Effects of 2,3-Dimercapto-1-propanesulfonic Acid (DMPS) on Tissue and Urine Mercury Levels following Prolonged Methylmercury Exposure in Rats

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Methylmercury, a potent neurotoxicant, accumulates in the brain as well as the kidney during chronic exposure. We evaluated the capacity of 2,3-dimercapto-1-propanesulfonic acid (DMPS), a tissue-permeable metal chelator, to reduce brain, kidney, and blood Hg levels and to promote Hg elimination in urine following exposure of F-344 rats to methylmercury hydroxide (MMH) (10 ppm) in drinking water for up to 9 weeks. Inorganic (Hg\(^{2+}\)) and organic (CH\(_3\)Hg\(^+\)) mercury species in urine and tissues were assayed by cold vapor atomic fluorescence spectroscopy (CVAFS). Following MMH treatment for 9 weeks, Hg\(^{2+}\) and CH\(_3\)Hg\(^+\) concentrations were 0.28 and 4.80 \(\mu g/g\) in the brain and 51.5 and 42.2 \(\mu g/g\) in the kidney, respectively. Twenty-four hours after ip administration of a single DMPS injection (100 mg/kg), kidney Hg\(^{2+}\) and CH\(_3\)Hg\(^+\) declined 38% and 59%, whereas brain mercury levels were slightly increased, attributable entirely to the CH\(_3\)Hg\(^+\) fraction. Concomitantly, Hg\(^{2+}\) and CH\(_3\)Hg\(^+\) in urine increased by 7.2- and 28.3-fold, respectively, compared with prechelation values. A higher dose of DMPS (200 mg/kg) was no more effective than 100 mg/kg in promoting mercury excretion. In contrast, consecutive DMPS injections (100 mg/kg) given at 72-h intervals significantly decreased total mercury concentrations in kidney, brain, and blood. However, the decrease in brain and blood mercury content was restricted entirely to the CH\(_3\)Hg\(^+\) fraction, consistent with the slow dealkylation rate of MMH in these tissues. Mass balance calculations showed that the total amount of mercury excreted in the urine following successive DMPS injections corresponds quantitatively to the total amount of mercury removed from the kidney, brain, and blood of MMH-exposed rats. These findings confirm the efficacy of consecutive DMPS treatments in decreasing mercury concentrations in target tissue and in reducing overall mercury body burden. They demonstrate further that the capacity of DMPS to deplete tissue Hg\(^{2+}\) is highly tissue-specific and reflects the relative capacity of the tissue for methylmercury dealkylation. In light of this observation, the inability of DMPS to reduce Hg\(^{2+}\) levels in brain or blood may explain the inefficacy of DMPS and similar chelating agents in the remediation of neurotoxicity associated with prolonged MMH exposure.

Key Words: 2,3-dimercapto-1-propanesulfonic acid (DMPS); chelation; mercury; methylmercury; mercuric ion; brain; blood; kidney.

Methylmercury, a potent neurotoxicant, readily penetrates the blood-brain barrier through specific carrier-mediated transport systems (Miura et al., 1994). CNS damage by methylmercury results from preferential accumulation in focal parts of the brain including the cerebellum and visual cortex, where it is slowly dealkylated to Hg\(^{2+}\) (Berlin, 1986). Degeneration and atrophy of the sensory cerebral cortex, leading to paraesthesia, ataxia, and hearing and visual impairment in the adult during chronic methylmercury exposure have also been reported (Miura et al., 1994). Prenatal exposure can cause cerebral palsy, psychomotor retardation, delayed development, and cognition changes (Clarkson, 1997). During prolonged exposure, methylmercury may also accumulate in the kidney and promote renal toxicity (Woods et al., 1991).

A specific concern associated with methylmercury exposure in humans is the need for effective therapy in dealing with intoxication. In this respect, chelation therapy is the most commonly used and seen as the least invasive (Aposhian, 1983). Chelating agents compete with the in vivo binding site for the metal ion through the process of ligand exchange (Jones, 1994). The toxic metal bound to the chelating agent is excreted from the body through the urine or feces. Among chelating agents currently available, the sodium salt of 2,3-dimercapto-1-propane-sulfonate (DMPS) has been found to be highly effective, particularly with respect to promoting mercury elimination following inorganic or elemental mercury exposure (Aaseth et al., 1995; Aposhian 1983; Garza-Ocanas et al., 1997; Gonzalez-Ramirez et al., 1995; Maiorino et al., 1996). Remaining to be confirmed is the efficacy of DMPS in reduction of mercury body burden resulting from organic mercury exposure, and in particular, its value in the removal of inorganic Hg (Hg\(^{2+}\)) arising from dealkylation of methylmercury in target tissues.

In the present studies, we evaluated the efficacy of DMPS in reducing Hg\(^{2+}\) and CH\(_3\)Hg\(^+\) from kidney, brain, and blood mercury levels following prolonged methylmercury exposure in rats. As part of this assessment, we performed mass balance calculations to demonstrate the quantitative relationship between mercury removed from each tissue and that excreted in the urine. We also employed linear regression analysis to...
assess the strength of association between tissue mercury concentrations and urinary mercury levels in relation to DMPS treatments.

**MATERIALS AND METHODS**

**Materials.** Male Fischer-344 rats (200–225 g) were purchased from Simonsen Labs (Gilroy, CA). Methylmercury (II) hydroxide (CH$_3$HgOH, MMH) was purchased from Alfa Aesar (Ward Hill, MA), 2,3-Dimercaptopropanesulfonic acid (DMPS) was obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals were reagent grade and were purchased from standard commercial sources. Distilled deionized water was used for preparation of all aqueous solutions.

**Animal treatment.** Immediately upon receipt, all animals were placed in individual, wire bottom, hanging cages and given deionized water and food (Wayne Rodent Blox rat chow) *ad libitum*. This diet has been shown to be free of mercury content. Lighting was set at a 12-h light/dark cycle, and the temperature of the animal housing facility was kept at 20°C. Following 1-week acclimation, the animals were separated into 2 groups (described below) and given either deionized water or drinking water containing 10 ppm MMH. This dose of MMH was selected from previous studies (Fowler and Woods, 1977; Woods and Fowler, 1977) as sufficient to permit significant target tissue accumulation without evidence of overt neurotoxicity or renal injury during the course of the treatment regimen.

**Study design.** Two prolonged methylmercury-exposure studies were undertaken. Study 1 was designed to evaluate the efficacy of DMPS dosage on mercury clearance from brain and kidney. In this study, 30 rats were divided into 2 groups of 18 and 12 each. The larger group of 18 animals was placed on a continuous regimen of drinking water containing 10 ppm MMH, whereas the remaining 12 animals were placed on deionized water (dH$_2$O) as controls. After 9 weeks, all 30 rats were transferred to individual metabolism cages for 24-h urine collections. Immediately thereafter, the 18 animals that had received MMH for 9 weeks were divided randomly into 3 groups of 6 each. The first 2 groups of 6 animals received single ip injections of DMPS at either 100 mg/kg or 200 mg/kg, respectively, whereas the third group received saline injections. Concurrently, the 12 control rats that had been maintained for 9 weeks on dH$_2$O were divided into 3 groups of 4 each and given comparable injections of 100 or 200 mg/kg DMPS or saline. Subsequently, all rats were returned to individual metabolism cages for post-treatment 24-h urine collections. All animals were then sacrificed, and tissues were collected for mercury assessment.

The second study (Study 2) was designed to assess the effect of consecutive DMPS treatments on mercury clearance from brain, kidney, and blood of MMH-exposed rats. In this study, 30 rats were placed on a continuous regimen of drinking water containing 10 ppm MMH for 6 weeks. The shorter MMH exposure time in Study 2 was selected based on the observation from Study 1 that the equilibrium between organic and inorganic mercury species in kidney cortex is achieved by 6 weeks of MMH exposure (described in Results). Hence, the prechelation concentrations of both Hg$^{2+}$ and CH$_3$Hg$^+$ in renal cortex are sufficient following 6 weeks of MMH exposure to permit evaluation of the efficacy of DMPS chelation in clearing both organic and inorganic mercury species from kidney, as well as from brain and blood.

To determine the effects of multiple DMPS treatments on tissue mercury levels, animals were given up to 3 DMPS injections over a period of 3–7 days prior to sacrifice. For this study, the 30 MMH-exposed rats were divided into 2 groups of 18 and 12 animals, respectively. The first group of 18 rats received a single ip injection of 100 mg/kg DMPS, whereas the remaining 12 were given a saline injection, as controls. All 30 animals were then transferred to individual metabolism cages for 24-h urine collections. Rats were denied food but were provided dH$_2$O *ad libitum* during the urine collection period. Following urine collections, 6 animals from the DMPS-treatment group and 4 rats from the control group were sacrificed, and brains, kidneys, and blood were retrieved for mercury analyses. Seventy-two hours after the first injection, the remaining 12 DMPS-treated rats were given a second 100 mg/kg DMPS injection, while the remaining 8 saline-treated rats were given a second saline injection. After 24 h, 6 of the DMPS-treated rats and 4 of the control rats were sacrificed and tissues collected. Seventy-two h after the second injection the remaining animals were given a third DMPS or saline treatment. Twenty-four h after the third injection all remaining rats were sacrificed and tissues collected. Between DMPS treatments, animals were held in metabolism cages without food but with dH$_2$O for 24-h urine collections and then returned to their hanging cages and permitted food and water *ad libitum* for 48 hours. No animals were deprived of food for more than 24 hours during the treatment period. In all studies, animals were anesthetized by carbon dioxide (CO$_2$) and then sacrificed by decapitation. Blood was obtained by cardiac puncture into heparinized tubes prior to sacrifice. Brains and kidneys were harvested surgically immediately following sacrifice. All tissues were preserved at −80°C until mercury analysis.

**Collection of urine samples.** Animals were placed in hanging metabolism cages for 24 h with free access to drinking water (containing either MMH or dH$_2$O) but not food. The metabolism cages were fitted with metal funnels attached to the bottom with a plastic ping-pong ball placed at the hole of the funnel to allow urine but not feces to pass through. Aluminum foil-covered, polypropylene 125-ml volumetric flasks were placed under the funnels to collect the urine without allowing evaporation. At the end of 24 h the urine volume was measured. The urine was then acidified with a drop of 6 N HCl and frozen at −20°C until mercury analysis.

**Mercury determination.** Urinary mercury was measured using a modified version of the digestion method by Corns et al. (1994). 2.5 ml of HCl and 2-ml bromate/bromide solution was added to 2.5-ml urine sample and allowed to sit overnight in a 20-ml glass scillation vial. Hydroxylammonium chloride (20%) was then added to decolorize the sample and to stop the digestion process. The fully digested sample was transferred to a 50-ml borosilicate glass volumetric flask, and distilled water was added to a total volume of 50 ml. The total (organic and inorganic) mercury content of the sample was then analyzed by cold vapor atomic fluorescence spectroscopy (CVAFS) using a PSA Merlin Mercury Analysis System (Questron Corp., Mercerville, NJ). For inorganic mercury (Hg$^{2+}$) determinations, a 0.5-ml sample of urine was digested overnight in 2 ml of HNO$_3$. The sample was then diluted to a volume of 20 ml with dH$_2$O, and the total Hg$^{2+}$ content was measured using CVAFS. The organic mercury content of the sample was determined by the difference in the total and inorganic mercury values.

The total and inorganic mercury concentrations in kidney and brain tissues were analyzed by CVAFS following digestion and preparation of tissues as described by Atallah and Kalman (1993). The organic mercury content of tissue samples was again determined by the difference in the total and inorganic mercury concentrations.

Total and organic mercury concentrations in blood samples were directly measured by ethylation-GC-CVAFS after alkaline digestion-solvent extraction, as described by Liang et al., 2000. Inorganic mercury was calculated as the difference between total and organic mercury.

For each of the above cited procedures, validation of Hg$^{2+}$ and CH$_3$Hg$^+$ analysis was confirmed by concomitant measurement of control urine or tissue samples containing a range of known concentrations of Hg$^{2+}$, CH$_3$Hg$^+$ or total mercury (Hg$^{2+}$ + CH$_3$Hg$^+$) derived from standard reference materials. Mean recoveries of Hg$^{2+}$ and CH$_3$Hg$^+$ from spiked tissues were on the order of 95–100% with no detectable cross contamination of mercury species, consistent with findings reported by original authors. All procedures employed specific quality control protocols, including pre-analysis of all reagents and materials used in the analyses, precluding potential Hg$^{2+}$ or CH$_3$Hg$^+$ contamination from sources other than biological samples under evaluation.

**Statistical analyses.** Data are presented as means ± standard error of the mean (SEM). Statistical analyses were conducted using Student’s *t*-test with 1-tailed distribution. *p* values less than 0.05 were considered significant. Correlational analysis was performed using the Excel function (Microsoft, Redmond, WA) and expressed as the correlation coefficient.
RESULTS

DMPS Promotes Urinary Excretion of Both Organic and Inorganic Mercury Species

In Study 1 we evaluated the effects of DMPS dosage on kidney and brain mercury levels and corresponding urinary excretion of inorganic and organic mercury species following prolonged MMH exposure in rats. As shown in Table 1, inorganic and organic mercury concentrations in the urine of the MMH-exposed animals before DMPS treatment were approximately equivalent, consistent with previous observations (Woods et al., 1991). Following treatment with DMPS at either 100 or 200 mg/kg ip, urinary concentrations of both inorganic and organic mercury species increased significantly, compared to those of MMH-treated rats given a saline injection. The inorganic and organic mercury concentrations in urine of the animals given 100 mg/kg DMPS increased 9.5- and 15.1-fold, respectively, compared with those given saline injections. Notably, however, administration of 200 mg/kg DMPS did not promote a further increase in the concentration of either inorganic or organic mercury excreted in the urine, compared to that observed following treatment with 100 mg/kg DMPS. Also of interest is the observation that DMPS treatment at either the 100 or 200 mg/kg dose level resulted in a detectable increase in both inorganic and organic mercury concentrations in the urine of rats exposed only to dH₂O, compared to those receiving a saline injection. Organic mercury levels were significantly increased in urine of dH₂O-exposed rats following DMPS treatment, suggesting the efficacy of DMPS in the clearance of even very low levels of mercury derived from ambient exposures.

A Single Dose of DMPS Reduces Kidney but Not Brain Mercury Concentrations

Additional studies were undertaken to determine the corresponding effects of DMPS dosage on kidney and brain mercury levels. In previous studies (Woods et al., 1991) we have demonstrated that mercury accumulates in the kidney as well as the brain during prolonged MMH exposure. Moreover, methylmercury is demethylated to inorganic mercury (Hg²⁺) in the renal cortex, such that steady state equilibrium of organic and inorganic mercury species is sustained over a wide range of total mercury concentrations. Studies described here confirm this finding. As shown in Table 2, the mean concentrations of Hg²⁺ and CH₃Hg⁺ were 51.5 and 42.2 mg/g of renal cortex, respectively, in saline-injected rats, representative of prechelation kidney mercury concentrations following MMH exposure for 9 weeks. Treatment of MMH-exposed rats with DMPS at 100 mg/kg decreased both inorganic and organic mercury concentrations to 37.2 mg/g (73%) and 26.5 mg/g (63%) of prechelation levels, respectively. Treatment with DMPS at the higher dose of 200 mg/kg produced a somewhat greater reduction in the kidney concentrations of both inorganic and organic mercury species, to 33.4 mg/g (65%) and 18.9 mg/g (45%) of prechelation values, respectively. Treatment with DMPS at the higher dose of 200 mg/kg produced a somewhat greater reduction in the kidney concentrations of both inorganic and organic mercury species, to 33.4 mg/g (65%) and 18.9 mg/g (45%) of prechelation values, respectively. However, differences in effects of treatment at 100 versus 200 mg/kg were not statistically significant.

Unlike as observed with the kidney, treatment of 9-week MMH-exposed rats with a single dose of DMPS at either 100 mg/kg or 200 mg/kg was largely ineffective in reducing brain mercury levels. As shown in Table 2, the mean concentrations of Hg²⁺ and CH₃Hg⁺ were 0.28 and 4.8 µg/g of whole brain,
respectively, in saline-injected rats, representative of prechelation brain mercury concentrations following 9 weeks of MMH exposure. Of note, mercury in the brain was predominantly (95%) \( \text{CH}_3 \text{Hg}^+ \), indicative of the relatively slow dealkylation of MMH to \( \text{Hg}^{2+} \) in neuronal tissues (Berlin, 1986). Treatment of rats with a single dose of DMPS at 100 mg/kg appeared to slightly increase the brain concentrations of both organic and inorganic mercury constituents. However, the inorganic and organic mercury concentrations in brains of rats given a 200 mg/kg DMPS injection were not significantly different from concentrations found prior to chelation.

Consecutive DMPS Injections Promote Successive Urinary Elimination of Both Organic and Inorganic Mercury Species

We conducted Study 2 to assess the effects of consecutive DMPS treatments on mercury clearance from kidney, brain and blood of MMH-exposed rats and to measure the corresponding changes in urinary mercury levels. In these studies 30 rats were exposed to MMH in drinking water for 6 weeks, followed by 1, 2, or 3 consecutive DMPS treatments (100 mg/kg, ip) at 72-h intervals, as described in Materials and Methods. As shown in Figure 1, consecutive DMPS treatments were highly effective in promoting successive release of both organic and inorganic forms of mercury into the urine. The inorganic and organic mercury concentrations in the urine of MMH-exposed animals prior to DMPS injection were 1.13 and 1.17 mg/ml, respectively, compared with 0.01 and 0.00 mg/ml, respectively, in rats exposed only to \( \text{dH}_2\text{O} \). Following a single DMPS injection, the total mercury concentration in 24-h urine samples increased 18-fold, with 6- and 12-fold increases in inorganic and organic species, respectively, compared with that observed in urine of MMH-exposed rats receiving a saline injection.

### TABLE 2

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Treatment</th>
<th>Kidney (( \mu g/g ))</th>
<th>Brain (( \mu g/g ))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( \text{Hg}^{2+} )</td>
<td>( \text{CH}_3\text{Hg}^+ )</td>
</tr>
<tr>
<td>MMH</td>
<td>Saline</td>
<td>51.48</td>
<td>42.19</td>
</tr>
<tr>
<td>MMH</td>
<td>DMPS</td>
<td>(0.94)</td>
<td>(0.50)</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>DMPS</td>
<td>37.24*</td>
<td>26.52*</td>
</tr>
<tr>
<td>MMH</td>
<td>DMPS</td>
<td>(1.28)</td>
<td>(0.56)</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>DMPS</td>
<td>33.40*</td>
<td>18.88*</td>
</tr>
<tr>
<td>( \text{dH}_2\text{O} )</td>
<td>Saline</td>
<td>0.09</td>
<td>0.02</td>
</tr>
<tr>
<td>( \text{dH}_2\text{O} )</td>
<td>DMPS</td>
<td>(0.00)</td>
<td>(0.00)</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>DMPS</td>
<td>0.03*</td>
<td>0.03</td>
</tr>
<tr>
<td>( \text{dH}_2\text{O} )</td>
<td>DMPS</td>
<td>(0.00)</td>
<td>(0.00)</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>DMPS</td>
<td>0.02*</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Note. The mean mercury concentrations (± SEM) in the kidney and brain following 9 weeks of MMH exposure and after DMPS or saline injection are presented. Exposure and treatment regimens are as described in Table 1. The mercury levels are shown for each exposure and treatment group. *Significantly different from the saline-injected group (\( p \leq 0.05 \)).
second DMPS injection administered 72 h following the first was equally effectively in promoting mercury excretion, the total urinary mercury concentration increasing to 27 times that observed in MMH-exposed animals receiving 2 consecutive saline injections. A third DMPS injection given 72 h after the second promoted additional excretion of both organic and inorganic mercury species, the total urinary mercury concentration increasing another 18-fold that seen in urine of saline-injected controls. Quantitatively, mean total urine mercury concentrations following the first, second, and third DMPS injections were 24.8, 25.6, and 14.4 μg/ml, respectively. The sum of total mercury excreted in the urine following all 3 DMPS injections was 64.8 μg/ml, compared with 4.4 μg/ml after 3 consecutive saline injections. Creatinine adjustment of urinary mercury concentrations showed a slightly more pronounced mercury excretion trend associated with the number of DMPS injections when compared with that of the noncreatinine adjusted results, although no trend in creatinine excretion in the urine following DMPS administration was seen (not shown). DMPS treatments did not affect mean total urine 24-h excretion rates, which remained constant (12.2 ± 1.3 ml/24 h) throughout the treatment regimen.

**Consecutive DMPS Treatments Were Effective in Promoting Release of Mercury from the Kidney, Brain and Blood of MMH-Exposed Rats**

Figure 2 represents changes in kidney mercury concentrations following each of the 3 consecutive DMPS injections. Prior to chelation, the total mercury concentration found in kidneys of MMH-exposed rats given a saline injection was 75.6 ± 5.7 μg/g. The kidney total mercury concentration decreased significantly following each DMPS treatment. A single DMPS injection produced a decrease in total kidney mercury content to 55.2 μg/g, approximately 73% of that found in kidneys of saline-treated controls. Successive DMPS treatments produced additional reductions in total mercury content to 59 and 55% of remaining control levels, respectively. Organic and inorganic mercury constituents appeared to be cleared to a similar extent following consecutive DMPS treatments, suggesting comparable availability of Hg²⁺ and CH₃Hg⁺ for ligand exchange with thiol groups of DMPS in kidney cells. Notably, a slight (but not significant) increase in both inorganic and organic mercury levels was seen in kidneys of MMH-exposed animals following the first and second saline injections (stippled bars in Fig. 2). This trend possibly represents continued partitioning of mercury from blood to kidneys following cessation of mercury exposure.

The effects of consecutive DMPS treatments (100 mg/kg) on brain mercury levels are described in Figure 3. Similar to observations made in Study 1 (Table 2), brain total mercury content increased slightly within the 24-h period following a single DMPS injection. This increase appeared to be attributable principally to the organic mercury fraction. However, as shown in Figure 3, brain mercury levels decreased following subsequent DMPS treatments, the most notable decrease occurring after the third DMPS injection. Both total mercury and the organic mercury fraction were decreased to approximately 60% of levels found in brains of MMH-exposed rats receiving
3 consecutive saline injections. The inorganic mercury concentration remained largely unchanged with successive DMPS treatments. As noted previously, however, inorganic mercury comprises less than 10% of total mercury found in the brain of rats following prolonged MMH exposure.

Further studies were conducted to evaluate the effects of consecutive DMPS injections on blood mercury levels. As shown in Figure 4, mercury in the blood of MMH-exposed rats is present principally as the organic form, comparable to that observed in the brain. DMPS was highly effective in reducing
blood mercury levels. As with the brain, the decrease in blood mercury was attributable principally to the organic mercury content. A single DMPS injection reduced the blood total mercury concentration to 73% of that found in blood of MMH-exposed rats given a saline injection. Second and third consecutive DMPS treatments reduced the total blood mercury content by an additional 50 and 40% of the remaining blood mercury levels, respectively. Inorganic mercury concentrations in both the saline- and DMPS-injected animals appeared to increase slightly with the first and second consecutive DMPS injections and to decline after the third. However, these changes were not statistically significant. Second and third DMPS injections decreased the organic mercury fraction in blood to 53 and 61%, respectively, of that in blood of MMH-exposed rats given a saline injection.

The Increase in Urinary Mercury Corresponds Quantitatively to Reduction in Tissue Mercury Concentrations following DMPS Treatment

Mass balance analysis was performed to calculate the amount of total mercury removed from kidney, brain and blood following each DMPS injection compared with the amount of total mercury eliminated in the urine. The amount of mercury eliminated following each DMPS treatment was calculated in μg of mercury excreted in 24 h of urine collection after injection. Computations are presented in Table 3. Following consecutive DMPS treatments, the average concentration of mercury excreted after the previous injection was added to the amount found in the present 24-h collection to determine the total cumulative amount of mercury excreted following each consecutive injection. The mercury eliminated from each tissue was calculated by taking the average mercury concentration found in the tissue of the saline-treated animals (representing the prechelation mercury content), and subtracting the average mercury concentration found in the tissue following DMPS treatment. This value was then multiplied by the mean mass of the tissue for kidney (2.0 g) and brain (1.8 g), or the volume of the blood of the average adult male rat (13.5 ml) (Davies and Morris, 1993). This value represents the total amount of mercury that was eliminated from the tissue following DMPS injections. As shown in Table 3, the amount of mercury excreted in the urine following the first DMPS injection accounted for approximately 76% of the total amount of mercury eliminated from kidney, brain, and blood. The amount of mercury excreted in the urine following the second and third DMPS injections, however, exceeded the amount of mercury eliminated from kidney, blood, and brain, possibly reflecting depletion of mercury from other body stores (e.g., the hepatobiliary system or fatty tissues) with consecutive DMPS treatments.

Urinary Mercury Content following Successive DMPS Treatments Corresponds to Mercury Body Burden

Finally, linear regression analysis was performed and correlation coefficients (r) were calculated to assess the strength of the association between tissue mercury body burden and urinary mercury concentrations before and after successive DMPS treatments. Computations are presented in Table 4. Tissue mercury levels measured in saline-injected rats were employed as prechelation mercury levels. For these assessments, mercury body burden was defined as the amount of total mercury removed from (1) the kidney, (2) the kidney and brain combined, and (3) the kidney, brain and blood combined (prechelation – postchelation values) after 6 weeks of MMH exposure (Study 2). Mercury excretion following consecutive DMPS injections was calculated by adding the average total 24-h mercury concentration in the urine after the first, second, or third consecutive DMPS injection. As seen in Table 4, all 3 measures of mercury body burden were found to be strongly inversely correlated with the amount of mercury excreted in the urine following the first DMPS injection (r ~ -0.65 to -0.84), as well as for kidney and kidney plus brain following the second DMPS treatment (r ~ -0.79 to -0.85). Weaker associations were found with all 3 measures, however, following the third DMPS treatment. The correlation was inversely proportional, indicating that, as the mercury excreted in the urine increases, the mercury present in tissues decreases. Notably, these strong correlations were retained for all 3 measures of...
body burden when evaluated in relation to $\text{CH}_3\text{Hg}^+$ alone, consistent with the ability of DMPS to effectively reduce the organic mercury concentration of all 3 tissues. In contrast, the addition of brain and blood to the kidney as measures of body burden resulted in weaker associations for total mercury and $\text{Hg}^{2+}$, reflecting the lack of effect of DMPS on $\text{Hg}^{2+}$ in these tissues. As expected, the correlation between mercury body burden and prechelation urinary mercury excretion (i.e., saline-injected) was weak in each case. These findings support the established view that the post-DMPS chelation urinary mercury concentration is more representative of mercury body burden than prechelation levels (Aposhian et al., 1995; Echeverria et al., 1998).

**DISCUSSION**

Numerous studies have documented the efficacy of DMPS in promoting the elimination of mercury and other heavy metals in animals and human subjects (Aaseth et al., 1995; Aposhian et al., 1992; Cherian et al., 1988; Clarkson et al., 1981; de la Torre et al., 1996; Garza-Ocanas et al., 1997; Hurlbut et al., 1994; Keith et al., 1997). Particular attention in this respect has been paid to DMPS as an effective chelator of inorganic mercury derived from exposure to elemental or inorganic mercury compounds. Few studies, however, have sought to determine the efficacy of DMPS in clearance of mercury species from target tissues following prolonged exposure to organic mercury compounds. The present studies demonstrate that DMPS effectively clears both organic and inorganic mercury species from target tissues of rats following exposure to MMH for prolonged periods. However, the efficacy of DMPS in this respect appears to reflect of the relative capacity of the specific tissue for metalmercury dealkylation. Moreover, the efficacy of DMPS as a mercury chelator appears to be related to consecutive administration in consistent dosages, rather than to the magnitude of the dose received.

The dissociation of methylmercury into organic and inorganic species in the kidney during prolonged exposure to MMH in rats has been previously described (Woods et al., 1991). Of note, an equilibrium between $\text{Hg}^{2+}$ and $\text{CH}_3\text{Hg}^+$ concentrations in the kidney cortex is readily achieved in a dose- and time-dependent manner during the course of continuous MMH exposure, and this equilibrium is maintained by the kidney over a wide range of total mercury concentrations to approximately 100 $\mu$g/g cortex. Inasmuch as both $\text{Hg}^{2+}$ and $\text{CH}_3\text{Hg}^+$ may participate in renal toxicity, it is notable that DMPS is equally effective in depleting both species from kidney tissue. Analysis of the data presented here shows a direct correlation between the increases in the urinary concentrations of both organic and inorganic mercury fractions and the decline in the concentrations of each species in kidney following DMPS treatment. These findings are consistent with the very high ligand exchange rates ($10^9$/s) of both $\text{Hg}^{2+}$ and $\text{CH}_3\text{Hg}^+$ for thiol groups (Martin, 1986), and suggests comparable availability of organic and inorganic Hg species for exchange with DMPS in kidney cells.

Consistent with the effects on renal mercury content, DMPS effectively reduced blood total mercury concentrations over the course of consecutive treatments. Unlike in the kidney, however, less than 5% of total mercury in the blood was present as $\text{Hg}^{2+}$. The effect of DMPS in lowering total blood mercury was restricted entirely to the organic mercury constituent, which decreased significantly following the second and third consecutive DMPS injections. Previous studies (Planas-Bohne, 1981) have demonstrated that DMPS is moderately effective in removing methylmercury from blood following administration to rats. However, the effects of DMPS on the inorganic Hg fraction derived from demethylation of methylmercury in blood cells was not reported. This is the first report to our knowledge to assign the effect of DMPS on blood mercury to the organic fraction.

The dissociation of methylmercury into organic and inorganic species in the brain during prolonged exposure of rats to MMH was also noted in the present study, although the formation of $\text{Hg}^{2+}$ from MMH was substantially lower than that observed in kidney. In this respect, the changes in total brain mercury content observed during the course of MMH exposure appeared to largely reflect the $\text{CH}_3\text{Hg}^+$ constituent, similar to that observed in blood. Berlin (1986) reported that approximately 5–10% of methylmercury is demethylated to $\text{Hg}^{2+}$ in the brain, consistent with this observation. Of note, DMPS was effective in decreasing total brain mercury content in MMH-
exposed rats only following 3 consecutive treatments. Similar findings have been reported with respect to elemental mercury exposure (Cikrt et al., 1996). The initial increase in brain mercury content seen following a single DMPS injection is of interest, inasmuch as it suggests that DMPS may facilitate redistribution of CH$_3$Hg$^+$ from blood to brain until blood mercury levels are concomitantly depleted by subsequent DMPS treatments, or until DMPS accumulates in sufficient concentrations in the CNS to effect significant mercury chelation. Notably, the partitioning of DMPS into the CNS is relatively slow due to its polarity and limited lipid solubility (Jones, 1994), consistent with this idea. The failure of DMPS to cause significant depletion of Hg$^{2+}$ from the brain may reflect the slow rate of demethylation of methylmercury in the CNS and consequent low concentrations of Hg$^{2+}$ relative to those of CH$_3$Hg$^+$ available for exchange with DMPS. Alternatively, the efficacy of DMPS in extracting CH$_3$Hg$^+$ may reflect the greater availability of organic mercury following distribution from the blood to the CNS, as compared with that of Hg$^{2+}$, which most likely is formed following compartmentalization of methylmercury to specific regions of the CNS that may be poorly accessible to DMPS. The latter possibility raises concerns regarding the efficacy of DMPS or similar chelating agents in the remediation of neurotoxicity associated with methylmercury, since principal adverse effects may be attributable to Hg$^{2+}$ accumulation following partitioning of methylmercury to specific CNS foci during prolonged exposure (Aschner and Aschner, 1990; Friberg and Mottet, 1989). In contrast to the present findings, Aposhian et al. (1996) reported that DMPS did not alter brain mercury levels in rats treated by ip injection with HgCl$_2$. This distinction may reflect differences in the capacity of DMPS complexes of Hg$^{2+}$ versus those of CH$_3$Hg$^+$ to partition from blood into the CNS.

Although total mercury tissue levels increased significantly during the course of MMH exposure, the mercury concentration in the urine increased only slightly during this period (Table 1 and Fig. 1). These findings are consistent with the view that prechelation urinary excretion is not well correlated with mercury body burden (Aposhian et al., 1995). In contrast, urinary mercury levels subsequent to DMPS chelation have been shown to constitute a better approximation of Hg body burden, particularly in the case of elemental mercury exposure (Echeverria et al., 1998). Mass balance calculations performed in the present investigation extend these findings to organic mercury exposure, since the amount of mercury excreted in the urine following consecutive DMPS treatments accounted for essentially all of the mercury found in blood and the 2 principal target tissues, kidney, and brain. Notably, essentially all of the inorganic mercury excreted postchelation was of renal origin, since DMPS treatment did not significantly reduce Hg$^{2+}$ concentrations in either blood or brain. These findings support the view that postchelation urinary mercury levels are an accurate measure of mercury body burden attributable to prolonged methylmercury exposure.

In conclusion, the present studies demonstrate the efficacy of DMPS in depleting mercury body burden and confirm previous observations regarding the utility of postchelation urinary mercury levels as an accurate measure of body burden attributable to prolonged methylmercury exposure. The finding that DMPS readily depletes both Hg$^{2+}$ and CH$_3$Hg$^+$ from kidney, but only CH$_3$Hg$^+$ from brain and blood, suggests that the capacity of DMPS to remove Hg species reflects the tissue-specific dealylation rate of MMH and, hence, the relative amount of species present. These findings may explain the relative inefficacy of DMPS and similar chelators in the remediation of neurotoxicity associated with organic mercury exposure, in which Hg$^{2+}$, a principal mediator of toxicity, is present in low concentrations.

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REFERENCES


