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The Effect of Methylmercury, Tetraethyl Lead, and Sodium Arsenite on the Humoral Immune Response in Mice

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The Effect of Methylmercury, Tetraethyl Lead, and Sodium Arsenite on the Humoral Immune Response in Mice. BLAKLEY, B. R., SISODIA, C. S., AND MUKKUR, T. K. (1980). *Toxicol. Appl. Pharmacol.* **52**, 245–254. Male mice were exposed to methylmercury, tetraethyl lead, or sodium arsenite for 3 weeks in the drinking water at three or four concentrations ranging from 0.5 to 10.0 ppm. The humoral component of the immune system was subsequently evaluated using hemagglutination, radial immunodiffusion, and the Cunningham plaque assay (both primary and secondary immune response). Each evaluation of the immune response demonstrated immunosuppressive effects at low metal concentrations. A maximum immunosuppressive effect was produced in most instances at a metal concentration less than 2.0 ppm. Metal concentrations in the liver and kidney showed that a dose–effect relationship existed at all metal exposure levels; however, the maximal immunosuppressive effect was observed in spite of increasing tissue concentrations of the metal.

Heavy metals such as lead, cadmium, or mercury (Koller, 1973) are environmental contaminants for which immunosuppressive effects have been demonstrated. Lead acetate has produced a marked potentiation of endotoxin shock. Truscott (1970) demonstrated this effect in chickens and Cook, Hoffman and De Luzio (1975) demonstrated a similar response in rats. Interferon production was also suppressed by lead acetate (Vengris and Mare, 1974). Antibody titers to various infectious agents were suppressed by lead compounds (Koller, 1973; Stankovic and Jugo, 1976). Oral administration of lead acetate to mice produced a decrease in IgM and IgG antibody formation (Koller and Kovacii, 1974). Increased mortality from bacterial disease challenge in lead-exposed mice was shown by Hemphill *et al.* (1971).

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There have been limited reports of various arsenic compounds exhibiting immunosuppressive effects on interferon activity. Gainer and Pry (1972) demonstrated that moderately high doses of sodium arsenate increased mortality in mice exposed to several viral agents. An organic arsenical, *p*-arsanilic acid did not enhance the mortality. Gainer (1972) also showed that sodium arsenite inhibited interferon production at high concentrations. Low concentrations enhanced the specific antiviral activity of the interferon which was present.

Methylmercury, a cumulative environmental contaminant, produced increased susceptibility to infectious agents (Koller, 1975), decreased antibody concentrations (Koller *et al.*, 1977), and a reduction in IgM and IgG formation (Ohi *et al.*, 1976).

In the present study, the immunosuppressive effects of low-level exposure to methylmercury, sodium arsenite, or tetra-

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ethyl lead were investigated in mice using several humoral immunological tests including hemagglutination (HA), radial immunodiffusion (RID), and the Cunningham plaque assay. Low-level dose–response relationships involving humoral immunosuppression were evaluated using these techniques.

METHODS

Groups of adult (about 5–6 weeks old) male white Swiss cross mice[2] (20–25 g) were acclimatized for 1 week before exposure to tetraethyl lead,[3] sodium arsenite,[4] or methylmercury[5] in deionized drinking water at concentrations ranging from 0.5 to 10.0 ppm metal content. The control mice were given deionized water. Since the tetraethyl lead was not soluble in water at the desired concentrations, it was emulsified with 0.7 g of Tween 80/4 liters of emulsion (10 ppm lead). The tetraethyl lead control mice were given deionized water containing Tween 80 (polyoxyethylene sorbitan monoleate).[4]

The mice were maintained in a temperature controlled room (20°C) with a constant cycle of 12 hr light and 12 hr dark. The various groups of mice (four per immunologic test) containing 8–10 mice per group, were exposed to the particular metal for 3 weeks. The antigenic stimulus was then administered and the immunologic status was evaluated at the appropriate time interval.

Sheep red blood cells (SRBC) were used as an antigenic stimulus on HA and Cunningham plaque assay studies. *Escherichia coli* lipopolysaccharide endotoxin (LPS)[6] was used as an antigenic stimulus for HA and RID studies. The immunizing dose of LPS was 100 μg/mouse.

The Cunningham plaque assay technique was employed as described by Jerne *et al.* (1974). The number of plaque-forming cells was determined using the method of Cunningham and Szenberg (1968). The primary immune response (19 S or IgM antibody) was determined 5 days after ip inoculation with 0.2 ml of a 10% SRBC suspension. The secondary immune response (7 S or IgG antibody) was determined 10 days after similar ip inoculations with SRBC on Days 0 and 7. The second inoculation of SRBC was necessary in the case of the secondary response studies. The reagent constituents for the primary Cunningham assay included 20 μl SRBC (25% suspension in Hanks' BS containing 5% fetal calf serum), 20 μl guinea pig complement,[7] 60μl Hanks' BS containing 5% fetal calf serum and 100 μl of the appropriate spleen cell suspension. The secondary Cunningham assay reagents contained 20-, 20-, 90-, and 50-μl volumes, respectively. In addition, 20 μl of enhancing serum diluted 1:20 with Hanks' BS containing 5% fetal calf serum was required for the secondary Cunningham assay. The enhancing serum was rabbit antimouse IgG. The plaque production was visually enumerated under fluorescent lights against a dark background.

The hemagglutination technique employed was a modification of the method described by Chen (1952). HA studies using LPS as an antigenic stimulus required prior sensitization of the SRBC to LPS. A 2.5% suspension of SRBC was incubated at 37°C for 1 hr with an equal volume of LPS solution (100 μg/ml in buffered saline). The sensitized SRBC were washed with buffered saline (pH 7.2) and used immediately for HA studies. HA studies using SRBC as an antigenic stimulus did not require sensitization of the SRBC. The HA titers were determined 28 days after exposure to the specific antigen.

RID was used to quantitate the concentration of anti-LPS antibody present 28 days after exposure to LPS. The method employed was a modification of the method described by Fahey and McKelvey (1965). Exactly 16.5 ml of a 1% Bio-Cert agar[4] in 0.15 M NaCl containing 100 μg/ml LPS was poured on a 10 × 12-cm glass plate. Exactly 0.05 ml of test serum was placed in 4-mm (diameter) wells punched out on the agar plate. The square of the antigen–antibody precipitation ring diameter was proportional to the concentration of anti-LPS antibody in the serum. Sheep IgG which was purified by gel filtration contained 19.6% IgG specific for anti-LPS which was determined indirectly by optimal specific antibody precipitation and protein analysis of the supernatant and precipitate. This IgG was used to prepare the standard curve. Protein concentrations were determined using the method described by Zak and Cohen (1961). Bovine serum albumin was used as a protein standard.

Liver and whole kidney samples were collected immediately prior to immunological testing. Equal sized representative samples were taken from each group and analyzed for the appropriate metal. The method for mercury analysis was as described by Uhte *et al.* (1970). Analysis was conducted on an Evans Electroselenium Atomic Absorption Flame Photometer at a wavelength of 253.7 nm. Tissue samples from animals

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[3] ICN K & K Laboratories, Inc., Cleveland, Ohio.

[4] Fisher Scientific Company, Fairlawn, N. J.

[5] Methylmercuric chloride, Alfa Inorganics, Ventron, Beverly, Mass.

[6] Lipopolysaccharide B *E. coli*. 026:B6, Difco Laboratories, Detroit, Mich.

[7] Cappel Laboratories, Downingtown, Pa.

TABLE 1

WEIGHT GAINS AND WATER CONSUMPTION IN MICE EXPOSED TO TETRAETHYL LEAD, METHYLMERCURY, OR SODIUM ARSENITE FOR 3 WEEKS

Metal	Concentration in drinking water (ppm)	Weight gain (g/mouse/day)	Weight gain (% control)	Water consumption (ml/mouse/day)	Water consumption (% control)
Methylmercury	Control	0.18 ± 0.011[a]	100	4.88[b]	100
	0.5	0.22 ± 0.019	122	4.60	94
	2.0	0.16 ± 0.014	89	4.60	94
	10.0	0.19 ± 0.017	106	4.33	89
		$p = 0.084$[c]			
Tetraethyl lead	Tween control	0.18 ± 0.012	100	4.98	100
	0.5	0.17 ± 0.013	94	5.57	112
	1.0	0.16 ± 0.011	89	4.45	89
	2.0	0.15 ± 0.014	83	4.30	86
	10.0	0.14 ± 0.010	78	3.95	79
		$p = 0.168$[c]			
Sodium arsenite	Control	0.18 ± 0.011	100	4.88	100
	0.5	0.19 ± 0.013	106	4.69	96
	2.0	0.17 ± 0.010	94	4.47	92
	10.0	0.17 ± 0.015	94	4.37	90
		$p = 0.311$[c]			

[a] Mean ± SE.
[b] Average water consumption.
[c] Probability of no treatment effect for weight gain.

administered lead were digested using the procedure[8] described by Cominco Ltd. (1967). The digested residue was analyzed using standard atomic absorption spectroscopy techniques on a Unicam SP 1900 at 2833 Å. Arsenic samples were analyzed using the methods of Fisher Scientific Company (Technical Data Sheet TD-142, 1960) and Stolman (1961) which employed a colorimetric analysis. Two pooled tissue samples (eight or nine) at each exposure level were necessary for arsenic analysis because the tissue concentrations were approaching lower detection limits.

Analysis of variance was used for statistical description of the data. Three appropriate statistical descriptions were determined for each study. A simple analysis of variance was conducted to determine the presence of group differences. An analysis of variance comparing the responses of the control group and the corresponding combined metal exposed groups in each study was determined. A third analysis of variance was conducted, comparing the responses of the two high-exposure groups in each study. The latter two treatment comparisons are a special type of analysis variance known as orthogonal contrasts (Ostle and Mensing, 1975).

[8] Tissue samples (about 1 g) were placed in acid-cleaned 100-ml beakers. An acid mixture containing HNO_3 (10 ml), H_2SO4 (3 ml), and $HC10_4$ (2 ml) was added to the tissue. The tissues were heated on a hot plate and allowed to digest to near dryness, at which point, the residue was diluted to a 10-ml volume with distilled-deionized water containing 2.5% HNO_3.

RESULTS

The mice in this study, exposed to chronic low levels of the heavy metals showed no evidence of clinical toxicity. Table 1 shows weight gains and water consumption for mice treated with tetraethyl lead, sodium arsenite, and methylmercury. The statistical probability of a treatment effect as determined by weight gains was not significant

TABLE 2

THE EFFECT OF METHYLMERCURY, TETRAETHYL LEAD, OR SODIUM ARSENITE ON THE PRODUCTION OF ANTIBODY-PRODUCING CELLS (PRIMARY IMMUNE RESPONSE)

Metal	Concentration in drinking water (ppm)	PFC per 10^6 spleen cells[a]	Percentage control	Probability
Methylmercury	Control	502.2 ± 46.3[b] (9)[c]	100	<0.0001[d]
	0.5	288.1 ± 32.2 (10)	57	<0.0001[e]
	2.0	220.4 ± 50.1 (10)	44	0.97[f]
	10.0	213.8 ± 36.5 (10)	42	
Tetraethyl lead	Tween control	1253.1 ± 122.6 (10)	100	<0.0001
	0.5	574.5 ± 74.8 (8)	46	<0.0001
	1.0	504.7 ± 51.3 (10)	40	0.77
	2.0	391.8 ± 33.4 (10)	31	
Sodium arsenite	Control	1036.7 ± 71.7 (9)	100	<0.0001
	0.5	493.0 ± 26.2 (10)	48	<0.0001
	2.0	577.7 ± 77.7 (10)	56	0.83
	10.0	514.4 ± 67.3 (10)	50	

[a] Plaque-forming cells per 10^6 spleen cells—primary response.
[b] Mean ± SE.
[c] Number of animals.
[d] Probability of no group differences.
[e] Probability of no treatment effect (control vs three treatment groups)—orthogonal contrast.
[f] Probability of no treatment different between the two highest exposure groups—orthogonal contrast.

with each metal. Individual water consumption data were not determined; therefore, statistical analysis was not possible.

All animals appeared normal at necropsy except for two incidental findings. One mouse exposed to methylmercury (10 ppm) had a pulmonary papillary adenocarcinoma with evidence of metastasis. This tumor is commonly seen in laboratory rodents. Another mouse exposed to tetraethyl lead (10 ppm) had a large facial abscess due to excessive fighting. The immunological studies were not affected in either instance since the experimental values obtained in each case were within their respective group variances.

The Effect of Methylmercury, Tetraethyl Lead, or Sodium Arsenite on the Production of Antibody-Producing Cells

Methylmercury suppressed the production of antibody-producing cells (plaque-forming cells). The suppression of the primary immune response which measures the specific IgM-producing cells is summarized on Table 2. The immunosuppression produced by methylmercury attained a maximal level of expression by 2 ppm since statistical comparison of the plaque-forming cells produced at 2 and 10 ppm showed that no further change had taken place ($p = 0.97$). The maximal immunosuppressed values were about 40–50% of the control value.

The effect on the primary immune response obtained with tetraethyl lead or sodium arsenite exposure shows a similar immunosuppressive behavior (Table 2). A maximal degree of primary immunosuppression was reached by 2 ppm in both instances. The maximal suppression was approximately 50% of the corresponding control value.

The secondary immune response, which was measured by indirect determination of IgG-producing cells, was suppressed by

TABLE 3

THE EFFECT OF METHYLMERCURY, TETRAETHYL LEAD, OR SODIUM ARSENITE ON THE PRODUCTION OF ANTIBODY-PRODUCING CELLS (SECONDARY IMMUNE RESPONSE)

Metal	Concentration in drinking water (ppm)	PFC per 10^6 spleen cells[a]	Percentage control	Probability
Methylmercury	Control	1091.0 ± 52.3[b] (10)[c]	100	0.0041[d]
	0.5	888.5 ± 39.0 (10)	81	0.0015[e]
	2.0	796.1 ± 56.7 (10)	73	0.881[f]
	10.0	834.8 ± 79.9 (9)	76	
Tetraethyl lead	Tween control	871.1 ± 98.5 (8)	100	0.0012
	0.5	559.9 ± 72.7 (10)	64	0.0046
	1.0	489.4 ± 42.6 (8)	56	0.81
	2.0	570.9 ± 58.2 (9)	66	
Sodium arsenite	Control	1091.0 ± 52.3 (10)	100	<0.0001
	0.5	605.4 ± 46.9 (10)	55	<0.0001
	2.0	838.1 ± 32.8 (10)	77	0.182
	10.0	708.5 ± 48.9 (8)	65	

[a] Plaque-forming cells per 10^6 spleen cells—secondary response.
[b] Mean ± SE.
[c] Number of animals.
[d] Probability of no group differences.
[e] Probability of no treatment effect (control vs three treatment groups)—orthogonal contrast.
[f] Probability of no treatment difference between the two highest exposure groups—orthogonal contrast.

TABLE 4

THE EFFECT OF METHYLMERCURY OR TETRAETHYL LEAD ON HEMAGGLUTINATION TITERS USING SHEEP RED BLOOD CELLS AS AN ANTIGENIC STIMULUS

Metal	Concentration in drinking water (ppm)	Antibody titer[a]	Percentage control	Probability
Methylmercury	Control	497.8 ± 56.2[b] (9)[c]	100	0.0105[d]
	0.5	384.0 ± 42.7 (10)	77	0.0042[e]
	2.0	284.4 ± 56.7 (9)	57	0.766[f]
	10.0	320 ± 0 (10)	64	
Tetraethyl lead	Tween control	416.0 ± 48.9 (10)	100	0.02
	0.5	368.0 ± 48.0 (10)	89	0.058
	1.0	304.0 ± 16.0 (10)	73	0.754
	2.0	240.0 ± 32.7 (9)	58	

[a] Highest serum dilution showing hemagglutination.
[b] Mean ± SE.
[c] Number of animals.
[d] Probability of no group differences.
[e] Probability of no treatment effect (control vs three treatment groups)—orthogonal contrast.
[f] Probability of no treatment difference between the two highest exposure groups—orthogonal contrast.

TABLE 5

THE EFFECT OF METHYLMERCURY OR TETRAETHYL LEAD ON HEMAGGLUTINATION TITERS USING *Escherichia coli* LIPOPOLYSACCHARIDE AS AN ANTIGENIC STIMULUS

Metal	Concentration in drinking water (ppm)	Antibody titer[a]	Percentage control	Probability
Methylmercury	Control	116 ± 15.1[b] (10)[c]	100	0.0026[d]
	0.5	80 ± 10.3 (10)	69	0.0009[e]
	2.0	64 ± 6.5 (10)	55	0.99[f]
	10.0	64 ± 6.5 (10)	55	
Tetraethyl lead	Tween control	92 ± 15.8 (10)	100	0.0351
	0.5	60 ± 6.7 (10)	65	0.0117
	2.0	56 ± 6.5 (10)	61	0.99
	10.0	56 ± 6.5 (10)	61	

[a] Highest serum dilution showing hemagglutination.
[b] Mean ± SE.
[c] Number of animals.
[d] Probability of no group differences.
[e] Probability of no treatment effect (control vs three treatment groups)—orthogonal contrast.
[f] Probability of no treatment difference between the two highest exposure groups—orthogonal contrast.

methylmercury, tetraethyl lead, or sodium arsenite (Table 3). Each metal produced a significant and consistent amount of immunosuppression (about 50–70% of the corresponding control). A similar maximal effect was seen at 2 ppm which was comparable to the primary immunosuppression response seen.

The Effect of Methylmercury or Tetraethyl Lead on Hemagglutination Titers

Methylmercury and tetraethyl lead suppressed HA titers in which SRBC were used as an antigenic stimulus (Table 4). Maximal immunosuppressive effects were produced with methylmercury at 2 ppm. No statistically significant changes were seen at the higher concentration of 10 ppm. The maximum degree of immunosuppression attained was about 60% of the control value, which compares favorably with antibody-producing cell studies. A similar response was seen with tetraethyl lead, though concentrations above 2 ppm were not tested. The maximum degree of immunosuppression attained was about 60% of the control value which was consistent with antibody producing cell studies.

Similar studies using LPS as an antigenic stimulus showed that methylmercury or tetraethyl lead produced immunosuppression (Table 5). A similar response was produced by both metals. The immunosuppression attained its maximal effect by 2 ppm, with no apparent change at the higher exposure concentration (10 ppm). In each instance, the magnitude of the suppressed titers was about 60% of the corresponding control.

The Effect of Methylmercury or Tetraethyl Lead on a Specific Antibody Concentration Determined by Radial Immunodiffusion

The RID protein concentrations were determined on mice exposed to methylmercury or tetraethyl lead using LPS as an antigenic stimulus (Table 6). Highly significant immunosuppressive effects were observed with both metals. A statistically significant maximal response was produced

at a metal concentration of 2 ppm. The magnitude of the immunosuppression was in each instance, about 50% of the comparable control value.

Tissue Concentrations of Mercury, Lead, or Arsenic

The mean tissue concentrations of mercury in liver and kidney samples are summarized in Table 7. In both tissues, the concentrations of mercury increased as the concentration of methylmercury in the drinking water increased.

The mean tissue concentrations of lead in liver and kidney samples are summarized in Table 7. In general, tissue concentrations of lead increased as exposure to tetraethyl lead in the drinking water increased.

Arsenic tissue concentrations were obtained from the average of two pooled groups at each treatment level because the tissue concentrations of arsenic were very low. The average tissue concentrations of arsenic are summarized in Table 7. All tissue concentrations were well below toxic concentrations, though a general increase in arsenic content was seen as the exposure level increased (Buck *et al.*, 1973). No maximal concentration was reached in the tissues which would correspond to the maximal immunosuppressive effect demonstrated by the immunologic techniques. The immunosuppressive action of methylmercury, tetraethyl lead, or sodium arsenite cannot be correlated directly with the corresponding tissue concentrations of mercury, lead, or arsenic, particularly at the higher concentrations since a linear dose–response was not produced.

DISCUSSION

The present study has demonstrated the humoral immunosuppressive action of tetraethyl lead, methylmercury, and sodium arsenite. The dose–effect relationships determined for methylmercury, tetraethyl lead, and sodium arsenite using several immunological techniques produced similar immunosuppressive effects in essentially all instances. The similarity of effects was both qualitatively and quantitatively consistent. The maximal immunosuppressive effect was produced at similar metal concentrations

TABLE 6

THE EFFECT OF METHYLMERCURY OR TETRAETHYL LEAD ON THE PROTEIN CONCENTRATION OF *Escherichia coli* LIPOPOLYSACCHARIDE ANTIBODY DETERMINED BY RADIAL IMMUNODIFFUSION

Metal	Concentration in drinking water (ppm)	Protein concentration (mg/ml)	Percentage control	Probability
Methylmercury	Control	7.58 ± 0.61[a] (10)[b]	100	<0.0001[c]
	0.5	4.61 ± 0.29 (10)	61	<0.0001[d]
	2.0	3.53 ± 0.42 (10)	47	0.921[e]
	10.0	3.33 ± 0.46 (10)	44	
Tetraethyl lead	Tween control	6.66 ± 0.65 (9)	100	<0.0001
	0.5	4.73 ± 0.32 (10)	71	<0.0001
	2.0	3.93 ± 0.25 (10)	59	0.781
	10.0	3.40 ± 0.31 (10)	51	

[a] Mean ± SE.
[b] Number of animals.
[c] Probability of no group differences.
[d] Probability of no treatment effect (control vs three treatment groups)—orthogonal contrast.
[e] Probability of no treatment difference between the two highest exposure groups—orthogonal contrast.

TABLE 7

TISSUE CONCENTRATIONS OF MERCURY, LEAD, OR ARSENIC IN MICE EXPOSED TO METHYLMERCURY, TETRAETHYL LEAD, OR SODIUM ARSENIC FOR 3 WEEKS[a]

Metal	Concentration in drinking water (ppm)	Whole kidney[b] (μg/g)	Probability	Liver[b] (μg/g)	Probability
Methylmercury	Control	0.17 ± 0.02[c] (14)[d]	<0.0001[e]	0.02 ± 0	<0.0001[e]
	0.5	2.47 ± 0.33 (18)	<0.0001[f]	0.41 ± 0.09	<0.0001[f]
	2.0	28.96 ± 4.83 (19)	<0.0001[g]	6.80 ± 0.88	<0.0001[g]
	10.0	80.78 ± 4.11 (13)		22.55 ± 1.92	
Tetraethyl lead	Tween control	3.68 ± 0.89 (14)	0.0009[e]	1.26 ± 0.37 (14)	<0.0001[e]
	0.5	6.60 ± 1.12 (17)	0.0032[f]	5.27 ± 0.79 (17)	<0.0001[f]
	1.0	5.49 ± 1.01 (18)	0.023[h]	10.52 ± 0.58 (18)	<0.0001[g]
	2.0	8.60 ± 1.47 (18)	0.203[g]	12.51 ± 0.98 (18)	
	10.0	9.65 ± 1.80 (10)		23.00 ± 1.06 (10)	
Sodium arsenite	Control	0.13 (18)[i]		0.04 (18)[i]	
	0.5	0.16 (17)		0.05 (17)	
	2.0	0.18 (18)		0.05 (18)	
	10.0	1.16 (17)		0.59 (17)	

[a] Values have been corrected for 70.8% recovery of lead.
[b] Wet weight.
[c] Mean ± SE methylmercury and tetraethyl lead only.
[d] Number of tissues (animals).
[e] Probability of no group differences.
[f] Probability of no treatment effect (control vs metal exposed groups)—orthogonal contrast.
[g] Probability of no treatment difference between the two highest exposure groups—orthogonal contrast.
[h] Probability of no treatment effect (1.0 vs 2.0 ppm tetraethyl lead)—orthogonal contrast.
[i] Average of two pooled samples sodium arsenite only.

(about 2 ppm). No statistically significant differences in the responses were seen when statistical comparisons (orthogonal contrasts) were determined on the highest two exposure groups in each immunologic technique. The magnitude of the maximal effect seen was generally about 50–60% of the comparable control value.

No previous studies have been conducted demonstrating the effects of tetraethyl lead or other alkyl lead compounds on the immune system. The immunologic effects of lead nitrate or lead acetate have been studied. Koller *et al.* (1976) demonstrated that a single exposure to lead (4 mg/mouse) caused alteration in the immune system. Administration of lead acetate orally at lower levels for extended time periods up to 70 days resulted in lower antibody titers (Koller, 1973; Stankovic and Jugo, 1976). Koller and Kovacii (1974) demonstrated that lead acetate caused a decrease in production of antibody-producing cells. Both IgM and IgG responses were suppressed by oral administration of lead acetate (13.75, 137.5, and 1375 ppm). No maximal inhibitory effect was demonstrated; however, the maximum concentration of lead necessary to attain the maximal effect may not have been reached. Direct comparisons between inorganic lead compounds and tetraethyl lead cannot easily be made since the absorption and distribution of the compounds are dramatically different, as well as the nature of their toxic effects.

The immunosuppressive effects of methylmercury are consistent with previous studies. Koller *et al.* (1977) showed suppression of antibody titers in rabbits exposed to methylmercury (1 and 10 ppm) against

A1PR8 influenza virus. Ohi *et al.* (1976) conducted a study in mice exposed to 0.5 or 5 ppm methylmercury. They demonstrated impaired IgM and IgG responses. Mice exposed to 1 or 10 ppm methylmercury showed an increased mortality when exposed to an encephalomyocarditis virus (Koller, 1975). These findings are consistent with the present study, though no clearly defined maximum immunosuppressive effect was demonstrated in the previous studies.

Gainer and Pry (1972) demonstrated that sodium arsenite affected interferon activity and production. They observed enhanced encephalomyocarditis mortality in mice exposed to arsenicals. No studies have been reported demonstrating the effects of arsenicals on antibody production.

The concentrations of metal found in the various tissues increased statistically (arsenic concentrations were not statistically analyzed) as the dose administered increased except for the high tetraethyl lead concentration (10 ppm) in the kidney. Since a statistically significant tissue concentration difference was seen in the liver (at all exposure levels) and in the kidney (1–2 ppm) in the presence of a maximal immunosuppressive response at these exposure levels, the observed relationship between tissue concentrations and immunosuppression remains valid. The lack of a more pronounced difference in the 2 and 10-ppm kidney concentrations may suggest the kidney is a poor tissue model for chronic tetraethyl lead accumulation studies. In contrast to the continual increase in tissue concentrations, the immunosuppressive response reached a maximal response much earlier. The tissue concentrations of the various metals with a few exceptions, were within concentration ranges considered nontoxic, yet immunosuppressive effects were seen at these concentrations (Buck *et al.*, 1973).

The protein concentrations in mouse sera determined by RID appear to be higher than expected values. This difference may be attributed to the choice of protein standard (bovine serum albumin) and method of protein estimation. Since all protein estimations are similarly affected, the comparison between values to determine the presence of immunosuppression is still valid because the differences are all relative.

Methylmercury, tetraethyl lead, and sodium arsenite exhibited their immunosuppressive effects at very low concentrations. The quantitative and qualitative changes were similar with each metal. This consistency of result suggests that the immunosuppressive action of these metals may have a common denominator. If a common mechanism was responsible for the immunosuppressive effects of the three metals, it may involve protein synthesis, ultimately gamma globulin synthesis. Alterations in macromolecular synthesis, particularly DNA or RNA have been demonstrated with lead chloride (Skred and Habazin-Novak, 1975). Shenker *et al.* (1977) demonstrated altered DNA and protein turnover in the presence of lead, cadmium and chromium. The effects on protein synthesis may also be reflected in decreased interferon production (Vengris and Mare, 1974). The direct inactivation of enzymes containing sulfhydryl groups may also suggest a general effect.

It has been reported that IgG response in lead or cadmium-exposed mice is affected more than the IgM response (Koller *et al.*, 1976). They concluded that T-helper cells in the thymus may be more directly involved, since they are in part responsible for the enhancement of the IgG response. The present study does not support this T helper-cell hypothesis, since the IgG and IgM responses were both affected in a similar manner. The LPS immune studies add further support. The LPS immune response is known to be T helper-cell independent, yet a similar immunosuppression was produced. The direct involvement of the T helper cell is open to some question.

The evidence presented in this study suggest a common underlying mechanism. This

mechanism may involve an impairment of antibody processing by the macrophage (impairment of a specific macrophage function). Lead has been shown to impair macrophage function (Vos, 1977). It may also be possible that the metal causes suppression of a particular lymphocyte subpopulation composed of B or T-lymphocytes which play a role in the immune response.

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