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Methylmercury: Decreased Antibody Formation in Mice[1,2] (39859)

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Methylmercury is an environmental contaminant that was recognized as a general public health hazard after epidemics of poisoning occurred in Minamata and Nigata, Japan (1, 2) during 1953 to 1960. Since that time, several other incidents of human poisoning due to methylmercury have been reported. Seed grain treated with methylmercury fungicide is a primary source of human exposure either by direct consumption of contaminated grain that has been processed into flour and used for baking (3) or indirectly from eating meat of animals fed the treated grain (4, 5). Contaminated fish is another source of human exposure to methylmercury (6). These incidents have provided data for comparison of acute and chronic toxicity in humans (7).

Methylmercury compounds are almost completely absorbed from the gastrointestinal tract and readily pass through the blood-brain and placental barriers (8, 9). Inorganic mercury is transformed into methylmercury by microorganisms in aquatic sediments (10) as well as by microbial flora in the human intestine (11). Methylmercury at a concentration of 10 ppm is considered potentially toxic to people and animals. In the United States and Canada, fish which contain mercury concentrations of less than 0.5 ppm may be sold.

It has been shown that methylmercury from environmental sources can readily produce toxicity in human beings. Most environmental exposures reported to date involved relatively high methylmercury concentrations and produced clinical signs of mercury poisoning. However, it is equally important to determine whether subclinical doses of methylmercury affect human health. Experiments with animals revealed that subclinical doses of methylmercury increased mortality of mice challenged with an infectious virus (12). Furthermore, rabbits fed 10 ppm methylmercury for 14 weeks and then exposed to an influenza virus demonstrated decreased circulating antibody titers as compared to those of controls (13).

In this study, we report that methylmercury significantly suppresses antibody synthesis in mice exposed to a nonpathogenic antigen.

Materials and methods. CBA/J male mice, 28 days old were given 1, 5, or 10 ppm methylmercury for 10 weeks as methylmercuric chloride incorporated into Oregon State University (OSU) rodent chow. Controls were given regular OSU rodent chow. The mice were housed in polycarbonate cages (five per cage) and given feed and deionized drinking water ad libitum.

Sheep red blood cells (SRBC) were collected in Alsever's solution (1:1) and stored at 4° for at least 1 week. The SRBC were washed 3 × prior to use in medium 199 (M199) (Flow Laboratories).

After 10 weeks on the prescribed diets, the mice were immunized by inoculation ip with 5×10^8 SRBC in 0.2 ml of physiological saline (PBS) to measure the primary antibody response (IgM). For the secondary response (IgG), the mice were reimmunized with an ip inoculation of 5×10^8 SRBC in 0.2 ml of PBS on Day 7.

Ten mice per group were sacrificed by cervical dislocation on Days 3-6 (primary response) and Days 8-11 (secondary response). The spleen from each mouse was removed and teased through nylon mesh into cold M199. The dissociated cells were then centrifuged at 200*g* for 8 min, washed once in M199, counted in a hemocytometer, and diluted to 7×10^6 cells/ml.

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The plaque assay used in this experiment was that of Cunningham and Szenberg (14) as modified by Koller *et al.* (15). To measure the primary response, 0.35 ml of the diluted spleen cells (7×10^6 cells/ml) were mixed with 0.10 ml of 20% SRBC, 0.025 ml of guinea pig complement (Colorado Serum Co.) absorbed with SRBC, and 0.025 ml of M199 for a final volume of 0.50 ml. Rabbit anti-mouse IgG serum (Cappel Laboratories) was substituted for the 0.025 ml of M199 for measuring the secondary response. This antiserum was heat inactivated and absorbed with SRBC. The optimum dilution of antiserum was determined to be 1:20 (final concentration was 1:400).

Two chambers on a modified microscope slide were filled (30–40 μl) with the above mixture using a microliter syringe. The chambers were sealed with petroleum jelly and incubated at 37° for 45–60 min. Plaques were counted using a 6.3 or 10 × planacromatic objective on a light microscope.

Tissue residue analysis for mercury was performed on kidneys after digestion in nitric acid and 30% hydrogen peroxide and analyzed by cold vapor technique using a Varion Model 1200 atomic absorption spectrophotometer.

Results. Antibody synthesis was decreased at all levels of mercury exposure after a single inoculation of antigen (Fig. 1, primary response). The threefold decrease in numbers of plaques of antibody-producing cells from animals that received 10 ppm methylmercury was highly significant ($P < 0.01$). Antibody synthesis as measured by the secondary immune response tended to be suppressed in animals fed 1 or 10 ppm methylmercury, but these values were not statistically significant as compared to those of controls (Fig. 2, secondary response). Antibody response in the 5 ppm group was similar to that of controls.

Tissue residues of mercury increased as dietary methylmercury concentrations increased (Table I).

Discussion. Methylmercury, 10 ppm, fed to mice for 10 weeks after weaning significantly ($P < 0.01$) suppressed the primary immune response (IgM). The secondary response (IgG) was impaired, but not significantly. Studies by other investigators (16) demonstrated suppression of both primary and secondary immune responses in mice that had been exposed to methylmercury during embryonic development and until 9

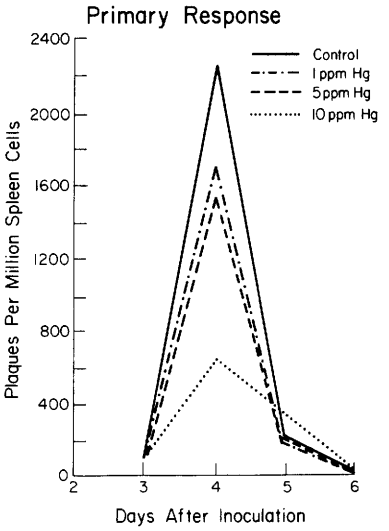

FIG. 1. Primary immune response. Methylmercury, 10 ppm, produced a highly significant ($P < 0.01$) decrease in antibody-forming cells at Day 4 as determined by one-way analysis of variance (LSD at Day 4 = 594).

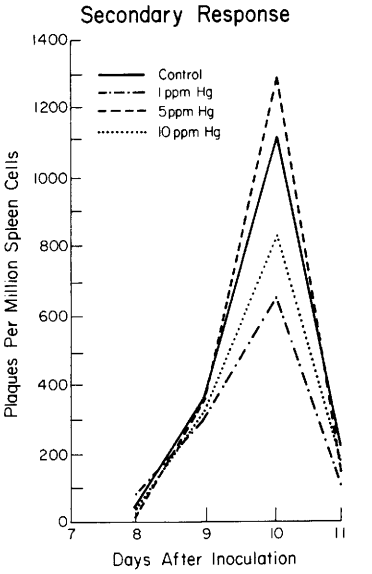

FIG. 2. Secondary immune response. Antibody synthesis was inhibited by 1 and 10 ppm methylmercury, but was not statistically significant by one-way analysis of variance (LDS at Day 10 = 637).

TABLE I. RENAL CONCENTRATIONS OF MERCURY FROM MICE FED METHYLMERCURY FOR 10 WEEKS.[a]

Methylmercury in feed (ppm)	Total mercury in kidney (ppm)
0	0.17
1	11.32
6	31.44
12	42.86

[a] Kidneys were analyzed as pooled samples of 10 kidneys.

weeks of age. However, splenic lymphocytes from mice that had been given methylmercury at weaning age and continued on methylmercury for 12 weeks did not elicit a statistically significant reduction in antibody synthesis. Accordingly, the secondary immune response of mice in our study was not significantly suppressed. Rabbits exposed to 10 ppm methylmercury also had a greater suppression of primary than secondary immune response (13). These results indicate that methylmercury affects the B-cell or plasma-cell synthesis of IgM antibody more than IgG antibody production. It also appears that exposure to methylmercury during early development may more greatly affect the immune response than exposure occuring after weaning.

In a previous study, mortality rates of mice exposed to 1 or 10 ppm methylmercury in the feed for 10 weeks and then inoculated with encephalomyocarditis virus (EMCV) were greater than those in control animals given the same dose of virus (12). Mortality was greater in the 10 ppm than in the 1 ppm group. It was postulated that suppression of the immune system by methylmercury contributed to increased mortality. This study supports that hypothesis, since suppression of the primary antibody response was observed in mice exposed to both 10 and 1 ppm methylmercury. Antibody suppression was greater in the 10 ppm group. Therefore, inhibition of antibody synthesis by methylmercury, even at subclinical concentrations, could contribute to increased mortality when an animal is exposed to an infectious agent.

Extropolation of these and previous data to people suggest that subclinical doses of methylmercury may be indirectly harmful to health. The embryo or neonate may be especially susceptible since exposure occurs during critical periods of development of the immune system.

Summary. Antibody synthesis was decreased in mice fed 1, 5, or 10 ppm methylmercury for 10 weeks. The primary immune response was significantly suppressed, while the secondary response was impaired but not significantly. These results indicate that methylmercury affects the B-cell or plasma-cell synthesis of IgM antibody.

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