Influence of 2,3-Dimercaptopropane-1-Sulfonate (DMPS) and Meso-2,3-Dimercaptosuccinic Acid (DMSA) on the Renal Disposition of Mercury in Normal and Uninephrectomized Rats Exposed to Inorganic Mercury

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ABSTRACT
The effects of the water-soluble chelating agents 2,3-dimercapto-1-propane sulfonate (DMPS) and meso-2,3-dimercaptosuccinic acid (DMSA) on the renal disposition of inorganic mercury were studied in normal and uninephrectomized (NPX) rats injected (i.v.) with a nontoxic 0.5-μmol/kg dose of mercuric chloride (HgCl₂). When a 100-mg/kg dose of either DMPS or DMSA was injected (i.p.) 24 and 30 hr after treatment with HgCl₂, the renal concentration and burden of inorganic mercury decreased markedly in both normal and NPX rats during the 24 hr after the first dose of the respective chelating agent was administered. Treatment with DMPS was more effective than treatment with DMSA in reducing the renal burden of mercury in both groups of rats. The fall in the renal concentration and burden of mercury in both normal and NPX rats was due primarily to a decrease in the content of mercury in the renal cortex and outer stripe of the outer medulla. However, the decrease in the concentration of inorganic mercury in the outer stripe was significantly greater in NPX rats than in normal rats. Both chelating agents caused urinary excretion of mercury to increase significantly in normal and NPX rats. In association with the increased renal release of mercury in NPX rats, the urinary excretion of mercury per gram of kidney was significantly greater in NPX rats than in normal rats. These data indicate that the renal handling of DMPS and DMSA may be altered significantly after a substantial reduction in renal mass. Findings from the present study also show that treatment with DMPS, but not with DMSA, causes the content of mercury in the liver and cellular fraction of blood to decrease in normal and NPX rats. These findings indicate that there are significant differences in the extrarenal handling of these two chelating agents. The findings in the present study suggest that DMPS and DMSA are very effective agents in reducing the renal (and whole body) burden of inorganic mercury in normal and NPX rats.

Experimental evidence from rats indicates that the renal accumulation of inorganic mercury increases after renal mass is reduced significantly and compensatory renal growth has occurred (Zalups et al., 1987; Zalups and Diamond, 1987; Zalups and Lash, 1990; Zalups, 1991; Zalups and Cherian, 1992). This increase is due to enhanced accumulation of inorganic mercury in the outer medulla (Zalups et al., 1987; Zalups and Diamond, 1987), specifically in the outer stripe of the outer medulla (Zalups and Lash, 1990; Zalups, 1991; Zalups and Cherian, 1992). Recent histochemical findings have indicated that the increased accumulation of inorganic mercury in the outer stripe of the outer medulla is due to enhanced uptake and accumulation of inorganic mercury along pars recta (S3) segments of proximal tubules (Zalups, 1991). The increased renal accumulation and altered intrarenal distribution of mercury that occur in rats after unilateral nephrectomy appear to be related to a shift that occurs in the dose-effect relationship for the nephropathy that is induced by low nephrotoxic doses of mercuric chloride (Zalups and Diamond, 1987; Zalups and Lash, 1990).

In a recent study, it was shown that the water-soluble chelating agent DMPS can provide significant protection to both normal (sham-operated) and NPX rats from the nephropathy induced by a nephrotoxic dose of HgCl₂ when the DMPS is administered within 1 hr after treatment with HgCl₂ (Zalups et al., 1991). When a 10-mg/kg dose of DMPS was used, NPX rats were provided a greater level of protection than normal (sham-operated) rats. Data from this study indicate that the increased protection was related to increased elimination of inorganic mercury from renal tissue and increased urinary excretion of mercury. It was speculated that increased secretion of DMPS occurs along hypertrophied segments of the proximal tubule as a result of the compensatory adaptive mechanisms that ensue after a substantial reduction of renal mass.

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ABBREVIATIONS: DMPS, 2,3-dimercaptopropane-1-sulfonate; DMSA, meso-2,3-dimercaptosuccinic acid; NPX, uninephrectomized; HgCl₂, mercuric chloride.
Because compensatory renal growth has profound effects on renal transport and metabolic processes (Meyer et al., 1991), it is possible that the renal effects and handling of DMPS and other chelating agents are significantly altered after a reduction of renal mass. Thus one of the principal aims of the present study is to evaluate and compare the effects of DMPS and DMSA on the renal burden and intrarenal distribution of inorganic mercury in normal and NPX rats treated with a nontoxic dose of HgCl₂. DMSA, like DMPS, is a water-soluble di-thiol chelating agent that has been used clinically on patients exposed to mercurials (Aposhian, 1983; Aposhian and Aposhian, 1990; Aposhian et al., 1992). Both compounds have been shown to be effective in reducing the renal burden of mercury in normal rats (Planas-Bohne, 1981). Particular focus will be placed on how the effectiveness of these two chelating agents in reducing the renal concentration of mercury and increasing the urinary excretion of mercury is influenced by unilateral nephrectomy and compensatory renal growth.

Materials and Methods

Animals and Operative Procedures

Male Sprague-Dawley rats weighing 175 to 200 g were purchased from Harlan Sprague-Dawley (Indianapolis, IN). After several days of acclimation, the animals were divided into two main groups: one group of rats to be nephrectomized and the other to serve as a control.

Unilateral nephrectomy (removal of the right kidney) was performed according to the procedures established previously in this laboratory (Zalups et al., 1990; Zalups and Lash, 1990; Zalups et al., 1992). After surgery, NPX rats and control rats were housed individually in plastic cages for 12 days to allow for the completion of the rapid phase of compensatory renal growth in the NPX rats (Zalups et al., 1990; Zalups and Lash; Zalups et al., 1992). Water and a commercial laboratory diet for rats (Teklad 6% mouse/rat diet, Harlan Sprague-Dawley, Indianapolis, IN) were provided ad libitum during all phases of the study.

Groups and Treatments

On the morning of the 13th day after surgery, all the NPX and control rats were given a single intravenous 0.5-μmol/kg nontoxic dose of HgCl₂ delivered in 2 ml of 0.9% aqueous sodium chloride per kilogram of body weight. The injection solution also contained 5 μCi/ml of [³²⁰Hg]Cl₂ (Buffalo Materials Corp., Buffalo, NY), which had a specific activity of 2.03 mCi/mg at the time of experimentation. Before administration of the dose of HgCl₂, each animal was anesthetized lightly with ether and a small incision was made through the skin in the midventral region of the thigh to expose the femoral vein and artery. After the fascia around the femoral vein was trimmed, the dose of HgCl₂ was administered into the vein. Afterwards, the opposite ends of the incised skin were approximated using two sterile 9-mm surgical wound clips.

Once all the animals had been administered the dose of HgCl₂, they were randomly assigned to 12 groups. Three groups of NPX rats and three groups of control rats were used in experiments designed to evaluate the effects of DMPS on the renal burden and intrarenal distribution of mercury. The other three groups of NPX rats and three groups of control rats were used in experiments designed to evaluate the effects of DMSA on the renal burden and intrarenal distribution of mercury. After each animal was administered the dose of HgCl₂, it was placed in a plastic metabolic cage and kept there until it was anesthetized for the procurement of tissues and organs.

DMPS experiments. One group of NPX rats and one group of control rats were anesthetized with sodium pentobarbital (100 mg/kg) 24 hr after the rats were given the 0.5-μmol/kg dose of HgCl₂. These animals were used to establish a base line for the renal burden and intrarenal distribution of mercury 24 hr after injection.

Also after the first 24 hr, one group of NPX rats and one group of control rats were given a 100-μg/kg (0.48-μmol/kg) dose of DMPS intraperitoneally. The remaining NPX and control rats were injected (i.p.) with the same volume (2 ml/kg) of vehicle (0.9% aqueous sodium chloride) used to administer the dose of DMPS. Six hours later (that is, 30 hr after injection of HgCl₂), the animals treated with DMPS were given a second 100-μg/kg dose of DMPS (i.p.). The NPX and control rats given the injection of vehicle also received a second injection. At the end of 48 hr after the injection of HgCl₂, the NPX and control rats treated with either DMPS or vehicle were anesthetized with sodium pentobarbital, and organs and tissues were procured to study the effects of DMPS on the renal, hepatic and hematological disposition of mercury.

DMSA experiments. The DMSA experiments were modeled identically after the DMPS experiments, except that a group of NPX rats and a group of control rats were given an intraperitoneal 100-μg/kg (0.55-μmol/kg) dose of DMSA in 2 ml/kg 0.9% saline (instead of DMPS) 24 and 30 hr after they had received a single intravenous 0.5-μmol/kg dose of HgCl₂.

Collection and Handling of Urine and Feces

All urine and feces excreted by each animal during the experimental periods in the present study were collected on a 24-hr basis. Each 24-hr sample of urine or feces was first weighed. The entire amount of feces excreted during each 24-hr period was placed and sealed in 16 × 95 mm polypropylene gamma-counting tubes. From the total volume of urine excreted during each 24-hr period, a 1-ml sample was obtained and then placed and sealed in a 12 × 75-mm polystyrene gamma-counting tube. The amount of mercury excreted in the feces and urine by each animal was determined by standard gamma-counting techniques.

Collection of Tissues and Organs

After each animal in the present study was anesthetized with sodium pentobarbital, two 1-ml samples of blood were obtained from the inferior vena cava with a syringe and a 20-gauge needle. One of the samples was placed directly into a 12 × 75-mm polystyrene gammacounting tube. The other sample was placed in a 1.5-ml polypropylene microcentrifuge tube and centrifuged at 3000 × g for 7 minutes. Subsequently, the plasma fraction was separated from the cellular fraction. Each fraction was placed individually in a tared polystyrene gammacounting tube. Separation of the two fractions of blood made it possible to determine the distribution of mercury between the plasma and the cellular fractions of blood.

After the samples of blood were drawn, the kidney(s) and liver were excised and weighed quickly. The left kidney was sliced in half along the transverse plane. One of the halves was placed in a tared gammacounting tube. A 3-mm transverse slice of kidney was obtained from the other half. Samples of cortex, outer stripe of the outer medulla, inner stripe of the inner medulla and inner medulla were obtained from this slice of kidney by careful dissection. A 1-g sample of liver was also obtained. All samples of renal and hepatic tissues were placed and sealed in tared 12 × 75 mm gamma-counting tubes.

Determination of the Content of Mercury in Samples of Tissue, Urine and Feces

The radioactivity of ³²⁰Hg in the samples of tissues, organs, urine, feces and injection solution was determined by counting the samples in a 1282 Compugamma CS deep-well gamma spectrometer (Pharma- cies-LKB, Gaithersburg, MD) operating at a counting efficiency of 50% for ³²⁰Hg. The actual content of inorganic mercury (Hg⁺⁺) in each sample was calculated by dividing the radioactivity of ³²⁰Hg in the sample (dpm) by the specific activity of ³²⁰Hg in the injection solution (dpm/μmol). The concentration of inorganic mercury in the samples of tissues is expressed as percent of the administered dose per gram of tissue. The total content of mercury in the whole liver and kidney(s) is expressed simply as percent of the administered dose. Urinary excretion of mercury and fecal excretion of mercury are expressed as percent of the administered dose excreted in 24 and 48 hr.

Statistics

All values are expressed as mean ± S.E.M. Differences between means for individual sets of data obtained from corresponding groups
of NPX and control rats treated with DMPS or saline were evaluated statistically by first using a 2 × 2 two-way analysis of variance (ANOVA). When F values obtained with the ANOVA were found to be statistically significant (P < .05), the Tukey’s protected-t post hoc multiple comparison test was used to determine which means were significantly different from one another. The same analyses were used to evaluate differences between means for individual sets of data obtained from corresponding groups of NPX and control rats treated with DMSA or saline.

Differences between means for data obtained from the NPX and control rats, used to establish base-line data for the renal burden and intrarenal distribution of mercury 24 hr after injection of mercury for the DMPS or the DMSA experiments, were evaluated statistically using the unpaired Student’s t test for two independent samples.

Because data expressed as a percent or fraction of some total value do not statistically fit a normal or Gaussian distribution, all scores expressed as a percent of the dose of mercury in the present study were transformed by the arcsine transformation before any statistical analyses were performed. The arc sine transformation normalizes percent data by taking the arc sine of the square root of n, where n is the decimal fraction of the percent score.

The level of significance (P < .05) for all statistical analyses performed in the present study was chosen a priori.

Results

DMPS Experiments

Renal disposition of mercury 24 hr after injection of HgCl₂ (before treatment with DMPS). Compensatory renal growth had occurred in the NPX rats 24 hr after treatment with the intravenous 0.5-μmol/kg nontoxic dose of HgCl₂ (table 1). The weight of the remnant left kidney in the NPX rats was significantly greater than the weight of the left kidney in the control rats by the morning of the 14th day after surgery.

The concentration of mercury in the left kidney was significantly greater in the NPX rats than in the control rats 24 hr after injection of HgCl₂ (table 1). The increase in the renal concentration of mercury in the NPX rats was due to an increase in concentration of mercury in the outer stripe of the outer medulla. No significant differences in the concentration of mercury were detected in any other zone of the kidney.

Despite the occurrence of compensatory renal growth and increased renal concentration of mercury, the content of mercury in the total renal mass of the NPX rats was significantly less than that in the control rats (table 1). Approximately 45% of the administered dose of mercury was present in the combined renal mass of the control rats injected with the 0.5-μmol/kg dose of HgCl₂.

Disposition of mercury in blood and liver 24 hr after injection of HgCl₂ (before treatment with DMPS). There was no significant difference in either the concentration or the content of mercury in the blood or liver between the NPX and control rats 24 hr after they received the intravenous dose of HgCl₂ (table 2).

Effects of DMPS on the renal disposition of mercury. There were no significant differences in body weight between any of the groups of rats. Body weights ranged from 222 ± 8 g in the control animals treated with saline to 238 ± 4 g in the NPX rats treated with DMPS. However, the remnant left kidney in the NPX rats was significantly greater than the left kidney in the corresponding group of control rats, indicating that compensatory renal growth had occurred in the NPX rats. The remnant kidney in the NPX rats treated with saline and DMPS weighed on average between 1.34 and 1.40 g, whereas the left kidney in the control animals treated with saline and DMPS weighed on average between 0.95 and 0.97 g.

The concentration of mercury in the remnant kidney of the NPX rats treated with two injections of saline was significantly greater than the concentration of mercury in the corresponding group of control rats (fig. 1). The concentration of mercury in the left kidney of both the NPX and the SO rats was similar to that measured 24 hr after injection of HgCl₂ (table 1). In the NPX rats, the concentration of mercury averaged around 30% of the administered dose of mercury per gram of kidney, compared with 25% of the dose per gram of kidney in the control rats. Despite the increased concentration of mercury in the remnant left kidney of the NPX rats, the content of mercury in the total renal mass was significantly greater in the control rats. The renal burden of mercury in the control rats was about 48% of the dose of mercury, and the renal burden of mercury in the NPX rats was about 40% of the dose (fig. 2).

As was the case 24 hr after injection of HgCl₂, the increased concentration of mercury at the level of the whole kidney in the NPX rats was due to a specific increase in the concentration of mercury in the outer stripe of the outer medulla (fig. 1). The concentration of mercury in the renal outer stripe of the outer medulla in the control rats treated with saline was about 30% of the administered dose of mercury per gram of tissue. In the NPX rats, the concentration of mercury in the renal outer stripe was around 42% of the dose per gram of tissue. No significant differences in the concentration of mercury in the

### TABLE 1

Renal disposition of mercury in normal and NPX rats 24 hr after the i.v. administration of a 0.5-μmol/kg nontoxic dose of HgCl₂

<table>
<thead>
<tr>
<th>Group</th>
<th>Left Kidney Weight (g)</th>
<th>Right Kidney Weight (g)</th>
<th>[Hg] in Left Kidney (% dose/g)</th>
<th>Content of Hg in Left Kidney (% dose)</th>
<th>Content of Hg in Total Renal Mass (% dose)</th>
<th>[Hg] in Cortex (% dose/g)</th>
<th>[Hg] in ISOM (% dose/g)</th>
<th>[Hg] in ISOM (% dose/g)</th>
<th>[Hg] in Inner Medulla (% dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n = 4)</td>
<td>0.95 ± 0.02</td>
<td>0.99 ± 0.05</td>
<td>23.16 ± 1.11</td>
<td>21.90 ± 0.84</td>
<td>45.25 ± 1.53</td>
<td>30.20 ± 1.07</td>
<td>23.87 ± 2.27</td>
<td>1.51 ± 0.44</td>
<td>0.75 ± 0.09</td>
</tr>
<tr>
<td>DMPS Exp. NPX (n = 2)</td>
<td>1.42 ± 0.18</td>
<td>28.31 ± 2.50</td>
<td>39.38 ± 1.54</td>
<td>39.38 ± 1.54</td>
<td>31.46 ± 4.37</td>
<td>37.78 ± 5.07</td>
<td>1.08 ± 0.19</td>
<td>1.07 ± 0.19</td>
<td>23.87 ± 2.27</td>
</tr>
<tr>
<td>Normal (n = 5)</td>
<td>0.95 ± 0.02</td>
<td>0.97 ± 0.05</td>
<td>23.21 ± 2.33</td>
<td>22.08 ± 2.32</td>
<td>44.63 ± 4.24</td>
<td>34.53 ± 2.88</td>
<td>19.91 ± 2.62</td>
<td>4.80 ± 0.23</td>
<td>—</td>
</tr>
<tr>
<td>DMPS Exp. NPX (n = 3)</td>
<td>1.48 ± 0.12</td>
<td>29.86 ± 1.98</td>
<td>43.78 ± 4.27</td>
<td>38.32 ± 3.17</td>
<td>31.02 ± 3.56</td>
<td>5.40 ± 1.71</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. The NPX rats were injected with the 0.5-μmol/kg dose of mercuric chloride 12 days after surgery. OSOM = outer stripe of the outer medulla. * = significant different (P < .05) from the mean for the corresponding normal rats. Animal body weights ranged from 222 ± 1 to 233 ± 15 g. There were no significant differences in body weights between any of the groups of animals.
cortex, inner stripe of the outer medulla or inner medulla were found between the two groups of rats. Accumulation of mercury in the inner stripe of the outer medulla and inner medulla in both the NPX and the control rats was minimal compared with that in the cortex and outer stripe of the outer medulla.

In both the NPX and the control rats treated with the two 100-mg/kg intraperitoneal doses of DMPS, the concentration of mercury in each zone of the kidney was significantly lower than that in the corresponding NPX and control rats treated with two injections of saline (fig. 1). The most striking differences in the concentration of mercury were found in the cortex and outer stripe of the outer medulla. The concentration of mercury in the cortex of the NPX and control rats treated with saline was about 31% of the dose per gram of tissue, whereas the concentration of mercury in the cortex of the NPX and control rats treated with DMPS was around 5% of the dose per gram of tissue. By far, the greatest difference in the concentration of mercury was detected between the outer stripe of the NPX rats treated with saline and the outer stripe of the NPX rats treated with DMPS. In the NPX rats treated with DMPS, the concentration of mercury in the outer stripe was only around 4% to 5% of the dose per gram of tissue, as compared with 42% of the dose per gram of tissue in the NPX rats treated with saline. No differences in the concentration of mercury were detected in any zone of the kidney between the NPX and control rats treated with the two injections of DMPS.

The renal burden of mercury in the control rats treated with DMPS was about 7 times lower than that in the control rats treated with saline (fig. 2). In the NPX rats treated with DMPS, the renal burden of mercury was about 6 times lower than that in the NPX rats treated with saline.

Effects of DMPS on the disposition of mercury in blood and liver. There was no significant difference between the saline-injected NPX and control rats with respect to the content of mercury in the blood and liver 48 hr after administration of the 0.5-μmol/kg dose of HgCl₂ (fig. 3). About 1% of the dose of mercury was present in the entire blood volume, which was estimated to be 6% of body weight. A little over 7% of the dose of mercury was present in the liver of the two groups of rats.

No significant differences in the content of mercury in the blood and liver were detected between the NPX and control rats treated with DMPS (fig. 3). However, the concentrations of mercury in the blood and liver of the NPX and control rats treated with DMPS were significantly lower than those of the corresponding NPX and control rats treated with saline. The total amount of mercury in the blood of both the NPX and the control rats treated with DMPS was less than 1% of the administered dose. In the liver, between 5% and 6% of the dose was present.

By the end of 48 hr after the injection of the 0.5-μmol/kg dose of HgCl₂, between 40% and 45% of the mercury in blood of the NPX and control rats treated with saline was present in the plasma (fig. 4). The remainder of mercury in blood was in the cellular fraction. In the animals treated with DMPS, around 55% of the mercury in blood was present in the plasma, which is statistically greater than that found in the plasma of the NPX and control rats treated with saline.

Effects of DMPS on the urinary excretion of mercury. During the initial 24 hr after administration of the 0.5-μmol/kg dose of HgCl₂, all groups of rats excreted between 3% and 6% of the administered dose of mercury in the urine (fig. 5).

By the end of 48 hr after the injection of HgCl₂, the NPX and control rats had excreted only about 6% to 8% of the dose of mercury in the urine (fig. 5). Both NPX and control rats treated with DMPS excreted several times more mercury in the urine than the corresponding NPX and control rats treated with saline. Moreover, the control rats treated with DMPS excreted significantly more mercury than the NPX rats treated with DMPS. The control rats excreted about 50% of the dose of mercury in the urine, whereas the NPX rats excreted about 43% of the dose of mercury in the urine.

Effects of DMPS on the fecal excretion of mercury. The NPX rats that were treated with DMPS 24 hr after administration of the 0.5-μmol/kg dose of HgCl₂ excreted slightly more mercury in the feces during the initial 24 hr after the injection of HgCl₂ than the corresponding control animals (fig. 6). The NPX rats excreted about 13% of the administered dose of mercury in the feces, and the corresponding group of control rats excreted about 10% of the dose.

At the close of the first 48 hr after the injection of HgCl₂, the NPX rats treated with saline had excreted significantly more mercury in the feces than the control rats treated with saline (fig. 6). The NPX rats treated with saline had excreted about 14% of the administered dose of mercury, whereas the corresponding control rats had excreted about 11% of the dose. By contrast, the NPX and control rats treated with DMPS excreted significantly more mercury in the feces than corresponding NPX and control rats treated with saline. Moreover,
the NPX rats treated with DMPS excreted significantly more mercury in the feces than the corresponding group of control rats treated with DMPS. About 18% of the dose of mercury was excreted in the feces by the NPX rats treated with DMPS, whereas only about 14% of the dose was excreted in the feces by the control rats treated with DMPS.

DMSA-Experiments

Renal disposition of mercury 24 hr after injection of HgCl₂ (before treatment with DMSA). In the NPX rats associated with the DMSA experiments that were studied 24 hr after treatment with the intravenous 0.5-μmol/kg dose of HgCl₂, compensatory renal growth occurred (table 1). The remnant left kidney in the NPX rats was significantly greater in mass than the mass of the left kidney in the control rats 14 days after surgery.

As in the DMPS experiments, the concentration of mercury in the left kidney was significantly greater in the NPX rats than in the control rats 24 hr after injection of HgCl₂ (table 1). This increase in the renal concentration of mercury was due to an increase in concentration of mercury in the outer stripe of the outer medulla. Significant differences in the concentration of mercury were not detected in any other zone of the kidney.

The increase in renal accumulation of mercury in the NPX rats was sufficient to make the total renal burden of mercury
TREATMENT = IN

Fig. 4. Distribution of mercury in blood of NPX and control rats treated with DMPS (100 mg/kg, i.p.) or saline (2.0 ml/kg, i.p.) 24 and 30 hr after they received a single intravenous 0.5-μmol/kg nontoxic dose of HgCl₂. The percentage of mercury in blood present in the cellular and plasma fractions of blood was determined 48 hr after injection of HgCl₂. Each value is a mean ± S.E.M. generated from four rats. * = significantly different (P < .05) from the corresponding mean for the same type of rat not treated with DMPS.

Fig. 5. Amount of mercury excreted in the urine (% of the administered dose) by NPX and control rats treated with DMPS (100 mg/kg, i.p.) or saline (2.0 ml/kg, i.p.) 24 and 30 hr after they received a single intravenous 0.5-μmol/kg nontoxic dose of HgCl₂. The amount of mercury excreted in the urine was measured 24 and 48 hr after injection of HgCl₂. Each value is a mean ± S.E.M. generated from four rats. * = significantly different (P < .05) from the corresponding mean for the control rats treated in the same manner. + = significantly different (P < .05) from the corresponding mean for the same type of rat not treated with DMPS.

Fig. 6. Amount of mercury excreted in the feces (% of the administered dose) by NPX and control rats treated with DMPS (100 mg/kg, i.p.) or saline (2.0 ml/kg, i.p.) 24 and 30 hr after they received a single intravenous 0.5-μmol/kg nontoxic dose of HgCl₂. The amount of mercury excreted in the feces was measured 24 and 48 hr after injection of HgCl₂. Each value is a mean ± S.E.M. generated from four rats. * = significantly different (P < .05) from the corresponding mean for the control rats treated in the same manner. + = significantly different (P < .05) from the corresponding mean for the same type of rat not treated with DMPS.

NPX and control rats in the DMSA experiments studied 24 hr after they received the intravenous dose of HgCl₂ (table 2).

Effects of DMSA on the renal disposition of mercury. No significant differences in body weight were found between any of the groups of rats in these experiments. Animal body weight ranged from 235 ± 3 g in the control animals treated with saline to 242 ± 4 g in the NPX rats treated with DMSA. The remnant left kidney in the NPX rats was, however, significantly greater in mass than the left kidney in the corresponding group of control rats, indicating that compensatory renal growth had occurred in the NPX rats. The remnant kidney in the NPX rats treated with saline and DMPS weighed on average between 1.35 and 1.45 g, whereas the left kidney in the control animals treated with saline and DMPS weighed on average between 0.93 and 0.97 g.

In NPX rats treated with two injections of saline, the concentration of mercury in the remnant kidney was significantly greater than the concentration of mercury in the left kidney of the corresponding group of control rats (fig. 7). The concentration of mercury in the remnant kidney of the NPX rats averaged around 27% of the administered dose of mercury per gram of kidney, whereas in the control rats, the concentration of mercury in the kidney was about 21% of the dose per gram of kidney. Despite the increased concentration of mercury in the remnant left kidney of the NPX rats, the content of mercury in the total renal mass was significantly greater in the control rats. The renal burden of mercury in the control rats was about 46% of the dose of mercury, and the renal burden of mercury in the NPX rats was about 36% of the dose (fig. 8).

Increases in the concentration of mercury in the remnant kidney of the NPX rats were due to a specific increase in the concentration of mercury in the outer stripe of the outer medulla (fig. 7). The concentration of mercury in the outer stripe of the outer medulla in the control rats treated with saline was about 18% of the administered dose of mercury per gram of tissue. In the NPX rats, the concentration of mercury in the

statistically equivalent to the renal burden of mercury in the control rats (table 1). Approximately 44% to 45% of the administered dose of mercury was present in the combined renal mass of either the NPX or the control rats injected with the 0.5-μmol/kg dose of HgCl₂.

Disposition of mercury in blood and liver 24 hr after injection of HgCl₂ (before treatment with DMSA). No significant differences were found in either the concentration or the content of mercury in the blood or liver between the
outer stripe was around 40% of the administered dose per gram of tissue. Significant differences in the concentration of mercury in the cortex, inner stripe of the outer medulla and inner medulla were not found between the NPX and control rats treated with saline. Very little mercury accumulated in the inner stripe of the outer medulla or inner medulla in both the NPX and the control rats relative to that in the cortex and outer stripe of the outer medulla.

The concentration of mercury in each zone of the kidney in the NPX and control rats treated with the two 100-mg/kg intraperitoneal doses of DMSA was significantly lower than that in the corresponding NPX and control rats treated with two injections of saline (fig. 7). Major differences in the concentration of mercury were found in the cortex and outer stripe of the outer medulla. The concentration of mercury in the cortex of the NPX and control rats treated with saline was about 27% to 33% of the dose of mercury per gram of cortex, whereas that in the cortex of the NPX and control rats treated with DMPS was around 11% to 14% of the dose per gram of cortex. The greatest difference in the concentration of mercury was detected between the outer stripe of the NPX rats treated with saline and the outer stripe of the NPX rats treated with DMSA. In the NPX rats treated with DMSA, the concentration of mercury in the outer stripe was around 15% of the dose of mercury per gram of tissue. No differences in the concentration of mercury were detected in any zone of the kidney between the NPX and control rats treated with the two injections of DMPS.

With respect to the renal burden of mercury, it was less than 2 times lower in the control rats treated with DMSA than in the control rats treated with saline (fig. 8). In the NPX rats treated with DMSA, the renal burden of mercury was a little over 2 times lower than that in the control rats treated with saline.

Effects of DMSA on the disposition of mercury in blood and liver. No significant differences in the content of mercury in the blood or liver were found between the NPX and control rats treated with saline or DMSA 48 hr after administration of the 0.5-μmol/kg dose of HgCl₂ (fig. 9). Approximately 1% of the dose of mercury was present in the entire blood volume in all four groups of rats, and 7% to 9% of the administered dose of mercury was present in their liver.

In all four groups of rats, 42% to 46% of the mercury in the blood (48 hr after administration of the 0.5-μmol/kg dose of HgCl₂) was in the plasma (fig. 10). The small differences in the distribution of mercury between the cellular and plasma fractions of blood between the four groups of rats were not statistically significant.

Effects of DMSA on the urinary excretion of mercury. All groups of rats excreted about 5% of the administered dose of mercury during the initial 24 hr after administration of the 0.5-μmol/kg dose of HgCl₂ (fig. 11). During the initial 48 hr after the injection of HgCl₂, the NPX
Fig. 9. Content of mercury (% of administered dose of mercury) in the blood and liver of NPX and control rats treated with DMSA (100 mg/kg, i.p.) or saline (2.0 ml/kg, i.p.) 24 and 30 hr after they received a single intravenous 0.5-μmol/kg nontoxic dose of HgCl₂. The content of mercury in the blood and liver was determined 48 hr after injection of HgCl₂. Each value is a mean ± S.E.M. generated from four rats.

and control rats treated with saline excreted only about 7% to 8% of the dose of mercury in the urine (fig. 11). Both NPX and control rats treated with DMSA excreted several times more mercury in the urine than the corresponding NPX and control rats treated with saline. In addition, the NPX rats treated with DMSA excreted significantly more mercury in the urine than the control rats treated with DMSA. The control rats excreted about 30% of the dose of mercury, whereas the NPX rats excreted about 42% of the dose of mercury (fig. 12). By the end of 48 hr after injection of HgCl₂, all groups of rats had excreted 11% to 15% of the dose. The differences in the fecal excretion of mercury between the groups of rats were not statistically significant.

Discussion

Both DMPS and DMSA are water-soluble chelating agents that have been shown to be effective in reducing the renal burden of mercury after exposure to mercurials (Aposhian, 1983; Aposhian and Aposhian, 1990; Cherian et al., 1988; Zalups et al., 1991). When normal and NPX rats in the present study
were administered a 100-mg/kg dose of either DMPS or DMSA 24 and 30 hr after the intravenous injection of a nontoxic 0.5-µmol/kg dose of HgCl₂, significant alterations in the renal burden and intrarenal distribution of inorganic mercury occurred during the 24 hr after the first dose of DMPS or DMSA was administered. The renal concentration and burden of mercury fell significantly in both groups of rats treated with either chelating agent. However, treatment with DMPS was more effective than treatment with DMSA in reducing the renal concentration and burden of accumulated inorganic mercury, despite the fact that more moles of free sulfhydryl groups were administered when DMSA was given to the rats. These findings are consistent with those of a previous study, which show that, on a per mole basis, DMPS is more effective in reducing the renal burden of inorganic mercury than treatment with DMSA (Planas-Bohne, 1981). In the present study, most of the decrease in the renal concentration and burden of inorganic mercury that occurred in the normal and NPX rats treated with either chelating agent was due to substantial removal of inorganic mercury from both the renal cortex and outer stripe of the outer medulla. It is in these zones of the kidney that accumulation and retention of inorganic mercury predominate after exposure to mercury (Zalups et al., 1987; Zalups and Lash, 1990; Zalups and Cherian, 1992; Zalups et al., 1992).

The precise mechanisms by which DMPS or DMSA reduces the content of mercury in the renal cortex and outer stripe of the outer medulla are not yet well defined. The tremendous rate of reduction in the renal burden of mercury that occurred after treatment with either DMPS or DMSA in this study favors an explanation that involves some chelation process associated with the renal tubular transport of the chelating agent. The argument that the reduction in the renal burden of mercury caused by treatment with either DMPS or DMSA is due simply to mass action does not appear to be supported by the data collected in studies on the renal tubular accumulation of inorganic mercury. According to the principle of mass action, the renal intracellular burden of inorganic mercury should decrease as the extracellular content of mercury decreases. Data from a recent study (Zalups 1993a) show that this does not occur during the first few hours after treatment. In addition, data from a previous study (Zalups et al., 1987) and the present study show that the renal burden of mercury in normal or NPX rats (not given either DMPS or DMSA) does not decrease significantly between the first and second day after injection of inorganic mercury, despite the fact that the content of mercury in the blood decreases significantly. Another point that argues against mass action being the primary mechanism involved in the tremendous reduction in the renal burden of mercury after treatment with either DMPS or DMSA is the fact that much of the inorganic mercury that accumulates in proximal tubular cells is bound strongly to cellular proteins (Barfuss et al., 1990).

There is substantial evidence indicating that DMPS is secreted by a probenecid-sensitive organic anion transport system (Stewart and Diamond, 1987, 1988; Klotzbach and Diamond, 1988), which is presumably localized exclusively along the three segments of the proximal tubule (Irish and Grantham, 1981). It is interesting that inorganic mercury that accumulates in the renal cortex and the outer stripe of the outer medulla is also localized almost exclusively along segments of the proximal tubule (Zalups, 1991a, 1991b, Zalups and Barfuss, 1990). Thus, assuming that DMPS is secreted by segments of the proximal tubule, it seems likely that inorganic mercury localized within, or on, proximal tubular epithelial cells can bind to DMPS and be removed from the intracellular environment during the secretory passage of DMPS into the lumen of the proximal tubule.

DMPS also appears to be transported in other cells. There is evidence that transport of DMPS occurs in erythrocytes (Wildenauer et al., 1982; Reuther et al., 1982) and hepatocytes (Aposian, 1990). In the present study, transport of DMPS by erythrocytes provides an explanation for the reduction of mercury in the cellular fraction of blood in both the normal and the NPX rats treated with DMPS. In addition, the hepatic transport of DMPS explains the significant decrease in the hepatic content of mercury and the increased fecal excretion of mercury that was detected in both the normal and the NPX rats treated with DMPS.

Recent data indicate that DMSA, unlike DMPS, is not transported from the blood into the bile by hepatocytes (Aposian, 1990). Findings in the present study are consistent with these data, in that treatment with DMSA did not have a significant effect on the hepatic burden of mercury and the fecal excretion of mercury in both normal and NPX rats. The present study also shows that treatment with DMSA did not cause a significant alteration in the content of mercury present in the cellular fraction of blood. This too supports the hypothesis that DMSA is not transported in erythrocytes. The lack of transport in extrarenal tissues is believed to be related to the negatively charged carboxyl groups on the DMSA molecule.

Because there has been a lack of evidence for extrarenal cellular transport of DMSA, many have assumed that no transport occurs in the kidney. However, the epithelial cells lining the segments of the proximal tubule do transport dicarboxylic acids, including succinate (Murier et al., 1992). Thus, it is likely that transport of DMSA does occur in the kidney. As stated above, the rapid decrease in the renal burden of mercury found in the normal and NPX rats treated with DMSA tends to support the hypothesis that DMSA chelates intracellular mercury and reduces the renal cellular burden of inorganic mercury by a transport-related process.

Data from the present study confirm previous findings showing that the renal accumulation of inorganic mercury increases in rats after unilateral nephrectomy and compensatory renal growth and that this increase is due to enhanced accumulation of inorganic mercury in the outer stripe of the outer medulla (Zalups and Lash, 1990; Zalups, 1991; Zalups and Cherian, 1992). When NPX and normal rats in the present study were treated with the same dose of either DMPS or DMSA, the renal concentration of inorganic mercury decreased to a greater extent in the NPX rats than in the normal rats. This enhanced decrease in the renal concentration of inorganic mercury in the NPX rats was due to a specific increase in the removal of inorganic mercury from the renal outer stripe of the outer medulla. Although the exact mechanism for the increased release of mercury from this zone of the kidney is not known, it is probably related to some aspect of compensatory renal growth. The pars recta segment of the proximal tubule, which is the main segment of the nephron in the outer stripe of the outer medulla that accumulates inorganic mercury (Zalups, 1991a, 1991b; Zalups and Barfuss, 1990), undergoes significant hypertrophy after renal mass is reduced (Meyer, et al., 1991; Zalups, 1993b). This segment of the nephron is also responsible for the increased accumulation of mercury that occurs in the outer stripe of the outer medulla after unilateral nephrectomy. In association with the cellular hypertrophy that occurs in the
pars recta, there is increased electrolyte and other solute transport (Meyer et al., 1991). Thus a potential mechanism for the increased removal of mercury from the outer stripe may involve increased transepithelial transport of the two chelating agents along hypertrophied pars recta segments of the proximal tubule.

Most of the mercury released from the kidney(s) of the normal and NPX rats treated with either DMPS or DMSA was excreted in the urine. After treatment with either DMPS or DMSA, NPX rats excreted significantly more inorganic mercury per gram of kidney than corresponding normal rats. This finding correlates well with the greater decrease in the renal concentration of mercury in the NPX rats.

The urinary data of the present study are also consistent with findings from previous studies showing increased urinary excretion of mercury after treatment with DMPS or DMSA (Gabard, 1976a,b; Planas-Bohne, 1981; Hursh et al., 1985; Cherian et al., 1988; Aposhian and Aposhian, 1990). One study (Cherian et al., 1988), demonstrated a correlation between the urinary excretion of mercury and the total animal burden of mercury in rats exposed to inorganic and mercury vapor.

In summary, the data from the present study show that treatment with DMPS or DMSA reduces the renal concentration and burden of inorganic mercury very rapidly and substantially in both normal and NPX rats injected with a nontoxic dose of \( \text{HgCl}_2 \). This is accomplished primarily by reducing the content of inorganic mercury in the renal cortex and outer stripe of the outer medulla. According to the present data, DMPS is more effective at reducing the renal concentration and burden of mercury than DMSA. At the same dose of either chelating agent, the renal concentration of mercury decreases to a much greater extent in NPX rats than in normal rats. This is because more mercury is released from the renal outer stripe of the outer medulla of NPX rats than from that of normal rats. The urinary excretion of mercury (per gram of kidney) is also significantly greater in NPX rats than in normal rats, presumably because of the increased removal of renal mercury caused by either chelating agent. Because the total renal mass can contain as much as 50% of an administered dose of mercury within as little as 3 hr after exposure (Zalups, 1993b), a significant reduction in the renal burden of mercury also provides a significant reduction in the total burden of mercury in the organism. Thus both DMPS and DMSA hold much promise as effective agents for reducing the renal and whole body burden of mercury.

References


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