A REVIEW OF RESEARCH ON TYPE C BOTULISM AMONG WATERBIRDS

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PREFACTORY NOTE

This paper will concern itself only with type C botulism as it affects wild waterbirds. Type C botulism in upland birds and poultry, and type E as it affects Great Lakes birds are sufficiently distinct in their ecological manifestations to be excluded from the present consideration.

Type C affects nearly every species of bird associated with western lakes and marshes but the bulk of the literature is concerned specifically with ducks, as they are the main game species killed.

A number of unpublished reports have been cited in this review. These progress reports reflect tentative opinions of the authors at the time but do not necessarily express their final or present conclusions.

TABLE OF CONTENTS

ABSTRACT

After investigating a variety of possible alternatives, including "alkali poisoning", Kalmbach and Gunderson (1934) determined that "duck sickness" was the result of ingestion of the toxin of *Clostridium botulinum* type C. Subsequent studies of ecological manifestations of botulism outbreaks and bacterial requirements led to an acceptance of a microenvironmental concept of toxin production which hypothesizes that all requisites for bacterial metabolism are available in particulate animal matter, notably invertebrate carcasses. Invertebrates associated with areas of outbreak are generally believed to provide the mode of transmission for the bacterium and its toxin to waterbirds. These conclusions are based largely on laboratory experimentation and have yet to be verified under field conditions.

Methods for detection, treatment and control of avian botulism are still largely post factum and temporary in nature. Determining effective, long-term controls provides a challenge for the future.

Sufficient commitments of time, funds and manpower are the keys to eventual understanding and control of avian botulism.

INTRODUCTION

In an emotional appeal to the duck hunter and waterfowl biologist alike, Madsen (1929) records his first-hand impression of an outbreak of a malady among waterbirds commonly referred to as "duck sickness."

"Can you imagine walking along a shoreline for twenty-five miles and passing an average of ten dead birds every time you step? Fifty birds for every rod! Imagine going to a small lake recently flooded and counting 4,800 teal, pintail and shovelers that you are certain have died within forty-eight hours! From 50 to 80 per cent of the ducks that were raised or found their way to Bear River Bay from July 5 to September 25, this year, died there. You can traverse hundreds of square miles of marsh and lake and never be out of sight of dead birds. You cannot build a blind except over the carcasses of dead birds that have crawled up in the tules during the early stages of the sickness and died."

The year was 1920, but this description of "duck sickness" compares closely with accounts of serious losses of waterbirds in the previous decade. Prior to 1910 losses of water-birds were obscured in the sheer numbers of birds present (Kalmbach, 1968) and the inaccessibility of outbreak areas (Madsen, 1929).

Estimates of losses in the 1910 and 1920 outbreaks were described as being in the millions; intervening outbreaks as hundreds of thousands. In later years, outbreaks appear to have been either smaller or better estimated. Some of the severe losses recorded after 1920 include: 100,000 at Lake Malheur, Oregon, in 1925; 100,000 to 300,000 in the Great Salt Lake Basin, Utah, in 1929 (Kalmbach, 1935); 65,000 to 150,000 at Tule and Lower Klamath Lakes, California in 1948 (McLeod, 1950); 50,000 to 60,000 at Whitewater Lake, Manitoba in 1949 (Bossenmaier, et al. 1954); and 140,000 in northern California in 1969 (Hunter, et al. 1970). However, the untallied but disastrous losses of 1910 attracted national attention and stimulated the Bureau of Biological Survey (later the Fish and Wildlife Service) to initiate investigations into the cause and control of the malady. These investigations, from 1914 to the present, have gradually uncovered and unraveled the highly complex ecological puzzle that is "duck sickness."

THE INITIAL SEARCH 1914-1927

At first everyone had a theory about the causative factor. Duck sickness was variously attributed to lack of grit, eating mud, cockleburs or sour grain, intoxication from fermented grain (Mays, 1940), sulfur fumes, sewage, lead poisoning, bacterial infection, parasites, toxic algae (Kalmbach and Gunderson, 1934), and dietary deficiency (Lewis, 1927). However, laboratory tests with these agents failed, in each case, to produce the full set of signs characteristic of duck sickness.

These agents dismissed, the Bureau's chief investigator, Alexander Wetmore, turned to observations of sick birds and the ecological factors associated with outbreaks of the malady. Wetmore (1918) concluded that reflooding of mud flats by wind or rain action was a major contributing factor. Reflooding brought about solution of large quantities of salts encrusted on the mud. Ducks rushing into such areas to feed on the insects washed up by the water, were thought to ingest sufficient quantities of calcium and magnesium salts to cause intoxication and death. This theory of "alkali poisoning" was held widely until the sickness was discovered in areas of lower salinity than the Great Salt Lake area studied by Wetmore (Monro, 1927; and Kalmbach, 1968). In 1927 investigations were reopened.

Studies by Sperry (1927) and Kalmbach (1930) pointed to a chemical toxin as the cause of the sickness. Giltner and Couch (1930) and Hobmaier (1930) verified the presence of *Clostridium botulinum* type C toxin in the tissues of sick birds and identified the bacterial toxin as the causative factor. Kalmbach (1932) demonstrated the presence of toxin in the mud, water, plants and dead insect larvae found in infected areas as well as in the blood streams of dying birds.

The majority of subsequent studies have assumed that duck sickness is a case of botulinus intoxication. The notable exception was the Twomeys' assertion that selenium buildup in the bird's liver was responsible for the sickness. Later studies of selenium involvement by Quortrup and Sudheimer (1942a) and Lakin, Quortrup and Hotchkiss (1944) produced contrary results and this theory was shelved. (In the case of selenium and earlier theories, controversies between principle researchers were worked out by letter. Contents of these letters, on file at the Bear River Research Station, in Brigham City, Utah, are often more illuminating than the subsequent published reports).

Cl. botulinum is an anaerobic bacterium whose various subspecific forms are designated as types. Death of waterbirds attributed to avian botulism are largely the result of ingestion of the toxin of types C_{alpha} and $_{beta}$, E and possibly F. Subtypes C_{alpha} and $_{beta}$ are not determined routinely (Jensen, personal communication), although C_{alpha} appears to be the typical form encountered in North America. Type C_{beta} has been reported once in New Jersey (Reilly and Boroff, 1967), but is more common in Australia and Africa. Type E is found in the Great Lakes region.

In North America the range of major outbreaks covers much of the area west of the Mississippi River from Saskatchewan and Alberta south into Mexico. East of the Mississippi incidence of the disease is more isolated: Minnesota and Manitoba (Bossenmaier, 1959), Manitoba (Manuwal, 1967), Ohio (Crisley, Dowell and Angelotti, 1968), Massachusetts (Austin and Austin, 1931), New Jersey (Reilly and Boroff, 1967), Virginia (Kalmbach and Gunderson, 1934), Georgia (Richardson, Brewer, and Holdeman, 1965), and Louisiana (Newsom, personal communication). How-ever, outbreaks in the East are probably more widespread and common than reported in the literature (C. Gruener, personal communication). Worldwide, avian botulism has been reported from Uraguay (Wetmore, 1918), Australia (Pullar, 1934; Rose, 1934; and Grubb, 1964), South Africa (Blaker, 1967), Sweden (Niléhn and Johannsen, 1965), Denmark (Muller, 1967) and Germany (Steiniger, 1960-61).

For many years toxin formation was assumed to occur as an intermediary anabolic process in which the toxin was not yet firmly bound to the cell. For this reason younger cells contained maximum amounts of toxin. Fragile young cells were believed to be more sensitive to changes in the osmolarity of their environment and lost their toxin more readily than older, well-formed cells (Dozier, 1924a). However, Boroff (1955) believes that *Cl. botulinum* autolyzes rapidly that "the toxin is at the maximum when most of the organisms have autolyzed." Jensen (personal communication) suggests that rupture of the cell wall may increase toxicity by making the toxin more readily available for absorption. But, "the toxin is much more stable when the cells are intact."

Type C toxin is absorbed from the bird's small intestine and acts specifically on the peripheral nervous system, preventing transmission of impulses from nerve to muscle by interfering with the output of acetylcholine at the neuromuscular junction (Smith and Holdeman, 1968). The result is an increasing paralysis of the muscles involved. In waterfowl paralysis usually affects, in turn, the muscles of the legs, wings, neck and pulmonary apparatus, terminating in complete prostration and death. The function of the salt gland also may be affected and result in osmotic imbalance of the blood plasma; a condition which may speed death (Cooch, 1964). Paralysis of the nictitating membrane is a characteristic sign of avian botulism, produced by no other disease to which birds are susceptible. This paralysis was demonstrated in 73 per cent of 743 birds examined (Sum. Proc. Bot. Conf., 1942). A detailed account of the symptomology and pathology of botulinus intoxication can be found in Wetmore (1915 and 1918) and Kalmbach and Gunderson (1934).

In an exhaustive work establishing the toxin of *Cl. botulinum* type C as the causative factor in duck sickness, Kalmbach and Gunderson (1934) ventured into a consideration of the ecological factors affecting bacterial growth and toxin production, and initiated the search for a suitable medium. Researchers of the next two decades followed these lines of investigation, gradually narrowing the number of factors to those exhibiting the most direct bearing on the disease. Several factors, present simultaneously, were believed to be responsible for the initiation, perpetuation and cessation of botulism outbreaks. These factors included: presence of the causative organism, temperature, alkalinity, anaerobic conditions, substrate or medium, climatic influences on the medium and concentrations and susceptibility of waterbirds in outbreak areas.

The Organism

Cl. botulinum type C is an anaerobic bacterium found in vegetative or spore form depending in part upon existing environmental conditions. In vegetative form the bacterium is a saprophyte, requiring dead organic material upon which to grow and produce toxin. Under adverse conditions the organism may persist as spores, viable from season to season or for years at a time. Type C spores are widespread in North America and may be considered ubiquitous in traditional outbreak areas. However, dispersal of the organism from sites of outbreak to cleaner areas may be facilitated by transport of spores by insects, birds, and wind (Kalmbach and Gunderson, 1934; and Gunderson, 1933).

Temperature

Optimum temperature for growth of the bacterium generally is recorded as 37°C although Hunter, et al. (1970) prescribe 25°C. The following table from Quortrup and Sudheimer (1942a and b) may explain this discrepancy.

Temperature	Toxin developed	Maximum growth developed	Maximum growth persisted
37°C	2 days	3 days	6 days
30°C	2 days	4 days	13 days
25°C	3 days	8 days	16 days
20°C	6 days	20 days	19 days

At the time Quortrup and Sudheimer (1942b) felt that these data indicated rapid growth at an ideal temperature (35-37°C) but slower growth and persistant toxicity over a longer period at lower temperatures. However, Jensen (personal communication) points out that "there is no dependable relationship between abundance of growth and toxicity in type C cultures."

Temperature correlations from field observations have shown the malady appearing after the hottest period of the summer and continuing into the cooler days preceding fall. Quortrup and Sudheimer (1942a) refer to experiments indicating that lower temperatures following summer highs may inactivate competing organisms, making conditions favorable for the more temperature tolerant botulinus bacterium. In Utah sudden cessations of outbreaks are often, but not always, associated with temperature drops in late September and October (Kalmbach and Gunderson, 1934).

Toxin production need not be restricted to a particular temperature range. While toxin is produced by young bacterial cells during periods of maximum growth at ideal temperatures, it may be released at other times by autolysis of mature cells (Dozier, 1942a; and Boroff, 1955). Dozier has reported laboratory studies in which toxin was produced or released during refrigeration. Quortrup and Sudheimer (1942a) also report toxin production under experimental cold situations but doubt that this would occur under natural conditions.

Alkalinity

The general conformity of botulism outbreaks to areas of alkaline waters (Kalmbach and Gunderson, 1934) and Bengston's (1924) assertion that a pH of 7.0 - 8.5 favored production of potent toxin has led many researchers to believe that optimum growth occurs about a pH of 8. Quortrup and Sudheimer (1942a) give the range for growth as pH 6-10.5: maximum growth being attained in 15 days at pH 6, 7 days at pH 8 and 18 days at pH 10.5. In a later study pH 8 is described as optimal for toxin production (Quortrup and Holt, 1941). Hunter, et al. (1970) support these figures by stating that the organism will grow only in neutral or moderately alkaline

substrates. However, laboratory tests by Mc-Kee, Bell and Hoyer (1958) indicate that the optimum pH for all stages of metabolism occurs within a rather narrow range on the acid side of neutrality (Table 1), with the greatest toxin production at pH 5.7.

McKee's findings have support from earlier studies. Quortrup and Sudheimer (1942b) expressed the belief that the botulin might be more powerful if produced under slightly "adverse" or acid conditions. Dozier (1924b) felt that slight acidity might be a stimulus for the initiation of metabolic activity, notably spore germination.

Looking at the problem of optimal alkalinity Dozier (1924a) points out that the medium does not affect the potency of the toxin except as it hinders or promotes growth of the bacterium. Quortrup and Sudheimer (1924a) admit that the pH of the ambient medium may not be critical if the bacterium is encapsulated within particulate animal matter, such as an invertebrate carcass, whose pH is closer to the bacterial optimum.

The significance of alkalinity in botulism outbreaks is not fully understood. Kalmbach and Gunderson (1934) point out that there is no combination of ions characteristic of alkaline waters collected in various outbreak areas. Jensen (personal communication) suggests that alkalinity may not be essential except as it favors the plant and animal life utilized as a medium by the botulinus bacterium.

Alkaline solutions will destroy free, preformed toxin: a fact that may account for the inability of investigators to demonstrate toxin in water samples, except those taken in close proximity to active production sites such as a decaying duck carcass (Quortrup and Sudheimer, 1942a; and Kalmbach and Gunderson, 1934). Gunderson (cited in Kalmbach and Gunderson, 1934) also reports that alkali "may sterilize the soil to the disadvantage of competing organisms, leaving the field to *Clostridium botulinum.*"

The preponderance of dead birds in less saline or alkaline waters of outbreak areas has been attributed variously to: destruction of toxin by high alkalinity (Kalmbach and Gunderson, 1934); retardation or stopping of biological functions related to the development of *Cl. botulinum* (Quortrup and Sudheimer, 1942b); or indication of an increased number of sources of toxin in less saline areas, a

larger or more varied animal fauna, or a preference of the birds for fresher water (Jensen, personal communication). Williams (1943) also noted that "most sickness areas were located at the mouths of inlets where the water would be freshest and . . . that sickness followed influxes of fresh water."

Finally, Cooch (1964) suggests that the total salinity of the infected area may be a critical factor as the salt load taken on during feeding may determine a bird's tolerance to sublethal doses of botulinus toxin (see salt gland research).

Anaerobic Conditions

Cl. botulinum is one of the strictest known anaerobes. The complete absence of oxygen required for growth prompted researchers to explore means by which such an environment could be induced and perpetuated under field conditions. Aerobic bacteria were known to produce anaerobic conditions in the process of breaking down vegetable matter. Thus attention was turned to decaying vegetation in hopes of demonstrating this medium to be a site of toxin production. Quortrup and Holt (1941) demonstrated toxic, oxygen-deficient water in association with decaying plant materials. This study provided the foundation for formulation of the "sludge-bed" theory of botulinus toxin production which states that the decay of vegetation brought about by flooding, in the case of upland plants, or evaporation, for aquatics, and the action of aerobic bacteria in consuming the available free oxygen provided a favorable environment for the growth and toxin elaboration of *Cl. botulinum.* However, an outbreak of botulism under these circumstances depended upon three other factors: a favorable pH, a proper medium and the ability of *Cl. botulinum* to produce toxin at a greater rate than it could be destroyed by other putrefactive bacteria, notably *Escherichia coli.* In the same set of laboratory experiments Quortrup and Holt also determined that common marsh plants provided a satisfactory medium for growth of the bacterium.

Coburn (1940b) refuted Quortrup's laboratory experiments as requiring special conditions not found in the marsh. He reported from his own experimentation that in no instance could *Cl. botulinum* compete with other putrefactive organisms in a vegetative medium. However, *Cl. botulinum* could com-

Table 1. Metabolism of *Clostridium botulinum* type C in relation to pH* from McKee, Bell and Hoyer, 1958.

Activity	pH Range	Optimum**	Other Studies
Germination	5.9 - 8.0	6.2 - 7.3	6.0 - 7.2 Dozier, 1924b 7.0 Wynne, et al., 1954
Growth in vegetative form	Not determined but probably broader than germination	6.6 Dozier, 1924b	6.0 - 8.2 Dozier, 1924b 7.0 - 8.5 Bengston, 1924
Inhibition of growth	5.0 - 5.2		
Greatest bacterial population	6.1 - 6.3	6.3	"compare favorably" with Dozier, 1924b
Lowest bacterial population	5.7 - 5.8		
Spore formation	5.8 - 6.3	5.8 - 6.1	6.2 Leifson, 1931
Toxin production	5.7 - 7.0	5.7 - 6.2	7.0 - 7.5 Bengston, 1924 7.2 Steine & Wentzel, 1950 8.0 Quortrup & Holt, 1941

*Data derived under conditions of controlled pH.

**Optimum level judged by amount of toxin produced.

pete and thrive on the animal life killed by aerobic bacteria as they depleted the oxygen supply. Quortrup and Sudheimer (1943b) resolved part of this conflict in a study demonstrating a symbiotic relationship between *Pseudomonas aeruginosa* and *Cl. botulinum.* In a vegetative medium *P. aeruginosa,* which does not destroy the botulin, can outgrow *E. coli* and thus permit *Cl. botulinum* to grow and produce toxin. Coburn, however, has been supported in his objection to the suitability of a vegetative medium by Hunter, et al. (1970) who concede that while there may be a symbiotic relationship between *Cl. botulinum* and certain aerobic bacteria, an animal source of protein appears necessary for bacterial growth and toxin elaboration.

Finally, in relation to the "sludge-bed" theory, McKee, Bell and Hoyer (1958) point out that since *Cl. botulinum* type C is favored by a pH at or below neutrality, "alkaline slurries of decomposing plant and animal life probably would not support growth."

The Medium

The search for the medium in which *Cl. botulinum* type C grew and produced toxin was paramount to determining how waterbirds ingested the botulin. Kalmbach and Gunderson (1934) listed three possible media: dead vegetative or animal matter and highly organic muds.

Vegetation:

There are few references to vegetation as a medium. Kalmbach and Gunderson (1934) reported toxin production in submerged grains. These results were questioned by Quortrup and Holt (1941) in their article on vegetative media. Coburn (1940b) in turn refuted Quortrup's studies and the probability that vegetation might be a major medium has been dismissed. However, marsh vegetation may play a substantial secondary role as a source of nutrition, support and cover for the animal life ultimately providing the medium for botulinus growth.

Animal Matter:

Decaying animal carcasses and particulate debris are accepted as sites of most botulinus growth and toxin production. *Cl. botuinum* was first isolated from the larvae of a green fly *Lucilia caesar* by Ida Bengston in 1923. Subsequently, toxin has been demonstrated in crustaceans, snails, earthworms, a wide variety of insects—both dead and alive, and decaying carcasses of birds, mammals and fish. (Robinson, 1929; Austin and Austin, 1931; Hobmaier, 1932; Kalmbach and Gunderson, 1934; Gunderson, 1935; and Bell, Sciple and Hubert, 1955). Toxin production in a given organism probably depends upon its protein content. "Very potent" toxin production results from use of all kinds of birds and mammals as well as chironomid (midge) larvae, sarcophagid fly larvae and free living nematods, as media (Sum. Proc. Bot. Conf., 1942). However, Jensen suggests (personal communication) that "highly toxic cultures can be produced in media containing no intact protein."

Mud:

The etiological role of mud is largely that of an incubator. The proceedings of the 1942 Botulism Conference noted an increase in average mud temperatures over averages for air and water during outbreaks. Coburn and Quortrup (1938) reported that "the greatest bacterial action takes place during the time required for 2 or 3 inches of water of the underlying silt and slime to evaporate to dryness"; time in which many annelids, crustaceans and insect larvae are trapped and killed, providing an excellent medium for the botulinus organism.

The mud underlying outbreak areas also have been implicated as a repository for preformed toxin. Giltner and Couch first cultured type C toxin from mud samples in 1930. Kalmbach and Gunderson (1934) reported a retention of toxin by mud samples 17 days after a dead duck had been removed from the area. Laboratory tests have demonstrated that type C toxin retained in the bacterial cell may remain potent for four weeks in mud of low organic content without subsequent addition of nutrient materials. This suggested that toxin was not rapidly inactivated and might build up gradually under natural conditions (Jensen, Allen and Capelle, 1963). Destruction of toxin might occur within a week in mud of high organic content. Thus, the composition of the mud might determine, in part, the carry-over of toxin responsible for winter or spring outbreaks (Jensen, Capelle and Gritman, 1964). Virulent forms of botulitic organisms might also survive many years as spores on dry mud flats surrounding areas of outbreak, to be released to produce highly toxic situations when these flats were reflooded by changes in water level (Jensen, 1969).

Climatic Influences

Climatic factors may play a major role in the production and release of toxin. On flat, western lakes a steady wind can push water over dry or drying mud flats (Coburn and Quortrup, 1938). As the wind ceases and the water recedes or evaporates, pools may be left on the flats. These pools, like fringe areas, trap and kill crustaceans and insects providing medium for the bacteria and an attractive lure for feeding waterbirds. Drying does not destroy botulinus toxin (Coburn and Quortrup, 1938). Thus flooded edges and pools not only allow further development of the botulin but also may become dangerous immediately if preformed toxin is stored in the area (Sum. Proc. Bot. Conf., 1942).

Pool formation and flooding may also be the result of rainfall. In California precipitation is a critical factor as heavy snowfall in the mountains may result in severe flooding of shallow lakes in the drainages below (Parrish and Hunter, 1969). These drainages contain areas of heavy waterfowl use and have been to the present day, the scene of major botulism outbreaks (Parrish and Hunter, 1969; and Hunter, 1970).

Sunlight has a minor but direct effect on stored toxins. Over time, direct exposure to sunlight causes the dissociation of that toxin stored in the top few millimeters of soil surrounding outbreak areas (Coburn and Quortrup, 1938).

Concentrations and Susceptibility of Waterbirds

Avian botulism could not occur without the presence of susceptible species of birds. The botulism season generally extends from mid-July to late-September in Utah and points north, and into November and December in California. During this period large numbers of ducks use the western lakes and marshes for molting and raising broods, and, with shorebirds, as migration stops and wintering areas. Nearly every avian species frequenting outbreak areas has been recorded succumbing to the disease. An annotated list of North American species affected can be found in Kalmbach and Gunderson (1934). Additional species include mottled duck, common egret, Louisiana heron, purple grackle, common crow (Newsom, personal communication) and others listed in Sciple (1953), Reilly and Boroff (1967) and Kinsey (1954). It should be noted that toxin has not been demonstrated in most of these species (Kalmbach and Gunderson, 1935; and Jensen, personal communication).

The susceptibility of avian species to botulism remains largely a topic of speculation. Susceptibility has been attributed to: food preferences and feeding habits; abundance by age, sex or species in affected areas; state of health; stage of molt; salt gland development; origin of migration; and immunity to the botulin. Differential feeding habits bring puddling ducks and mud-probing shorebirds into contact with toxin-laden materials and may account for the larger losses of these birds to the disease (Kalmbach and Gunderson, 1934; and Sciple, 1953).

Sciple (1953) reviewed the literature to 1952 and reported little species, sex or age differentiation in mortality. Mortality has been related to the proportion of the population present. However, high mortality-to-population ratios have been listed for cinnamon teal, widgeon (Williams, 1943) and shoveler (Kalmbach and Gunderson, 1934; and Williams, 1943). This mortality may be the result of the feeding habits of these species. Existing differentiation in mortality by sex appears to be related to the seasonal use of infected areas by the sexes and their respective periods of molt (Hammond, 1950).

In contrast to Sciple's reported lack of age differentiation, Kalmbach and Gunderson (1934) noted a marked incidence in mortality in juvenile redheads which they suggest might reflect differential feeding habits of the age classes. Louis (1941) reported an instance in Minnesota where 98 per cent of botulitic birds of several species examined were young of the year. In another instance a botulism outbreak during the hatching season claimed 95 per cent of the hatch (Coburn and Quortrup, 1938). This point appears to need further clarification.

Susceptibility may be based on the rate of ingestion or absorption of toxin. Internal parasites cause lesions in the stomach walls which may facilitate more rapid absorption of toxin (Hobmaier, 1932). Ingested alkali salts can increase the permeability of membranes (Gunderson, 1932) and may account for the intense irritation of the intestinal walls of botulitic birds observed by Wetmore (1915). Juveniles and adults in the molt may consume more animal matter; a case amply demonstrated for the former and generally accepted but not conclusively demonstrated for adults

(Krull, 1970; and Glover, 1970). Consumption of animal matter during these periods of growth may increase the bird's chances of ingesting toxin. Jensen (1969) suggests that other anaerobic bacteria may enhance absorption and thus increase the effect of type C toxin in strength and timing. Type E toxin may also enhance the rate of absorption of type C and result in higher mortality rates than would occur for type C alone (Jensen and Gritman, 1966; and Jensen, personal communication).

Some observers feel migrant birds are more susceptible to botulism than residents (Kalmbach and Gunderson, 1934; and Sciple, 1953). Cooch (1964) suggests that birds from areas of low alkalinity or acid conditions may have less developed salt glands than residents and may succumb more readily to the osmotic irregularities associated with botulinal impairment of the salt gland. Moreover, migrating birds may pick up large loads of toxin as a result of heavy feeding activities after long flights (Sciple, 1953).

Apparent and demonstrated immunity to type C toxin has been recorded for certain species frequenting outbreak areas. Blaker (1967) believes the greater flamingo of South Africa may have an inborn or acquired immunity to the toxin. Kalmbach (1939) found the turkey vulture highly resistant to oral and injected doses of type C toxin. Further studies with the vulture have shown that this resistance cannot be attributed to antibody action or failure of the botulin to escape the circulation and may be due to a lack of susceptibility of the motor end plates to the toxin (Cohen, et al., 1969).

The Microenvironment Concept

Much of the early research assumed that the botulinus organism was exposed to the environment at large. As described in the preceding pages, conditions for growth and toxin production were rigid and demanded that each of the following requirements be present simultaneously: complete anaerobic conditions; suitable temperature, alkalinity, and medium (variable from one author to another); little or no competition from other putrefactive organisms; and shallow water (up to four inches) on feather edges or in pools, which trap invertebrates and lure waterbirds. It was difficult to demonstrate all of these requirements simultaneously to the ex-

tent that they would have to occur if large scale outbreaks over extensive areas were to be explained. Likewise, occasional spring and winter outbreaks could not be explained under these strict requirements. A few researchers of this period had begun to suspect that the bacterium must have some way of circumventing this need for an all-favorable environment. Such a belief was expressed in a comment filed in a 1940 progress report:

"Some of the most potent toxin produced for experimental use has been prepared in the complete absence of any artificial means of anaerobiosis pH control or use of sterile media. This work, a simple repetition of confirmatory work of others, shows that under natural conditions in the field many of the supposed exact cultural requirements of the botulinus organism may be the result of its own productivity rather than a naturally favorable pre-existing environment (Coburn, 1940a)."

Perhaps the organism can produce its own environment. Where? As avian botulism is a form of food poisoning, a likely source would be the bird's food or water. Toxin can seldom be demonstrated in water samples, and plant material has not been shown to be a good medium. A demonstrated site of toxin production, animal matter (Bengston, 1923; Kalmbach and Gunderson, 1934; and Gunderson, 1935) is left as the leading candidate. Few of the avian species contracting botulism are carrion feeders and would not pick up toxin directly from decaying carcasses. However, emerging maggots become acceptable fare for ducks and shorebirds as they are blown or fall off these carcasses.

In the early 1950's attention turned to maggots and other common marsh invertebrates as possible sources of toxin production. The suitability of invertebrate carcasses as "circumscribed microenvironments" was demonstrated in the laboratory. This demonstration (Bell, Sciple and Hubert, 1955) led to the formulation of a "microenvironment concept" which hypothesizes:

"1. that *C. botulinum* type C germinates, reproduces, and synthesizes its toxin in small discrete particulate substances, possibly invertebrate carcasses;

2. that the particulate substances are in

no wise dependent upon the ambient medium for nurture of the bacteria, but contain all of the requisites within them; and

3. that the toxin is probably in the bacteria which reside in the particulate materials, rather than in the form of soluble, freely diffused toxin."

THE MODE OF TRANSMISSION 1955-1970

Invertebrates

The microenvironment concept coupled with a history of association between invertebrates and botulism stimulated an intensive program to determine the relationship of invertebrates and toxin production. In 1960, Jensen and Allen summarized a five year study in which they determined: 1. that carcasses of tendipedid (midge) larvae, oligochaetes, corixids and others would support toxin production when incubated in marsh mud; 2. that food habit studies of normal ducks revealed aquatic invertebrate remains ranging from trace amounts to 80 per cent of food in 44 per cent of the birds studied; and 3. that outbreaks of botulism when plotted against populations of aquatic invertebrates showed a tendency to occur following a decline or crash in the invertebrate populations —the severity of the outbreak correlating roughly with the extent of the decline in invertebrate numbers. The die-off was thought to provide a mass of medium for the growth and toxin production of *Cl. botulinum* (Jensen, 1969).

The study was continued another four years with the following results: 1. corixids, notonectids and other aquatic invertebrates served as well as midge (tendipedid) larvae for experimental toxin production; 2. studies of the relationship of invertebrate population fluctuations and botulism outbreaks were inconclusive, perhaps because the mobility of free-swimming species (corixids, notonectids, etc.) made detection of population fluctuations difficult; and 3. invertebrate carcasses probably contributed to the total production of botulinus toxin (Jensen, 1969).

Invertebrate carcasses not only provide the medium for toxin production experimentally, but may provide storage of the botulin. Toxic blowfly carcasses stored in open containers at 8 and 37°C respectively retained much of their toxin for four years: the 37°C sample becoming non-lethal to mice in the fifth year (Jensen, et al., 1968); but the 8°C sample retaining half of its first year's toxi-

city after six years of storage (Jensen, personal communication). Live blowflies are also a demonstrated source of toxin and have been implicated in die-offs of captive, experimental birds (Jensen, Allen and McDonald, 1957; and Jensen, Capelle and Gritman, 1964). Live invertebrates usually do not contain toxin at levels capable of inducing intoxication unless they are carrion feeders, such as blowflies, and probably only a small percentage of these are that toxic if one considers the low percentage of birds affected at any one time (Hunter, et al., 1970). This percentage of toxin-containing invertebrates might be small indeed if a sublethal dose of the botulin can be fatal in combination with a toxin-induced salt gland malfunction (Cooch, 1964).

A California research team has carried the hypothesis of invertebrate involvement even further by stating their belief that "terrestrial and aquatic invertebrates, flies and maggots are largely, if not solely, responsible for serving as vehicles for the ingestion of toxin by waterfowl" (Hunter, et al., 1970). This role of invertebrates is emphasized in the following summary (Hunter, et al., 1970) of the factors involved in botulism outbreaks.

"Initiation. Because of:

1. Terrestrial invertebrates drowned as land floods during warm weather.

2. Aquatic invertebrates or their larvae that die because of unfavorable environmental conditions resulting from lowered water level or inability of organisms to tolerate water conditions as it becomes stagnant.

3. Death of a bird, mammal or fish that results in the production of maggots.

Perpetuation. Because of:

1. Continued die-off of invertebrates and toxin formation within the carcasses.

2. Maggot cycle — maggots cause death of birds which produce more maggots.

3. Persistance of toxin as the result of favorable ecological conditions.

Subsidence. Because of:

1. Breakdown of toxin.

2. Toxin sources biologically developed so that they are no longer present.

3. Toxin sources no longer available because habitat changed.

4. Changed activities of waterfowl."

Each of these factors is discussed at length by Hunter, et al. (1970).

Fish

Historically, fish have been implicated as a medium from which the botulinus toxin can be transmitted to waterbirds through fly larvae (Jensen, Capelle and Gritman, 1965; and Austin and Austin, 1931). Jensen, et al. (1968) have found, moreover, that live carp are susceptible to type C toxin. Hunter, et al. (1970) also tested fresh water fish, including carp, but found them "highly" resistant to type C toxin. In Jensen's study, experimental birds, allowed to feed on small intoxicated fish, showed mild to moderate signs after eating two fish and died after consuming as many as four (Bur. Sport Fish. and Wildl., 1968). Reilly and Boroff (1967) record a number of authors who have found fish in the diets of waterfowl.

Salt Gland Research

A recent and frequently overlooked study re-emphasizes the role of alkali in avian botulism. Cooch (1964) verified the presence of functional salt glands in several species of waterbirds frequenting the Great Plains. Through experimentation he found that the toxin liberated by *Cl. botulinum* appeared to block the facial nerve and interfered with the function of the salt gland. Cooch believes that death from botulism can result either from a lethal dose of toxin or a sublethal dose in conjunction with an impairment of the salt gland. Such impairment can result in an increase in osmolarity of the blood plasma to intolerable levels. Cooch's theory has been reinforced by Blaker's (1967) study of botulitic waterbirds in South Africa.

Winter Botulism

Botulism outbreaks occurring outside the regular July to October season are unpredictable, seldom recorded, and remain little understood. Wetmore (1918) noted deaths of ducks in early spring as the water rose. Winter outbreaks were mentioned briefly in the proceedings of the 1942 Botulism Conference, where drifting ice was believed to stir up underlying strata of mud and release toxin.

In the last twenty years two severe winter outbreaks have been reported from California. In both cases, minimum temperatures of 42°F or less were believed to be too low to support bacterial growth. Carryover of existing toxigenic bacterial cells (Parrish and Hunter, 1969) and release of preformed toxin from the previous summer's outbreak (Rosen and Cowan, 1953) were suggested as possible toxin sources. Rosen and Cowan (1953) believed that rainstorms agitated bottom muds, and washed toxin from unrecovered duck carcasses into impoundments used by waterbirds. Diving ducks—ruddys, scaup and ringneck—were most frequently affected while water levels were high, but dead teal and shovelers became more prevalent as levels were lowered. Bottom muds were thus implicated as a toxin source. Rosen and Cowan further hypothesized that there was sufficient toxin in solution from agitation of bottom muds and drainage from carcasses to reach levels toxic to birds. This theory was not supported by toxicity tests of water samples and might be questioned in view of the instability of botulinus toxin in alkaline solutions (Quartrup and Sudheimer, 1942a). Parrish and Hunter (1969) did not indulge in speculation but noted that even in below freezing weather "the area remained extremely toxic, killing birds overnight."

MANAGEMENT PRACTICES

Methods for detection, treatment and control of avian botulism have evolved along with an understanding of the malady. The various methods described in the literature often reflect the knowledge of the disease in that day.

Detection

The earliest and most reliable method of detecting active, toxin-producing bacteria was

the presence of intoxicated birds. However, this detection after-the-fact did not fill the need for methods of predicting areas of outbreak for research or control purposes. Wetmore (1918) associated outbreaks with reflooding of mud flats and subsequent solution of existing alkali deposits. He was able to predict outbreaks in certain areas based on the force and direction of wind storms.

Knowledge that the causative organism was an anaerobic, saprophytic bacterium prompted attempts to detect situations favorable to its growth and toxin production. In the days of the sludge-bed theory Quortrup and Holt (1941) were able to demonstrate toxin in decaying vegetation mats and suggested agitation or removal of these mats as control measures. The advent of the micro-environment concept (Bell, Sciple and Hubert, 1955) and Sperry's (1946) demonstration of the importance of water levels (see control section) directed attention to invertebrates, particularly along feather edges of water bodies. Here again, toxin could be demonstrated in invertebrate carcasses incubated in marsh muds (Jensen and Allen, 1960a). The findings of Jensen and Allen also indicated that sites of toxin production were scattered throughout the marsh rather than being evenly distributed. The problem of detecting such scattered sites has been aptly described by Jensen (1969) who compares the Bear River Refuge to a 30,000 acre "test tube" containing dozens of nutrient materials heavily seeded with Cl. botulinum.

Researchers turned to chemical tests in an attempt to find a rapid means of detecting toxin. The standard demonstration of toxin in sick birds utilizes a mouse protection test (Quortrup and Sudheimer, 1943a) which can take up to six days to complete. Experimentation was carried out in hopes of developing a botulinal hemagglutination test which could detect toxin in the field in two hours (Jensen, Allen and Sperry, 1956; and Jensen, Allen and McDonald, 1958b). Problems were encountered in correlating hemagglutination results with toxicity and further research has been postponed.

In 1967 Hunter and Rosen described a fluorescent antibody technique which could detect Cl. botulinum type C and its toxin in particulate animal matter, vegetation and dead botulitic waterfowl in less than one hour —after a laboratory had been set up for this experimentation. Problems encountered with this technique included storage of the conjugate, nonspecific background fluorescence and possible subjective bias introduced by the investigator interpreting the slides (Hunter, personal communication). In his current research, Hunter prefers the non-biased mouse protection test. This still leaves detection of toxin production sites largely to chance, and prediction of outbreaks to the interpretation of prevailing ecological conditions by seasoned field workers.

Treatment

Attempts to salvage the victims of botulism outbreaks began in the earliest years of research. Wetmore (1918) held birds in pens with access to "fresh" water and reported a 77 per cent recovery rate for all birds collected. Slight to moderately affected birds showed a 90 per cent recovery rate. Kalmbach and Gunderson (1934) felt that holding birds in pens designed to provide shade from the midday sun and protection from predation was the most efficient means of salvaging sick birds. They considered the use of antitoxin but were unable to determine its effectiveness. Quortrup and Sudheimer (1942b) reported results of tests with antitoxin and three other methods at the 1942 Botulism Conference:

Treatment	Per cent Recovery
Antitoxin	85-89%
Potassium permanganate	76%
Water (dosing)	60-76%
No treatment (Penned in clean water)	74%

These data, however, do not indicate the severity of intoxication involved against which the results can be measured.

A 1955 report of the Cooperative Botulism Studies series (#30) records the following treatment agents and procedures from the literature: shade, potassium permanganate, calcium gluconate, sulfa drugs, albumen, removal of leeches from the mouth, magnesium sulfate, saline, glucose, strychnine, alcohol, sodium bicarbonate, milk, castor oil, formaldehyde, fresh drinking water (available or forced) and antitoxin. Evaluation of these procedures was not possible due to a frequent lack of controls and inadequate numbers of birds used.

Hospitalization studies were begun in 1953 to determine in more detail the effectiveness of treatment with type C antitoxin. Incoming birds were classified by degree of

intoxication for the purposes of data analysis (Coop. Bot. Studies #22, 1954):

"*Mild:* any duck which is definitely affected, but attempts to escape when placed on the floor.

Moderate: a duck just able to observe its surroundings, but unable to make effective movements to escape the observer.

Severe: a prostrate duck, unable to hold up its head."

Severity of intoxication may be determined also from the bird's temperature. Healthy ducks exhibit a temperature of 106.6°F, dropping to 101.8°F in affected birds and below 100°F in severely intoxicated pintails (Oglesby and Glover, 1954).

Use of antitoxin showed little effect (2.5 per cent more recoveries) on mildly intoxicated birds. Moderately and severely affected birds demonstrated a more marked response, 13.5 and 32.5 per cent more recoveries respectively (Jensen, Allen and Sperry, 1956). Another study indicated little difference (0.4 and 6.0 per cent) in the recovery rate of moderately and severely intoxicated ducks given 0.5 ml., 1.0 ml. and 2.0 ml. doses of monovalent type C antitoxin (50 units per ml.) (Jensen, Allen and McDonald, 1958b). Detailed results of these studies are presently being prepared for publication by Bear River Research Station personnel.

"Since the interval between ingestion of toxin and death becomes shorter as the dose of toxin increases . . . early treatment of sick birds (at time of capture, if possible) would influence recovery rates to a greater degree than does increasing the dose of antitoxin above the 0.5 ml. level" (Jensen, Allen and McDonald, 1958a). Smith (1955) substantiated this statement in a comparative study of four treatment methods: hospital innoculation, fresh water, field innoculation and a no treatment or control group. He found the highest recovery rate in the field innoculated group.

The use of antitoxin, while effective in increasing the recovery rates of severely affected birds or protecting endangered species, is costly when the time required for collection and injection is considered. It is a temporary control method and an effective public relations device but "contributes nothing to the elimination of the cause" (Jensen, 1969).

Control

Methods for control or prevention of botulism outbreaks closely follow the leading theories of the day. In the days of the "alkali theory" Wetmore (1918) recommended increasing summer water in the streams and lakes to dilute the alkali, or drainage of affected areas to make them unattractive to waterbirds. Having verified the causative organism as *Cl. botulinum,* Kalmbach and Gunderson (1934) sought to eliminate those conditions favoring growth of the bacterium. Proposed methods included flooding mud flats and stagnant areas to one or more feet of water, circulating and changing water to keep temperatures down, and drying mud flats where water control was not possible. Kalmbach and Gunderson were aware that carcasses provided an excellent medium for bacterial growth but were not able to judge the value of removing them. They suggested, rather, that an attempt be made to keep healthy birds away from the sick and dead. Such an operation had been instituted in 1921 by the Utah Game Department, which sent details of men to frighten waterfowl out of the infected areas (Madsen, 1929).

Coburn was aware of the detoxifying effects of aerobic bacteria in situations of decaying vegetable matter and he and Quortrup (1939) suggested the use of vegetation as a management tool. In addition to using vegetation for the production of "beneficial decomposition products" to control dispersion of toxin, they recommended that plantings be used as windbreaks, repellents to discourage use of dangerous areas and food patches as lures to safe areas. Other recommendations included the manipulation of aerating wind currents by use of planted windbreaks and artificial islands, the latter also serving as lures to move birds to deeper water. The collection, burning and burying of all animal carcasses was strongly stressed as was the removal of algal mats which might provide a medium and anaerobic conditions favorable to the botulinus organism.

The advent of the "sludge-bed" theory resulted in control methods facilitating the removal of rotting vegetation (tractor-drawn rakes) or the agitation of stagnant water with appropriate devices (i.e. water pumps).

Quortrup and Sudheimer (1942a) spoke of these methods as temporary controls and voiced the need for a permanent cure. They

felt that preventative measures lay in proper water manipulation. (A consideration of the role of water levels in the etiology of botulism outbreaks carries with it a certain degree of confusion stemming largely from different interpretations of the term "low water". A less confusing substitute, "shallow water" more aptly describes the situation under consideration. Shallow water, as used here, is less than one foot deep and generally less than four inches.) An early conception of the role of water levels is exemplified in the following statement from the proceedings of the 1942 Botulism Conference: "The duck sickness period generally coincided with the lowest water levels and is abruptly stopped when water becomes available, filling the units [at Bear River Refuge]" (Quortrup and Sudheimer, 1942b). One of the control measures emanating from the conference called for water levels to be maintained at a constant level (Sum. Proc. Bot. Conf., 1942). The danger of flooding feather edges was recognized, however.

In 1943 Jensen noted that the disease was associated with reflooding of drying mud flats after the level had been stabilized for several days, rather than a constant rise and fall action.

A water manipulation experiment on the Bear River Refuge (Williams, 1943) revealed three possible sickness areas: 1. shallow deltas and barren or vegetated muck over or through which water spills intermittently; 2. stationary or near stationary fringe areas on an exposed mucky bottom; and 3. areas of very shallow water over a mucky bottom having no mud exposure and subsequent reflooding. The results of three years of studies by Williams and others were reported in Sperry (1947). It was concluded "that duck sickness is directly related to the production of toxin in shallow alkaline impoundments with an exposed, nearly flat, lake bottom as a shoreline". The suggestion was made that a thorough examination be made of the effect of water levels on botulism outbreaks. Sperry undertook such an examination and concluded that while a constantly retreating shoreline was not dangerous, "a shoreline stabilized on an old lake bottom will produce . . . an outbreak as soon as wind or inflow pushes water back onto the flats and makes the previously stabilized feather edge a feeding ground for waterfowl." As a result of this study Sperry recommended: 1. the delivery of water into impoundments by deep channels that extend

far into the basin; 2. a steady downward reduction of levels until the impoundment is dry or until sufficient water is available to place the shoreline against steep banks or on nonlake bottom soils not previously submerged that year; and 3. a rate of reduction rapid enough to prevent reflooding by normal winds or the stabilization of shorelines.

Water manipulation, as described by Sperry, is not always possible or desirable. Such procedures presuppose a sufficient source of water for reflooding when needed, or an outlet for drainage—conditions not always present in botulism areas. Temporary destruction of waterfowl habitat during drawdown must be considered also (Jensen and Williams, 1964).

Jensen has concluded that botulism outbreaks are associated with high or flood water—not low (personal communication). This conclusion is based on observations of heavier outbreaks in years of abundant water (Jensen, et al., 1968) and a tentative understanding of the toxicity of *Cl. botulinum* strains. Laboratory tests have shown that *Cl. botulinum* type C loses its ability to produce toxin after several subcultures (Jensen and Allen, 1960b), and the speculation is that this may be due to mutation to atoxigenic strains (Jensen, et al., 1968). Jensen (1961) has expressed the belief that "the capacity of the bacterium to produce toxin is decreased by repeated subculture in invertebrates as it is in artificial media." If this is the case, bacteria in constantly flooded areas eventually will lose their ability to produce sufficient toxin to sicken or kill a duck. But, "birds affected with botulism obviously picked up a toxigenic strain" (Jensen, 1969). The occurrence of a severe outbreak may thus depend upon the reintroduction of highly toxigenic strains of *Cl. botulinum*. Iida and Inoue (1970) feel they have demonstrated that non-toxigenic strains of type C bacteria can be converted to toxigenic forms by phages contained in the lysates of toxigenic parent strains. They did not report whether the reverse was true also. A very high conversion rate, 96 percent, was reported for the st Tox+ phage. In addition they reported the conversion of a non-toxic type D strain to a toxic form of type C using a lysate from type C. The ramifications of this observation were not speculated upon but might be considered in areas such as South Africa where type D is more common than type C. Reintroduction of toxigenic strains also may be accomplished through

introduction of duck carcasses which reseed an area with toxin-producing organisms (Jensen, 1969) or by reflooding of new ground or areas dry for many years, which may harbor spores of virulent bacterial forms. Outbreaks occurring on the Bear River Refuge in 1965 may have resulted from reflooding of areas dry since the severe outbreaks of the early 1930's and the subsequent germination and reproduction of bacteria capable of producing highly potent toxin (Jensen, et al., 1968). Support for this hypothetical explanation may be found in Parrish and Hunter (1969) who report that during a severe outbreak in California in 1967-68 the primary problem area was a piece of land which had not been flooded previously for ten years.

In summary, it would appear that shallow water associated with feather edges may affect botulism outbreaks by concentrating and killing the aquatic invertebrates and plants which provide a culture medium for the bacterium and a lure for feeding waterbirds. On the other hand, high or flood water may provide conditions favorable to further toxin production on drying mud flats and release of preformed toxin present and allow germination of spores of highly toxigenic strains of the botulinus organism.

The "microenvironment concept" (Bell, Sciple and Hubert, 1955) and subsequent studies by Jensen and Allen (1960a) implicated aquatic or marsh invertebrates as possible transmitters of toxin to waterbirds and led to a study—Haddock, 1962—to determine ways of limiting invertebrate numbers. A complete kill of invertebrates was undesirable as it would reduce waterfowl food below desired limits and might favor a botulism outbreak by increasing the mass of decomposing carcasses. Haddock aimed for a kill of 30 to 60 percent of the invertebrates present before their seasonal peak. Malathion and DDT were tested on midge larvae populations with success. (At the time both of these chemicals were considered safe for wildlife—a case that has been repeatedly disproved for DDT.) Subsequent tests with malathion revealed that this chemical may inhibit the growth of botulinus bacteria in naturally-occurring media (Jensen and Allen, 1961; and Jensen, Allen and Capelle, 1962). The problem associated with direct use of pesticides on the botulinus bacterium are manifold. "There is no chemical agent presently known that will kill it [Cl. botulinum] and yet spare the other organisms that are important in the ecology of

the marsh" (Jensen, 1969). Other problems include placement of the poison so that it can reach the bacterium encased in an invertebrate carcass buried in the mud (Jensen, 1969) and the ramifications of using pesticides over large areas in a day when the public is conscious of the detrimental effects of such chemical agents.

Accounts of management practices being used today can be found in Parrish and Hunter (1969) and Hunter (1970). Limited outbreak prediction is possible by keeping track of ecological factors favoring bacterial growth, particularly fluctuations in water levels. If there is reason to expect a severe outbreak in a given area, the California plan (Hunter, 1970) calls for a preventative management program consisting of:

"1. Maintaining current maps of flooded areas.

2. Surveillance of flooded areas.

3. Contacting land owners and soliciting their cooperation in:
   a. Burning barley, straw or other vegetative materials prior to flooding.
   b. Maintaining ponded areas at a depth of 18-24" and avoiding shallow water and feather edges.
   c. Moving water from cell to cell, by pumps if necessary, whenever such action would stop an outbreak.
   d. Assistance in herding waterfowl away from toxic areas."

Once losses have been detected, outbreak management practices include:

"1. Pick up dead birds.

2. Keep the birds away from toxic areas by:
   a. Herding
   b. Frighting devices
   c. Enticing birds away from toxic areas by putting feed on safe areas.

3. Pick up and treatment of sick birds."

These procedures were conducted in California in 1969 with a loss of 140,000 waterfowl but an estimated saving of 500,000 others (Hunter, 1970).

The practice of assuming that decreases below predicted estimates result from one's own efforts is not unusual, but there is some doubt whether it is a sound procedure for

botulism outbreaks, except under controlled conditions. The history of most botulism areas indicates "that initially heavy losses gradually decline over a period of years whether anything is done or not" (Jensen, personal communication). Examples of areas where this has occurred include the Great Salt Lake Basin and the Lower Souris National Wildlife Refuge. Although not proven, Jensen (personal communication) believes that "the trend toward lower mortality within a marsh can be explained by a general decline in the toxigenicity of the bacterium."

Type C toxoid has been used and appears to be an effective temporary tool to protect captive exhibition and research flocks (Schwartz and Smart, 1963). However, Hunter, et al. (1970) report that such slight increases in resistance result from injections of commercial toxoid that they do not justify the expense and trouble. Immunization studies have also been carried out at the Bear River Research Station, but not on a large enough scale to evaluate the practice (Jensen, personal communication).

Finally, a recent study by Jensen and Micuda (1970) has indicated the possible effect of pesticides, notably malathion, on the susceptibility of waterfowl to type C toxin. Malathion, administered with type C toxin, appeared to delay the development of paralysis and decrease the death rate from botulism. Malathion is itself toxic to waterfowl and its use as a control measure is not anticipated, but studies with this chemical may aid in determining a safer and more effective control for botulism. Finding a safe, adequate means of permanently controlling avian botulism may prove quite difficult but poses a major challenge for future research.

DISCUSSION

"Man has altered greatly the extent and nature of conditions affecting duck sickness. Through reclamation, drainage, and deforestation there has resulted an increase in the rapidity and extent of seasonal runoff of rainfall. Lakes and marsh lands that formerly maintained more or less constant water levels now fluctuate, and during late summer become stagnant areas of shallow water, mud flats, and decaying material. Alkaline sinks have been formed by drainage from irrigated sections. About many of these, grain crops have lured waterfowl in great numbers and have tended thereby to concentrate the birds in or near infected areas that formerly did not offer them much inducement to rest and feed. There is little doubt that there has been a material increase in the factors and general conditions conducive to duck sickness since civilized man has entered the picture; and, unless measures are taken to prevent a further extension of conditions favorable to the organism, the ravages of this disease are likely to make a still greater annual drain on our diminishing waterfowl" (Kalmbach and Gunderson, 1934).

The solution to the problem of botulism is one of priorities. Having created, or at least contributed to, conditions favorable to botulism outbreaks we are now faced with determining whether we can or should provide the time, funding and manpower to bring about the control or destruction of the causative organism. Were this type of botulism a direct threat to the health of our own species there would be no question of provisions for research. But—what is the value of a duck and a shorebird? Can we afford the 1969 loss of 140,000 ducks to botulism in California? I do not belittle the efforts of researchers in the field today or in the past; they are acutely aware of the efforts that need to be made but are forced to work with the limited resources and manpower available. A plan is presently being initiated to increase research by providing a cooperative effort on the part of several groups and agencies in working out the innumerable, unanswered questions. Before any widespread, cooperative efforts are undertaken, however, there is an urgent need for a detailed compilation of what is definitely known about avian botulism and a deliniation of what aspects of the disease still require study. In a survey of the literature one is struck by unresolved conflicts over such basic information as: the stage of growth at which the bacterium releases toxin; what constitutes a suitable medium; the significance of environmental factors, particularly water levels; the varying susceptibility of avian species; and others.

Theoretical explanations are available for all phases of this ecological phenomenon, but they are based largely on laboratory tests and responses of captive, commercially produced birds. The reasons for this are apparent, but

there is an equally obvious need to verify laboratory findings under field conditions. A safe, dependable and effective control method might eliminate the need for this basic knowledge, but such a control is not forth-coming. Experimentation in the field is indeed difficult considering the minuteness of the causative organism, the vastness of the area over which it may occur and the complex interplay of environmental factors involved. It has been suggested that the development of an ecological modeling system into which field observations could be fed, might aid in determining the combinations of factors contributing to botulism outbreaks (R. Curnow, personal communication).

ACKNOWLEDGEMENTS

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