PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 151,339-342 (1976)

Humoral Antibody Response in Mice after Single Dose Exposure to Lead or Cadmium[1] (39205)

LOREN D. KOLLER, JERRY H. EXON, AND JUDY G. ROAN

(Introduced by J. L. Fryer)

Department of Veterinary Medicine and Environmental Health Science Center, Oregon State University, Corvallis, Oregon 97331

Several environmental contaminants have been demonstrated to be synergistic to infectious agents (1-6). Some of the compounds are apparently immunosuppressive since circulating antibody titers to infectious agents from animals exposed to lead, cadmium, mercury (7), DDT (8), and polychlorinated biphenyls (9) were significantly lower than those from the control animals. More recently, it was reported that chronic exposure to lead (10) and cadmium (11) produced a significant decrease in antibody synthesis.

Since a single dose of cadmium yielded circulating antibody titers that varied according to time of antigen inoculation (12), this study was designed (i) to determine the effect of a single dose of lead or cadmium administered 8 hr prior to antigen inoculation on antibody synthesis and (ii) to compare antibody response after oral and intraperitoneal routes of exposure.

Material and methods. Four groups of 80 Swiss Webster mice 28-days-old were given single doses of 4 mg of lead or 0.15 mg of cadmium, either ip (IP) or orally by intubation. An additional group of 80 mice served as controls and were given deionized water.

Eight hours after administration of the metal, all mice were inoculated IP with 0.2 ml of a 2% suspension of sheep red blood cells (SRBC). Ten mice from each group were killed on Days 3-6 to measure primary immune response (19 S or IgM antibody) and on Days 8-11 for the secondary response (7 S or IgG antibody) after a second inoculation of SRBC on Day 7. The spleen from each mouse was immediately removed, cut into pieces, and forced through a nylon mesh into sterile medium 199. The spleen cell suspension was counted in a hemocytometer by the trypan blue exclusion method and diluted to 1×10^7 spleen cells per milliliter. The number of plaque-forming cells (PFC) was measured according to a modification of Cunningham and Szenberg (13). Three pieces of double-sided tape 1/2-in. wide were laid across a clean microscope slide (75 x 25 mm), dividing it into two equal areas. Two clean coverslips (22-mm square) were placed on the tape so that two edges of each coverslip were firmly attached to the tape and formed two shallow chambers. A mixture containing 88% spleen cells (1×10^7), 2% SRBC, 5% complement, and 5% medium 199 was delivered by microliter syringe into the two chambers. The chambers were then sealed with vaseline and incubated at 37° for 30 min. The plaques were counted using a light microscope. The number of plaques per million spleen cells was calculated from the number of plaques observed per microliter of the initial mixture placed in each chamber.

The same procedure was used to assay secondary response (animals killed on Days 8-11). Medium 199 was replaced in the mixture by developing antiserum (Anti-mouse IgG, Microbiological Associates, Bethesda, Maryland, No. 52-122). The number of plaques per million spleen cells was corrected by determining the sensitivity of the developing antiserum and by determining the number of plaques that occurred in spleen suspensions from nonantigen (SRBC) stimulated mice.

One kidney from each mouse was collected at necropsy and stored at −70° for cadmium analysis. The kidneys were analyzed for cadmium content by atomic absorption spectrophotometry using a microcarbon furnace. The other kidney and liver

[1] Supported by U.S. Public Health Service Grants Nos. ES-00210 and ES-00040. Technical Paper No. 4059, Oregon Agricultural Experiment Station.

were fixed in 10% buffered formalin for microscopic examination.

Results. Compared to the control mice, the 19 S antibody to SRBC was markedly elevated after ip injection of lead or cadmium (Fig. 1) and after oral exposure to lead but was supressed in mice dosed orally with cadmium (Fig. 2). The secondary immune response (7 S antibody) was significantly reduced in those mice inoculated ip with lead (Fig. 3) and in those exposed orally to lead or cadmium (Fig. 4). Cadmium injected ip produced a slight increase in IgG antibody synthesis (Fig. 3).

Analysis for cadmium and lead in the kidneys revealed residues that were approximately 30-fold larger when the metals were injected ip than when administered orally (Table I). The lead and cadmium doses were not large enough to produce pathologic alterations during the experimental period.

Discussion. The immune response of mice to a single dose of lead or cadmium depended on the route of exposure. Lead, administered orally or ip, stimulated the formation of 19 S antibody to SRBC, while cadmium caused an increase in 19 S antibody formation when injected ip but resulted in a slight decrease when dosed orally. The 7 S antibody response was slightly

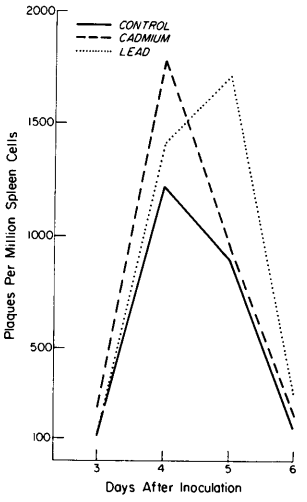

FIG. 1. Primary immune response, 4 mg of lead or 0.15 mg of cadmium inoculated ip. There was a highly significant ($P < 0.01$) increase of antibody-forming cells at Day 5 in mice exposed to lead as determined by analysis of variance. Cadmium increased IgM antibody production at Day 4 but was not significant at $P < 0.05$.

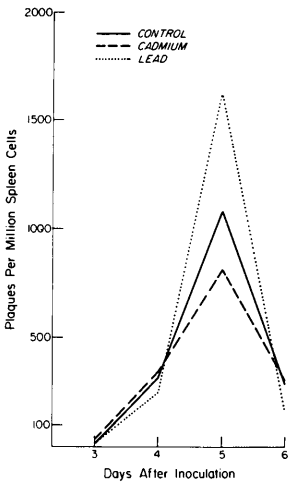

FIG. 2. Primary immune response, 4 mg of lead or 0.15 mg of cadmium given orally. Lead produced a highly significant ($P < 0.01$) increase in antibody-forming cells at Day 5 as determined by analysis of variance. Cadmium impaired antibody synthesis.

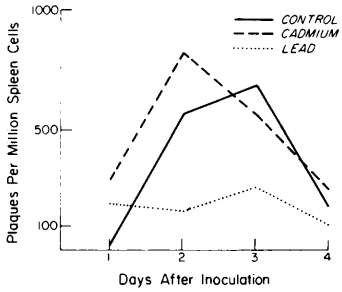

FIG. 3. Secondary immune response, 4 mg of lead or 0.15 mg of cadmium inoculated ip. Day 1 represents that day after the second inoculation of the antigen (SRBC), or Day 8 of the experiment. There was a highly significant ($P < 0.01$) decrease in IgG antibody-forming cells at Day 2 and a significant decrease ($P < 0.05$) at Day 3 in mice that were exposed to lead. Cadmium did not significantly alter antibody synthesis.

suppressed when lead was given orally but was markedly suppressed when lead was injected ip. Cadmium given orally decreased 7 S antibody but when injected ip resulted in a slight increase of antibody. However, IgG antibody was produced 1 day longer in those mice given cadmium and lead orally (Fig. 4) than in the controls, thereby increasing the actual amount of circulating antibody.

This study indicates that the route of exposure of cadmium produces different responses in a host. From 90 to 100% of ip-injected material will usually be absorbed

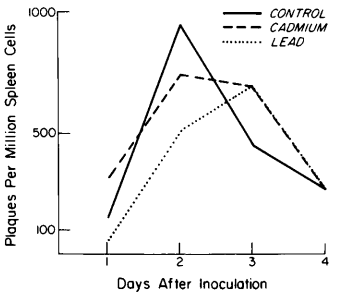

FIG. 4. Secondary immune response, 4 mg of lead or 0.15 mg of cadmium given orally. Day 1 represents that day after the second inoculation of the antigen (SRBC), or actually Day 8 of the experiment. Lead produced a highly significant ($P < 0.01$) and cadmium a significant ($P < 0.05$) decrease of antibody-forming cells at Day 2 as determined by analysis of variance. Antibody synthesis at Day 3 was greater in the lead- and cadmium-exposed mice than in the control animals; therefore, total antibody synthesis may have been similar for all three groups.

TABLE I. RENAL CONCENTRATION OF TOTAL LEAD AND CADMIUM IN MICE GIVEN SINGLE ORAL OR INTRAPERITONEAL (ip) DOSAGES OF LEAD (4 mg) OR CADMIUM (0.15 mg).[a]

Day postexposure	Mean ppm of renal residues[b]			
	Cadmium		Lead	
	Oral	ip	Oral	ip
3	0.18*	4.6	1.2	24.6
6	0.38	6.5	2.4	34.1
11	0.30	9.4	0.8	39.4

[a] The kidneys were collected 3, 6, and 11 days after exposure.

[b] Wet weight of 10 kidneys.

* Statistics were not computed due to pooling of samples.

compared to about 10% absorption from oral dosage. Tissue analyses confirmed that the kidneys contained larger residues of cadmium and lead after ip exposure than after oral exposure (Table I). Since responses vary according to route of administration, exposure to an environmental contaminant for experimental purposes should correlate with natural exposure of humans if data are to be extrapolated. Another aspect of this study indicated that immunologic responses in mice from a single exposure to lead or cadmium differed markedly from those reported for prolonged exposures (10, 11). The long term experiments produced immunosuppression, while the single dose exposure promoted IgM antibody formation.

Cadmium (12) and selenium (14–16) have promoted antibody production in animals when administered prior to an antigen. In the present study, the metals were administered 8 hr before antigenic stimulation of the host. Perhaps, after a single exposure, these metals stimulate mitogenic properties that increase antibody production but after prolonged exposure those properties are no longer stimulated. Lipopolysaccharide, dextran, and levan (17) are recognized B-cell mitogens that may function directly by activating T-cell helper function or indirectly through effects mediated by macrophages or biologically active factors (18). Whether lead and cadmium effect T-cell helper function is not currently known; however, lead has significantly reduced the ability of macrophages to phagocytize SRBC (19).

This study indicates that the route of exposure to environmental contaminants should simulate natural conditions since the immunologic response of mice varied considerably with the route of administration of the metal. Also, acute exposure enhanced IgM antibody synthesis, while chronic exposure has been shown to suppress antibody formation (10, 11). Acute responses can usually be toxic or produce short-term problems, whereas continuous exposure to low levels of enviromental pollutants can produce long lasting effects. Many of these latter effects, particularly those that are indirect, require further investigation to determine their significance on human health.

Summary. A single dose of lead administered orally or intraperitoneally (IP) to mice stimulated formation of IgM antibody. Cadmium caused an increase in IgM antibody formation when injected ip but resulted in a slight decrease when given orally. Lead, orally or ip, significantly reduced IgG antibody but the IgG antibody response decreased only when cadmium was given orally and increased when injected ip. Since responses vary according to route of administration, experiments should be designed so that experimental exposure will simulate natural exposure in order to develop comparable data.

1. Friend, M., and Trainer, D. O., Science **170,** 1314 (1970).
2. Gainer, J. H., Amer. J. Vet. Res. **33,** 2067 (1972).
3. Gainer, J. H., and Pry, T. W., Amer. J. Vet. Res. **33,** 2299 (1972).
4. Fairchild, G. A., Roan, J., and McCarroll, J., Arch. Environ. Health **25,** 174 (1972).
5. Koller, L. D. Amer. J. Vet. Res., In press.
6. Hemphill, F. E., Kaeberle, M. L., and Buck, W. B., Science **172,** 1031 (1971).
7. Koller, L. D., Amer. J. Vet. Res. **34,** 1457 (1973).
8. Wasserman, M., Wassermann, D., Kedar, E., and Djauaherian, M., Bull. Environ. Contam. and Tox. **6,** 426 (1971).
9. Koller, L. D., and Thigpen, J. E., Amer. J. Vet. Res. **34,** 1605 (1973).
10. Koller, L. D., and Kovacic, S., Nature (London) **250,** 148 (1974).
11. Koller, L. D., Exon, J. H., and Roan, J. G., Arch. Environ. Health, In press.
12. Jones, R. J., Williams, R. L., and Jones, A. M. Proc. Soc. Exp. Biol. Med. **137,** 1231 (1971).
13. Cunningham, A. J., and Szenberg, A. Immunology **14,** 599 (1968).
14. Spallholz, J. E., Martin, J. L., Gerlach, M. L., and Heinzerling, R. H., Proc. Soc. Exp. Biol. Med. **143,** 685 (1973).
15. Spallholz, J. E., Martin, J. L., Gerlach, M. L., and Heinzerling, R. H., Infec. Immunity **8,** 841 (1973).
16. Spallholz, J. E., Martin, J. L., Gerlach, M. L., and Heinzerling, R. H., Proc. Soc. Exp. Biol. Med. **148,** 37 (1975).
17. Ivanyi, L., and Lehner, T., Clin. Exp. Immunol. **18,** 347 (1974).
18. Newberger, P. E., Hamaoka, T., and Kutz, D. H., J. Immunol. **113,** 824 (1974).
19. Koller, L. D., and Roan, J. G., unpublished data.

Received July 21, 1975. P.S.E.B.M. 1976, Vol. 151.