

IMMUNOSUPPRESSIVE EFFECTS OF LEAD

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Immunosuppressive effects of lead were reported as early as 1966, when it was noted that lead increased the sensitivity of rats to bacterial endotoxins (Selye et al. 1966). Since then a substantial body of literature has demonstrated adverse effects of lead on the immune system in a variety of laboratory animals, but very little has been done in this area with avian species. Such immunosuppressive effects could be of significance to waterfowl populations, considering the potential for lead ingestion by waterfowl and subsequent exposure of these birds to disease agents.

For reviews of basic toxicological immunology see Vos (1977), Faith et al. (1980), Koller (1981), and Sharma (1981). Succinctly defined, immune response is the sum of phenomena resulting from interaction of an antigen with the cells of the immune system. The immune system is usually divided into the 2 broad categories of specific and nonspecific resistance. Specific immunity embraces the concept of immunological memory, which allows a more rapid and heightened host response to a second contact with an antigen. Nonspecific immunity includes phagocytosis by certain leukocytes and macrophages, the interferon system which acts against viruses, and the complement system.

Specific immunity is further divided into humoral and cell-mediated systems. Humoral immunity results from the action of B lymphocytes (named for the chicken bursa of Fabricius; Glick et al. 1956) and includes specific antibody protection against bacteria and some viruses, and immediate hypersensitivity reactions (e.g., anaphylactic shock). Cell-mediated immunity is regulated by T lymphocytes (named for the thymus gland) and includes protective immunity against viruses and some bacteria, delayed hypersensitivity reactions (e.g., tuberculin reaction), tumor and transplant rejection, and graft versus host reactions. T and B cells originate from precursors in bone marrow and must mature in the thymus, bursa, or bursa equivalent in mammals before they become functional. Although these 2 systems are distinct from one another, there is interaction and cooperation between the cell types in the expression of immunity.

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There also is cooperation by macrophages, which are apparently necessary to "process" antigens before induction of the immune response (Vos 1977). Much of the work thus far in testing effects of lead on immunity has focused on the humoral system, but there also is evidence of the suppression of cell-mediated and macrophage systems (Table 1).

Early reports noted the effect of lead on susceptibility to bacterial endotoxin. Selye et al. (1966) found the sensitivity of rats to several Gram-negative bacterial endotoxins increased about 100,000 times with an intravenous injection of 5 mg lead acetate. Highest mortality occurred when lead and endotoxin were given simultaneously, but increased mortality also was observed when endotoxin was administered 1 hour before, or up to 7 hours after, the lead. In young chickens, susceptibility to *Escherichia coli* endotoxin was increased 1,000 times by a simultaneous dose of 2.8 mg/100 g lead acetate (Truscott 1970). Similarly, rats receiving an acute intravenous dose of lead acetate were 1,000 times more susceptible to challenge with *E. coli* than were controls (Cook et al. 1975).

Hemphill et al. (1971) injected mice intraperitoneally with low levels of lead nitrate (100 or 250 µg daily) for 30 days, followed by challenge with *Salmonella typhimurium*. No signs of lead toxicity were observed, but within 7 days after challenge 54% of the mice which received 100 µg of lead nitrate, and all (100%) which received 250 µg, died, compared with only 13% of the controls. Analysis of these data suggested lead interfered with resistance to bacteria, allowing uninhibited growth followed by high levels of endotoxin production. The effects of lead, mercury, cadmium and nickel on viral-induced mortality were tested by Gainer (1977). After receiving metal salts in drinking water for 14 days, mice were challenged with encephalomyocarditis virus. Lead acetate (0.01-0.05 M) consumption produced the highest degree of susceptibility to this virus and a direct dose-response relationship was observed. As lead concentration increased, mortality rate also increased. This finding was attributed to reduced interferon and antibody activities.

In a study with swine, animals were fed 500 ppm lead (in the form of lead acetate) for 1-3 weeks, or 3,000 ppm lead (in the form of lead chloride) for 1-2 weeks (Lassen et al. 1980). When the animals receiving 500 ppm lead were challenged intraperitoneally with *Salmonella choleraesuis*, no increase in lead-induced mortality was observed. When pigs receiving 3,000 ppm lead were challenged orally with *S. choleraesuis*, over 90% of the lead-exposed animals died compared to 50% of the controls. The authors theorized that organisms administered intraperitoneally were quickly reduced in number by the reticuloendothelial system, while in orally-challenged

Table 1. Immunosuppression in animals exposed to lead.

Species	Immunosuppressive Effect ^a	Author
Rat	Increased susceptibility to bacterial endotoxins	Selye et al. (1966)
Chicken	Increased susceptibility to <i>Escherichia coli</i> endotoxin	Truscott (1970)
Mouse	Decreased resistance to <i>Salmonella typhimurium</i>	Hemphill et al. (1971)
Mouse	Reduced antibody to SRBC	Koller and Kovacic (1974)
Swine	Increased susceptibility to <i>Salmonella choleraesuis</i>	Lassen et al. (1980)
Mouse	Increased susceptibility to EMCV	Gainer (1977)
Rat	Reduced antibody to SRBC	Luster et al. (1978)
Mouse	Reduced antibody to SRBC	Blakley and Archer (1981)
Rat	Increased susceptibility to <i>Escherichia coli</i>	Cook et al. (1975)
Rabbit	Reduced antibody to PRV	Koller (1973)
Rat	Reduced antibody to BSA	Koller et al. (1983)
Rat	Suppressed delayed hypersensitivity reaction	Faith et al. (1979)
	Reduced thymus weight	
Rat	Increased sensitivity to shock	Filkins and Buchanan (1973)

^a SRBC = sheep red blood cells; EMCV = encephalomyocarditis virus; PRV = pseudorabies virus; BSA = bovine serum albumin.

pigs (a more natural type of exposure) bacteria were free to multiply within the intestinal tract before encountering host defense mechanisms. No significant reduction in humoral immunity was observed, and evidence was cited indicating the increased mortality may have been due to inhibition of macrophages and the cell-mediated system. Further evidence of macrophage inhibition was given by Blakley and Archer (1981) who reported lead-induced immunosuppression in mice when macrophage-depen-

dent antigens were used, but not when macrophage-independent antigens were used.

One approach which has been used to test the effects of lead on the humoral system is the measurement of antibody response after immunization with an antigen. Koller (1973) treated rabbits with lead acetate in their drinking water (2,500 ppm) for 70 days, then inoculated them with killed pseudorabies virus. Lead-exposed animals exhibited nearly a 10-fold decrease in antibody titer

compared with controls. In mice, antibody response to sheep red blood cells (SRBC) was markedly reduced after 56 days of oral exposure to lead (Koller and Kovacic 1974). During this study, mice were given lead in the form of lead acetate in their drinking water in concentrations of 1,375.00, 137.50, and 13.75 ppm. The number of spleen cells producing the antibody response was reduced in these animals with each respective dose. Antibody levels in response to SRBC also were depressed in rats which received chronic pre- and postnatal lead exposure (Luster et al. 1978). In the Luster et al. study, response to a thymus-independent antigen was not inhibited, suggesting that T lymphocytes, which cooperate in humoral immunity, were affected. Recently, a sensitive technique called enzyme-linked immunosorbent assay (ELISA) has been used to measure humoral response following lead exposure. Koller et al. (1983) reported that as little as 10 ppm lead in drinking water for 10 weeks produced marked antibody suppression, as determined by ELISA, in rats challenged with bovine serum albumin. These authors consider ELISA the method of choice for quantifying humoral response because it is simple, highly sensitive, and accurate.

Not all studies have demonstrated negative effects of lead on immunity. In chickens, subclinical lead exposure (<160 mg/kg/day for 35 days) did not affect interferon induction or antibody production in response to Newcastle disease virus (Vengris and Mare 1974). Barga (1980) reported that injection of lead acetate or oral dosage of lead shot had little effect on the outcome of acute or persistent duck plague infection in mallards. However, based on numerous reports of lead immunosuppression in mammals, it is apparent that further research is needed to determine if this effect could be leading to increased susceptibility of waterfowl to disease. Large waterfowl losses from diseases such as avian cholera occur each year, and some of these outbreaks closely follow or accompany lead poisoning mortality. Studies designed to simulate realistic routes and lengths of exposure, with care taken to select appropriate antigens and analytical methods, are needed. The ELISA technique is now being used for disease diagnostic work in poultry and could be adapted for studying humoral response in waterfowl. Perhaps the most realistic approach would be to challenge lead-exposed birds with common pathogens.

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Lou Locke: Before we take any questions, I'd like to go on to the next speaker because his paper addresses the subject of sublethal lead poisoning and deals with a question that was asked of me when I was in Texas testifying. I was asked by an attorney whether or not I would testify under oath that low levels of lead—sublethal amounts—could precipitate an avian cholera outbreak. In other words, does this laboratory information apply to the field, and could it be a factor in waterfowl mortality? Gary Wobeser will address this question in his paper, "INTERACTION BETWEEN LEAD AND OTHER DISEASE AGENTS."

INTERACTION BETWEEN LEAD AND OTHER DISEASE AGENTS

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Waterfowl throughout the world are exposed to lead, primarily through ingestion of lead shotgun pellets. Some of these birds die of lead poisoning, but the extent of this direct loss is difficult to determine. A large proportion of these birds are exposed to sublethal amounts of lead. The effects of such long term, low-level exposure are less well known and even more difficult to quantify. One such effect may be an increased susceptibility to infectious agents. This hypothesis is based on experimental evidence that lead interferes with the immune system in many animal species (Koller 1980).

This paper presents information from a study of interactions between ingested shotgun pellets and avian

cholera (*Pasteurella multocida*) infection, demonstrates the complexities involved in studying interrelationships between potential disease-producing agents, and stresses the need for great care in extrapolating experimental results to the natural environment.

METHODS

The study examined the effects of ingestion of 3 different shot types (lead, sintered lead-iron, and steel) on the susceptibility of mallards (*Anas platyrhynchos*) to *P. multocida* infection, hypothesizing that prior exposure to these metals might alter the birds' susceptibility to the bacterium. The procedure used a dose of lead shot that produced some evidence of clinical disease, but that was sublethal. The same number of lead-iron and steel shot was then used in the trials of these shot types.

One complication was that the toxicity of lead is influenced by diet (Jordan 1968). Mallards fed a balanced commercial ration were much more "tolerant" of lead than were birds fed a small-grain diet, and even the latter birds seemed more tolerant of lead than birds in the wild. The diet selected consisted of small grains (barley and wheat), realizing that difficulties might arise when extrapolating the results to natural conditions. With this diet consideration, a range of shot dosing regimens were developed which produced illness, but produced little mortality among experimental birds. The concentration of lead in the blood of these ducks peaked about 9 days post-dosing.

The next step was to establish a dose level and route of infection for *P. multocida* that would produce a predictable and low level of mortality in mallards. Ideally, birds should have been exposed to bacteria in their drinking water, or perhaps as an aerosol, to simulate natural conditions. However, exposure by those methods is difficult to quantify. A parenteral route (air sac injection) was used so that all birds received equivalent exposure to the bacterium. *P. multocida* is regarded as a single species, but the name embraces a range of biotypes and serotypes (Gillespie and Timothy 1981). This diversity was very evident in the trials. In our initial inoculation, we used strains of domestic poultry origin, one of which was known to be extremely pathogenic to turkeys. These strains had to be discarded because of very low or no pathogenicity for ducks under the test conditions, even when given in massive doses. Our second choice was an organism that was very appropriate for the study in that it had been isolated during an outbreak of avian cholera in Saskatchewan. However, this strain also proved useless for the study because it was so pathogenic that we could not establish a dose that did not cause excessive mor-