SHORT COMMUNICATIONS

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Brain Acetylcholinesterase Activity in Botulism-Intoxicated Mallards

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ABSTRACT: Brain acetylcholinesterase (AChE) activity in captive-reared mallards (*Anas platyrhynchos*) that died of botulism was compared with euthanized controls. AChE levels for both groups were within the range reported for normal mallards, and there was no significant difference in mean AChE activity between birds that ingested botulism toxin and died and those that did not.

Key words: Type C avian botulism, cholinesterase inhibition, organophosphorus and carbamate pesticides, mallards, *Anas platyrhynchos*.

More than one million waterfowl have died of avian botulism in the midwest, north central and western regions of the United States since 1980. In many of these regions, the use of various agricultural chemicals, including pesticides, has recently increased (Grue et al., 1988, 1989; Forsyth, 1989). The majority of these pesticides are organophosphorus (OP) or carbamate (CB) compounds; several of these agricultural chemicals, such as ethyl parathion and carbofuran, are highly toxic to both waterfowl and invertebrates. The potential for accidental pesticide introduction into waterfowl habitat is increasing because of widespread aerial application and proximity of wetlands to agricultural lands (Grue et al., 1988). In addition, waterfowl may be exposed to agricultural pesticides while feeding in treated fields, especially temporarily or seasonally flooded areas.

During a recent investigation of avian botulism mortality in North Dakota, birds were found with depressed brain acetylcholinesterase (AChE) activity indicative of OP or CB poisoning (National Wildlife Health Research Center (NWHRC), unpubl. data). This finding generated three hypotheses regarding the possible interactions of agricultural chemicals and avian botulism. First, concurrent botulism and pesticide poisonings may be coincidental, but unrelated. Second, direct application of agricultural pesticides to wetlands or runoff into wetlands may increase the likelihood of a botulism outbreak by creating a suitable substrate (dead invertebrates and dead birds) for botulism toxin production (Bell et al., 1955; Duncan and Jensen, 1976). Finally, botulism toxin may depress brain AChE, thus confounding the diagnosis of OP and CB poisoning. We evaluated the third hypothesis by determining brain AChE activity for mallards (*Anas platyrhynchos*) that ingested botulism toxin and died and for control birds that did not ingest toxin at a detectable level.

Captive-reared mallards, used as sentinels for botulism research at the Sacramento National Wildlife Refuge (Willows, California 95988, USA) were held in a 4-ac wetland enclosure during a botulism outbreak in late summer 1988. The enclosure was monitored daily to account for all sentinels. Any sick birds found were bled prior to euthanasia, and heart blood was collected from dead birds. Blood samples were centrifuged and the sera were tested for type C botulism toxin with mouse neutralization tests (Quortrup, 1946). Mallards with no signs of botulism that were surviving at the end of the outbreak were euthanized and used as controls. All carcasses were frozen. The brain was collected from the carcass of each bird, 15 birds with

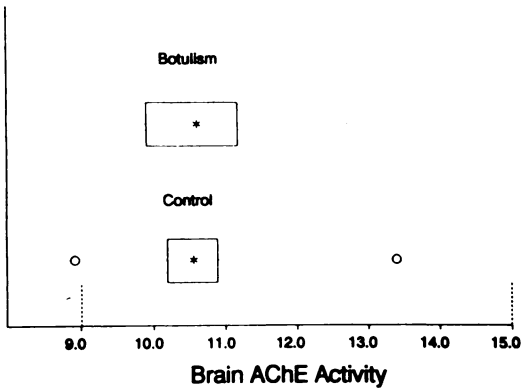

FIGURE 1. Brain AChE activity (μmoles of acetylthiocholine iodide hydrolyzed/min/g of tissue wet weight) for mallards that ingested botulism toxin and controls. Mean (*), quartiles (box), and values (O) beyond the 5 and 95 percentiles (·····). Range for mallards not exposed to OP or CB (Hill, 1988) is indicated by vertical dashed lines.

confirmed botulism and 29 controls. All birds had been held in the same wetland for at least 2 wk prior to death or euthanasia. AChE assays were conducted using the method described by Ellman et al. (1961), as modified by Hill and Fleming (1982). Reactions were measured with an Hitachi spectrophotometer (Model 100-20; Hitachi, Ltd., Tokyo, Japan).

AChE activity for botulism-intoxicated and control birds (Fig. 1) were within the range reported for mallards not exposed to OP or CB (9.0 to 15.0 μmoles of acetylthiocholine iodide hydrolyzed per minute per gram tissue, wet weight, Hill, 1988). The brain AChE activity of one control bird (8.9) was slightly below the minimum normal level of 9.0; however, the degree of depression was not substantial enough to suspect OP or CB exposure. Analysis of variance (SAS Institute, Inc., 1987) indicated no significant difference in mean AChE activity between botulism-intoxicated and control birds ($P = 0.75$) or between adults and juveniles ($P = 0.15$). However, a difference was found between males and females ($P = 0.06$). Mean AChE activity for males (10.4) was slightly lower than for females (11.0), but this difference was not clinically important in evaluating pesticide exposure.

Our findings suggest that ingestion of type C botulism toxin by waterfowl does not alter brain AChE activity. Extensive pharmacological research has shown that botulism neurotoxin primarily interferes with the release of acetylcholine from peripheral nerves (Simpson, 1981). Although there is one report of *in vitro* inhibition of AChE by type A botulism toxin (Marshall and Quinn, 1967), other investigators have found no interaction between botulism toxin and AChE with similar *in vitro* assays (Simpson and Morimoto, 1969; Sumyk and Yocum, 1968). In addition, inoculation of rats with lethal doses of type A botulism toxin had no effect on brain AChE activity (Simpson and Morimoto, 1969).

Diagnostic tests for type C botulism poisoning and for OP or CB poisoning in waterfowl are not confounded by one another. Botulism intoxication and pesticide exposure may occur simultaneously, and either toxicant can act as the proximate cause of mortality. Because both toxicants result in motor and neurological dysfunction, clinical signs in sick birds can be easily confused. Appropriate diagnostic tests for botulism intoxication and brain AChE inhibition are needed to determine the actual cause of death.

Hypotheses regarding the potential interaction of avian botulism and agricultural chemicals remain untested. Previous studies suggest that invertebrate die-offs can initiate a botulism outbreak by providing a suitable medium for growth of *Clostridium botulinum* and toxin production (Jensen and Allen, 1960). Waterfowl that subsequently ingest these toxic invertebrates can succumb to botulism intoxication. The application of a pesticide to a marsh could exacerbate this process. Botulism toxin also can be produced in carcasses of waterfowl that have died from direct exposure to agricultural chemicals. When maggots consume these toxin-laden carcasses and waterfowl subsequently ingest the toxic maggots, a carcass-maggot cycle of botulism is perpetuated. The extent to which agricultural chemicals pre-

cipitate botulism outbreaks via these mechanisms is unknown. Recent increases in the frequency and severity of botulism outbreaks in the Central Flyway (NWHRC, unpubl. data) may be coincidental to the increased use of agricultural chemicals. Even so, the potential interaction between the application of agricultural chemicals to wetlands and the risk of botulism outbreaks warrants further investigation.

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