinical signs

Depending on the amount of toxin ingested, and perhaps also on species susceptibility, the signs of botulism in waterfowl may progress from nothing more than slight difficulty in flying to complete flaccid paralysis of the limbs and neck, followed by acute respiratory distress and death. The clinical features may include eye disturbances such as rapid dilation and contraction of the pupil, paralysis of

the iris, immobilization of the nictitating membrane, disappearance of the eye closure reflex, and conjunctivitis. Paralysis of the neck muscles will result in drowning of birds that are on water. Ingestion of small doses of toxin may be followed by recovery after illness lasting up to a week.

Diagnosis

No characteristic lesion is seen at necropsy and diagnosis of botulism in waterfowl depends primarily on the demonstration and identification of type C toxin in the serum or tissues, or in the alimentary tract although this is less conclusive and usually less successful. The possibility of type E botulism should not be overlooked.

It must always be borne in mind that the alimentary tracts of birds dying from other causes may contain *C. botulinum*, especially if the immediate environment is heavily contaminated. If the organism is present in the gut, it may invade the carcase after death and produce toxin in the tissues. Thus, the demonstration of toxin in a carcase in which putrefaction has begun does not constitute a satisfactory diagnosis. Preferably several birds showing advanced symptoms consistent with a diagnosis of botulism should be killed to provide serum samples for examination. Some birds dying from botulism have insufficient toxin in their serum to permit a diagnosis. If the collection of blood presents difficulty, tissues, for example liver, may be homogenized in gelatine-phosphate buffer, though this is less satisfactory because of the dilution involved. After collection, the samples should be refrigerated without delay, or frozen if they cannot be examined within a day or so.

Intraperitoneal inoculation of mice with up to 1·0 ml of serum containing toxin will produce within four days—and usually much sooner—a characteristic 'wasp-waist', followed by progressive paralysis, respiratory distress and death. Very small amounts of toxin produce nothing more than a slight wasp-waist. If the initial test proves positive, the toxin in the remainder of the sample must be typed by neutralization tests with specific antitoxins in mice. To ensure complete specificity, the antitoxins should be diluted to the levels recommended by the supplier (Baird-Parker 1969). As little as 0·1 unit of type C antitoxin will effectively neutralize any toxin likely to be present in 1·0 ml of avian serum.

Where mortality is suspected retrospectively to have been due to botulism, it may be of interest to examine mud samples by methods such as those described by Smith & Moryson (1975). With experience, such methods may be expected always to give a positive result if several samples are examined from an area where the disease has occurred in recent years. While demonstration of type C in the mud does not confirm the original suspicion of botulism, failure to demonstrate it is good presumptive evidence that the mortality was due to some other cause.

Immunity

After recovery from botulism waterfowl remain susceptible to the disease. Rosen (1971) observed ducks that suffered three attacks within a single season. Haagsma (1974) found that Mallard failed to resist one minimal lethal dose of type C toxin after recovery from botulism, and even after multiple sublethal doses of type C toxin detectable levels of antitoxin were not produced. Shave (1970) reached similar conclusions from studies on pheasants. Lamanna (1970) failed to demonstrate antitoxin in the sera of normal persons and considered that the development of antitoxic immunity as a result of sublethal exposures to toxin was unlikely. Dolman *et al.* (1949) failed to immunise mice against *C. botulinum* toxin by administering formol-toxoid by mouth.

On the other hand, there is some evidence to suggest that an immune response can occur as a result of the ingestion of antigenic material. Thus, although Jansen, *et al.* (1970) failed to induce a primary immune response in rabbits by giving large quantities of type C toxin by mouth, in animals that were already basically immune a secondary type response resulted. Kaufmann & Crecelius (1967) reported experiments indicating that gulls may acquire immunity to type E toxin as a result of the repeated ingestion of sublethal doses over a period of time. Resistance to the toxins of *C. botulinum* has been observed in carrion-eating birds such as the Turkey Vulture *Cathartes aura* (Kalmbach 1939). Holdeman (1970) cited work by Pates and her colleagues showing that vulture serum contained a substance—probably antibody—that neutralized type C toxin, and that exposure of vultures to sublethal doses of type A toxin resulted in the production of antitoxin.

Vaccines consisting of toxoid with a reinforcer are used to protect laboratory workers against various types of *C. botulinum* toxin, and to protect cattle (Sterne & Wentzel 1950, 1952) and mink (Appleton & White 1959) against naturally occurring botulism. The im-

munization of pheasants and ducks against type C toxin has also been studied (Boroff & Reilly 1959, 1962; Rosen 1959; Fish *et al.* 1967) and vaccination is occasionally used to protect valuable collections of waterfowl. In many developed countries, stocks of antitoxin are held at central points for emergency use in the treatment of human botulism, and type C antitoxin is sometimes used in the treatment of naturally affected waterfowl (Kalmbach 1968; Rosen 1971). The international standards for the antitoxins type A-E have been discussed by Bowmer (1963).

Treatment

Individual rescue and treatment of birds can be an expensive exercise in terms of manpower. Sick birds that are removed to an uncontaminated environment and given toxin-free water and food will often recover. Treatment with type C antitoxin is often effective and may sometimes be economically feasible (Kalmbach 1968; Haagsma *et al.* 1972). The artificial administration of water by mouth is useful in diluting the toxin and flushing the intestinal tract (Rosen 1971). Birds in a state of collapse seldom respond to treatment.

Control

Control of botulism in waterfowl is often very difficult. It depends either on eliminating as many as possible of those factors favouring the precipitation of an outbreak, or upon keeping birds away from areas in which outbreaks are already in progress. Methods of control have been discussed by Kalmbach & Gunderson (1934), Hobmaier (1932), Quortrup & Holt (1941), Quortrup & Sudheimer (1942), McLean (1946), Sperry (1947), Rosen & Bischoff (1953) and Rosen (1971).

Various forms of water manipulation have been suggested: the permanent drainage of heavily contaminated marshes; the construction of embankments to eliminate shallow marginal water and thus prevent recession of the waterline in hot weather; maintenance of a constant water level by withdrawals from reservoirs; circulation of water by pumping. The early removal and burning of carcases during an outbreak is important and the removal of mats of drifting vegetation has also been considered helpful. It may be feasible to drain and clean small lakes such as those in public parks. Attempts have sometimes been made to keep birds away from dangerous areas by baiting elsewhere, or by putting them up with thunderflashes, flares or power boats and herding them by aircraft.

Surveys of the prevalence of *C. botulinum* in wetlands might with advantage be used in the planning stages of certain projects concerned with waterfowl. Surveys repeated at intervals would be of interest in relation to the management of refuges, as well as providing valuable epidemiological data. Thus, Smith & Moryson (unpublished) were unable to demonstrate type C in any of the eight refuges of the Wildfowl Trust in England and Scotland.

Public health and botulism in waterfowl

Despite the enormous annual mortality from botulism that occurs in waterfowl, the literature contains only three reports of type C botulism in man and none is entirely conclusive (Gilbert 1974). The reasons for this state of affairs are not well understood (Lamanna, 1970) and Gunnison & Meyer (1928) found type C toxin to be pathogenic for rhesus monkeys. In our present state of knowledge it would seem wise to regard *C. botulinum* type C as a potential human pathogen. It has been shown that types B and E are sometimes widespread in inland freshwater environments and it seems possible that they might increase under the influence of those factors that cause multiplication of type C. On general grounds, the unbridled proliferation of *C. botulinum* in any aquatic environment must be considered undesirable.

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Summary

A brief review is given of the bacteriology of the disease of botulism, its distribution, epidemiology, diagnosis, treatment and control in waterfowl.

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