Progressive Losses of Renal Mass and the Renal and Hepatic Disposition of Administered Inorganic Mercury

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The present study was designed to evaluate, in rats, the effect of progressive losses of renal mass, from a state where renal function is not compromised significantly to a state where the early stages of renal failure are detectable, on the disposition of administered inorganic mercury. As part of this evaluation, the intrarenal, hepatic, and hematological disposition of mercury and the urinary and fecal excretion of mercury were studied and characterized in control, uninephrectomized (NXP), and 75% nephrectomized (75% NXP) rats 1, 2, and 7 days after the intravenous injection of a nontoxic 0.5 μmol/kg dose of mercuric chloride. Clearance data showed that concentration of creatinine in the plasma was increased, whole animal glomerular filtration rate (GFR) was decreased, and the fractional excretion of sodium and potassium was increased in 75% NXP rats but not in NXP rats by the 12th day after surgery. These findings confirm that 75% nephrectomy in the rat causes changes that begin to compromise renal function significantly. Renal accumulation of mercury and the intrarenal distribution of mercury were significantly different between 75% NXP rats and NXP rats, presumably because of the differences in GFR and the renal clearance of mercury between the two groups of rats. Interestingly, the contents of mercury in the blood and liver were significantly greater in 75% NXP rats than in NXP or control rats. In addition, 75% NXP rats excreted significantly more mercury in the feces over the 7 days of study than did the other two groups of rats, indicating the hepatobiliary clearance of mercury was significantly greater in 75% NXP rats. Urinary excretion of mercury was also significantly greater in 75% NXP rats than in control rats or NXP rats. This enhanced urinary excretion of mercury may be related to polyuria that occurs in 75% NXP rats. In summary, the findings from the present study clearly indicate that the renal and hepatic handling of administered inorganic mercury in rats changes significantly when renal mass is reduced from about 50% to only about 25% of the original, total renal mass. Further studies are needed to better characterize the effects of 75% nephrectomy on both the disposition and the toxicity of inorganic mercury in renal and hepatic tissues and to determine the mechanisms responsible for the effects seen in this study.

There is a substantial body of evidence indicating that unilateral nephrectomy and compensatory renal growth have a significant effect on the renal accumulation and intrarenal distribution of both inorganic and organic forms of mercury in rats and rabbits (Zalups et al., 1987; Zalups, 1991a,b; 1993; Zalups and Cherian, 1992a,b; Zalups and Lash, 1990; Zalups et al., 1992). The most recent findings show that the combination of uninephrectomy and the accompanying compensatory renal growth causes the accumulation and/or retention of mercury (after exposure to low doses of inorganic or organic mercuric compounds) to increase in the renal outer medulla, specifically in the outer stripe of the outer medulla (Zalups, 1991a,b; 1993; Zalups and Cherian, 1992a,b; Zalups and Lash, 1990; Zalups et al., 1992). Moreover, recent histochemical findings indicate that this effect is most likely due to increased uptake and/or retention of mercury by the hypertrophied pars recta segment of proximal tubules located in the outer stripe of the outer medulla, particularly at the junction of the cortex and outer medulla (Zalups, 1991a).

Additional evidence indicates that the nephropathy induced by toxic doses of inorganic mercury is made more severe in rats as a result of unilateral nephrectomy (Houser and Berndt, 1986; Ramos-Frendo et al., 1979; Zalups and Diamond, 1987; Zalups and Lash, 1990; Zalups et al., 1988), presumably because of enhanced accumulation of mercury along the pars recta segment of proximal tubules, which is the main segment of the nephron involved in the nephropathy induced by toxic doses of inorganic mercury (Gritzka and Trump, 1968; Ganote et al., 1974; McDowell et al., 1976; Zalups and Diamond, 1987; Zalups and Lash, 1990; Zalups et al., 1991, 1988).

Despite the fact that a significant amount of scientific data has been collected on the effects of uninephrectomy and compensatory renal growth on the renal disposition and toxicity of mercury and other nephrotoxicants (Henry et al., 1983; Molland, 1976), very little is known about how losses in renal mass of greater than 50% affect the disposition and toxicity of mercury in the body. Since progressive losses of renal mass occur in many diseases in humans and other mammals, it is important to determine, from a risk assessment standpoint, the disposition of nephrotoxic metals like mercury (as well as other nephrotoxicants) when renal mass is reduced to the point where renal func-
tion is compromised significantly and chronic renal failure begins. An important point that needs to be considered with continued and significant losses in renal mass is that the renal clearance of numerous mercuric compounds (and other toxicants) will eventually be reduced (depending on how much functional renal mass is remaining), and consequently, there may be a greater risk of toxicity in organs and tissues that are generally not affected by the toxicants under more normal conditions. In addition, inasmuch as enhanced severity of the nephropathy induced by inorganic mercury has been detected after uninephrectomy and compensatory renal growth, it is possible that losses of renal mass of greater than 50% may cause an even more severe form of the nephropathy than that caused by uninephrectomy. Even if the severity in renal tubular injury is not much greater than that found after uninephrectomy, the net effect of the nephropathy may have far greater deleterious effects on renal function, since the number of functioning nephrons is significantly lower than that after uninephrectomy.

The primary objective of the present study is to begin evaluating, in rats, the effect of a reduction of renal mass that induces functional changes consistent with the early stages of chronic renal failure on the disposition and elimination of inorganic mercury. This study characterizes and compares the disposition of inorganic mercury in renal tissue, liver, and blood and compares and evaluates the urinary and fecal excretion of inorganic mercury in normal, uninephrectomized (NPX), and 75% nephrectomized (75% NPX) rats injected intravenously with a nontoxic 0.5 μmol/kg dose of mercuric chloride. A 75% reduction of renal mass was chosen, inasmuch as this surgical model of reduced renal mass has been shown to induce structural and functional changes in the rat that are consistent with the early stages of chronic renal failure (Zalups et al., 1985; Zalups, 1989; Zalups and Henderson, 1992).

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats weighing 175–200 g were used in the present study. The animals were purchased from Harlan Sprague-Dawley (Indianapolis, IN). Upon arriving from the vendor, the animals were allowed to acclimate to their new surroundings for several days. During this acclimation period the animals were allowed food and water ad libitum.

Groups. Three groups of four rats were used for each of three portions of the present study. The three groups of rats consisted of one control group, one group that underwent a uninephrectomy (right-sided), and one group that underwent an approximate 75% reduction of renal mass. The disposition of a nontoxic 0.5 μmol/kg dose of inorganic mercury injected intravenously was studied in the three groups of rats 1, 2, and 7 days after injection, with each day after injection representing one of the three portions of the study.

Surgery. Prior to surgery, animals were anesthetized with a 50 mg/kg intraperitoneal dose of sodium pentobarbital. The protocol used to perform a right-sided uninephrectomy was the same as that outlined in previous studies (Zalups et al., 1987, 1992; Zalups, 1991a, 1993; Zalups and Cherian, 1992a,b; Zalups and Lash, 1990).

The surgical procedure used to perform the 75% nephrectomy was more involved than the procedure used for the uninephrectomy. The first step after induction of anesthesia involved making a midline incision through the skin and underlying connective tissue from the xiphoid of the sternum to the pubis using a No. 11 scalpel blade. The next step involved moving the intestines to the left side of the body and then carefully freeing the right kidney from the perinephric fascia and fat, without damaging the liver or right adrenal gland. Afterward, the right renal artery and vein and right ureter were ligated with a single sterile 3-0 silk suture. The right kidney was excised by cutting through the right renal vessels and ureter distal to the ligature using a small pair of surgical scissors. Once the right kidney was removed, the left kidney was carefully freed from the left perinephric fascia and fat. The left kidney was then moved out from the body across the mid-line, in a manner that exposed the posterior surface of the organ, and was placed in a custom made Lucite cup mounted on a stainless steel rod attached to an assembly that can be adjusted in height. The left renal vessels and ureter were fed across an open edge of the Lucite cup so that the renal vessels and ureter would not be crimped. Using a dissecting microscope, the posterior branch of the left renal artery was identified near the hilus. With a pair of 45°-in. cross-action Dumont microdissecting forceps and gentle sharp dissection, a sterile 4-0 silk suture was threaded in between the renal vein and posterior branch of renal artery, passing through the fascia surrounding and covering the renal vessels. When the ligature was in place around the posterior branch of the left renal artery, it was then tied tightly. On average, one half of the left kidney changed to a darker color after the ligature was tightened, indicating a cessation, or great reduction, of blood flow to about one half of the kidney. Based on previous experiments in this laboratory, the removal of the right kidney and the tying off of the posterior branch of the left renal artery is sufficient to induce early changes associated with chronic renal failure. With the ligature tied, the left kidney was removed from the Lucite cup and was placed back into its normal retroperitoneal position. The abdominal muscles were sewn back together again using sterile 4-0 silk suture and the opposite ends of the incised skin were approximated using sterile 9-mm stainless steel wound clips.

Recovery from surgery. A period of 12 days was allowed for recovery from surgery and to allow for the completion of the rapid phase of compensatory renal growth in the uninephrectomized (NPX) rats and 75% nephrectomized (75% NPX) rats. It generally requires approximately 1 week for the completion of the rapid phase of compensatory renal growth in the rat.

During the initial 4 to 5 days after surgery, the control and NPX rats were pair-fed with respect to the 75% NPX rats. This was necessary due to the fact that the 75% NPX rats generally consume less food immediately after surgery than either control or regular NPX rats. Although the necessity for pair-feeding varies from one experiment to another, the 75% NPX rats tend to eat and gain weight in parallel to the NPX and control rats within the first 4–5 days. Water was provided ad libitum during the entire 12 days of recovery.

Evaluation of renal function after surgery and compensatory renal growth. On the morning of the 11th day after surgery, the three corresponding groups of control, NPX, and 75% NPX rats for each time period studied (1, 2, or 7 days) after injection of the 0.5 μmol/kg dose of mercuric chloride were placed in plastic metabolic cages for a 24-hr period to evaluate renal function. Renal clearance of creatinine and the fractional urinary excretion of sodium and potassium were used as indices for the evaluation of renal function.

Urine was collected from each animal for 24 hr. At the midpoint of the collection, a sample of blood was obtained from the end of the tail (which was incised) in a heparinized 1.5-ml microcentrifuge tube. The blood was spun down at 10,000g for 10 min and the plasma fraction was withdrawn and stored for analysis. At the end of the 24-hr clearance-period, the vol-
ume of urine excreted by each animal was determined gravimetrically and a 1-ml sample of urine was stored for analysis. The concentrations of creatinine, sodium, and potassium were measured in all samples of plasma and urine. The Lancer Creatinine II Rapid Stat Diagnostic Kit (Lancer Division of Sherwood Medical, St. Louis, MO) was used to determine spectrophotometrically the concentration of creatinine in the samples of plasma and urine. Standard methods for flame photometry were used to determine the concentrations of both sodium and potassium in the samples of plasma and urine (Zalups et al., 1985).

As a measure of renal function, the renal clearance of creatinine (Ccr), which is an estimator of glomerular filtration rate (GFR), was determined by using

\[ C_{cr}(GFR) = \frac{(U_{cr} \times P_{cr})}{V} \]

where \( U_{cr} \) and \( P_{cr} \) are the concentrations of creatinine (mg/dl) in the urine and plasma, respectively, and \( V \) is the rate of production, or flow, of urine in ml/min. Another index of renal function evaluated was the fractional excretion of sodium and potassium. The fractional excretion of a substance is the fraction of the filtered load of that substance that is excreted in the urine. Fractional excretion of sodium or potassium was expressed as a percentage and was determined by

\[ FE_{x} = \frac{(U_{x} + P_{x})(U_{cr} + P_{cr})}{100} \]

where \( FE_{x} \) represents the fractional excretion of either sodium or potassium, \( U_{x} \) and \( P_{x} \) are the concentrations of sodium or potassium (mEq/l) in the urine and plasma, respectively, and \( U_{cr} \) and \( P_{cr} \) are the concentrations of creatinine (mg/dl) in the urine and plasma, respectively.

**Injection of inorganic mercury.** On the morning of the 12th day after surgery, the corresponding groups of control rats, NPX rats, and 75% NPX rats received a single intravenous 0.5 μmol/kg nontoxic dose of mercuric chloride containing 200 μg/ml. The dose was administered in the right femoral vein while the animal was lightly anesthetized with ether. The injection solution contained nonradioactive inorganic mercury (0.25 μmol/ml; Aldrich, Milwaukee, WI), radioactive inorganic mercury (2.0 μCi/μg; 2.0 μCi/ml; Buffalo Materials Corp., Buffalo, NY), and sodium chloride (9 mg/ml). The animals received 2.0 ml of the injection solution per kilogram of body weight.

**Acquisition and handling of tissues.** Urine and feces were collected continuously during each of the periods after injection of mercuric chloride (1, 2, or 7 days) in 24-hr intervals. The amount of urine and feces excreted by each animal during each 24-hr interval was determined gravimetrically. Samples (1 ml) of urine were obtained from each collection and were placed on a 12 × 75-mm polystyrene gamma-counting tubes for the determination of the amount of mercury excreted in the urine. The entire amount of feces excreted in a 24-hr period was placed in 16 × 100-mm polypropylene gamma-counting tubes for determination of the amount of mercury excreted in the feces. At the close of 1, 2, or 7 days after the injection of the nontoxic dose of mercuric chloride, corresponding groups of control, NPX, and 75% NPX rats were anesthetized with a 100 mg/kg dose of sodium pentobarbital. Once anesthesia was induced, a 1-ml sample of blood was obtained from the inferior vena cava. Then the kidney(s) and liver were removed and weighed quickly. Representative samples of the whole kidney, the renal cortex, renal outer stripe of the outer medulla, combined renal inner stripe of the outer medulla and papilla, and liver were obtained and were placed and sealed in 12 × 75-mm polystyrene gamma-counting tubes for determining the concentration of mercury in these samples.

**Determinations of the content of inorganic mercury in tissues, urine, and feces.** The radioactivities of the samples of tissue, urine, feces, and injection solution were determined by counting the samples in a well-type gamma spectrometer equipped with a 3-in. sodium iodide crystal (1282 Compugamma CS; LKB-Wallac, Gaithersburg, MD) operating at a counting efficiency of approximately 50% for 203Hg. The content of mercury in each sample was calculated by dividing the activity of the sample (DPM) by the specific activity of the injection solution (DPM/nmol). The concentration of mercury in the samples of tissues is expressed as percentage of the administered dose per gram of tissue, while the entire content of mercury in the total renal mass, blood, and liver is simply expressed as percentage of the administered dose. As a technical note, the total volume of blood in the rat was estimated to be 6% of body weight. The total amount of mercury excreted in the urine or feces during any 24-hr period is expressed as percentage of the administered dose excreted per 24 hr. Cumulative excretion of mercury in the urine or feces was determined for each day studied after the injection of mercuric chloride.

**Statistics.** All values are expressed as mean ± SE for n = 4 animals per group except for the clearance data, where the values are expressed as a mean ± SE for n = 12 animals per surgical group (i.e., control, NPX, or 75% NPX). Differences between means for the corresponding three groups of animals for each parameter were evaluated by first performing a one-way analysis of variance (ANOVA). When statistically significant F values were obtained with the ANOVA, Tukey's protected t test was used to determine which means were statistically different from one another. Prior to performing the parametric statistical analyses, Levene's test for homogeneity of variance and the Kolmogorov–Smirnov test for normality were run. The one-way ANOVA was run only when the conditions for performing parametric statistical analyses were met. Moreover, all data expressed as a percentage were first normalized using the arcsine transformation, which takes the arcsine of the square root of the decimal fraction of the percentage score. This procedure was done because data expressed as a percentage of some total tend not to fit a normal or Gaussian distribution, which is a condition that must be met to run a parametric statistical analysis. The level for α for each statistical analysis was chosen a priori to be 0.05.

**RESULTS**

**Renal Clearance Data**

The concentration of creatinine in the plasma was significantly greater in the 75% NPX rats than in the control rats or NPX rats 11 days after surgery (Table 1). There was, however, no significant difference in the concentration of creatinine in the plasma between the control rats and NPX rats.

In association with the enhanced level of creatinine in the plasma, the whole animal GFR was significantly decreased in the 75% NPX rats, relative to that in the control rats or NPX rats (Table 1). GFR was statistically similar in the control rats and NPX rats. The 75% NPX rats were also polyuric with respect to the other two groups of rats. They excreted close to twice the volume of urine in 24 hr than the other two groups of rats (Table 1).

No significant differences in the content of sodium or potassium in the plasma were detected between the control rats, NPX rats, and 75% NPX rats. The values for the concentration of sodium and potassium in the plasma were all in the normal range. By contrast, the fractional excretion of both sodium and potassium was significantly greater in the 75% NPX rats than in the control rats or NPX rats. No significant differences in the fractional excretion of sodium.
or potassium were detected between the control rats and NPX rats.

**Body, Kidney, and Liver Weights**

There were no statistical differences in body weight between the corresponding groups of control rats, NPX rats, and 75% NPX rats on the day the rats were administered the intravenous, nontoxic, 0.5 μmol/kg dose of mercuric chloride (Table 2).

At all times studied after the injection of mercuric chloride, the left kidney in the NPX rats and the remnant left kidney in the 75% NPX rats were significantly greater in mass than the left kidney in the corresponding group of control rats (Table 2), which indicates that compensatory renal growth had occurred in the NPX rats and 75% NPX rats. In addition, the remnant left kidney in the 75% NPX rats studied 1 day after injection of the nontoxic dose of mercuric chloride was significantly greater in mass than the left kidney in the corresponding group of NPX rats.

**Renal Disposition of Mercury**

The concentration of mercury (μmol/gram tissue) in the left kidney was significantly greater in the NPX rats than in the corresponding control rats or 75% NPX rats 1 day after the injection of the 0.5 μmol/kg dose of mercuric chloride (Fig. 1a). At 2 or 7 days after the injection of mercuric chloride, however, the concentration of mercury was not significantly greater in the NPX rats than in the corresponding control rats or 75% NPX rats. By contrast, the concentration of mercury in the remnant left kidney of the 75% NPX rats 2 and 7 days after injection of mercuric chloride was significantly less than that in the left kidney of either the control rats or NPX rats.

The content of mercury in the total renal mass of the control rats studied 7 days injection was around 50% of the administered dose, which is lower than the levels (about 55% of the dose) measured 1 or 2 days after injection (Fig. 1b). At all times studied after injection of the nontoxic dose of mercuric chloride, the content of mercury in the total renal mass was significantly greater in control rats than in the corresponding NPX rats or 75% NPX rats. In addition, the content of mercury in the remnant left kidney of the NPX rats was significantly greater than that in the remnant left kidney of 75% NPX rats.

**Intrarenal Distribution of Mercury**

Only in the portion of the study where the disposition of inorganic mercury was studied 1 day after injection of the 0.5 μmol/kg dose of mercuric chloride was there a significant difference in the concentration (μmol/gram tissue) of mercury in the renal cortex between the control rats and NPX rats (Fig. 2a). Specifically, the concentration of mercury in the renal cortex was significantly greater in the NPX rats than in the control rats.
### TABLE 2
Kidney and Body Weight Data

<table>
<thead>
<tr>
<th>Length of study after injection with 0.5 μmol/kg HgCl₂ (days)</th>
<th>Group</th>
<th>Animal body weight (g)</th>
<th>Amount of Hg injected (nmol)</th>
<th>Weight of left kidney (g)</th>
<th>Weight of right kidney (g)</th>
<th>Weight of liver (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (n = 4)</td>
<td>273 ± 6</td>
<td>133 ± 3</td>
<td>1.11 ± 0.04</td>
<td>1.14 ± 0.02</td>
<td>11.75 ± 0.22</td>
</tr>
<tr>
<td>1</td>
<td>NPX (n = 4)</td>
<td>266 ± 4</td>
<td>130 ± 2</td>
<td>1.48 ± 0.02*</td>
<td>—</td>
<td>11.53 ± 0.31</td>
</tr>
<tr>
<td>1</td>
<td>75% NPX (n = 4)</td>
<td>257 ± 4</td>
<td>127 ± 2</td>
<td>1.66 ± 0.04**</td>
<td>—</td>
<td>11.12 ± 0.47</td>
</tr>
<tr>
<td>2</td>
<td>Control (n = 4)</td>
<td>270 ± 7</td>
<td>131 ± 3</td>
<td>1.19 ± 0.08</td>
<td>1.19 ± 0.09</td>
<td>11.35 ± 0.67</td>
</tr>
<tr>
<td>2</td>
<td>NPX (n = 4)</td>
<td>272 ± 6</td>
<td>134 ± 3</td>
<td>1.74 ± 0.06*</td>
<td>—</td>
<td>12.83 ± 0.48</td>
</tr>
<tr>
<td>2</td>
<td>75% NPX (n = 4)</td>
<td>256 ± 5</td>
<td>126 ± 3</td>
<td>1.84 ± 0.08*</td>
<td>—</td>
<td>11.80 ± 0.60</td>
</tr>
<tr>
<td>7</td>
<td>Control (n = 4)</td>
<td>286 ± 7</td>
<td>138 ± 4</td>
<td>1.26 ± 0.02</td>
<td>1.30 ± 0.01</td>
<td>12.83 ± 0.48</td>
</tr>
<tr>
<td>7</td>
<td>NPX (n = 4)</td>
<td>287 ± 6</td>
<td>140 ± 3</td>
<td>1.89 ± 0.14*</td>
<td>—</td>
<td>12.74 ± 0.47</td>
</tr>
<tr>
<td>7</td>
<td>75% NPX (n = 4)</td>
<td>265 ± 7</td>
<td>130 ± 3</td>
<td>1.74 ± 0.14*</td>
<td>—</td>
<td>12.20 ± 0.33</td>
</tr>
</tbody>
</table>

*Note:* The 0.5 μmol/kg dose of HgCl₂ was administered intravenously on the morning of the 13th day after surgery. NPX, uninephrectomized rats; 75% NPX, 75% nephrectomized rats. All values are mean ± SE.

*Significantly different (p < 0.05) from the mean for the corresponding group of control rats.

**Significantly different (p < 0.05) from the mean for the corresponding group of control rats and the corresponding group of NPX rats.

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Rats than in the control rats. By contrast, the concentration of mercury in the renal cortex of the 75% NPX rats was significantly lower than that in the corresponding groups of control rats or NPX rats at each time studied after the injection of mercuric chloride. As an additional point, the concentration of mercury in the renal cortex tended to decrease in all three surgical groups of rats over time.

One consistent finding was that concentration of mercury in the renal outer stripe of the outer medulla was significantly greater in the NPX rats than in corresponding control rats or 75% NPX rats at all times studied following the injection of the 0.5 μmol/kg dose of mercuric chloride (Fig. 2b). The concentration of mercury in the renal outer stripe of the outer medulla in the 75% NPX rats was also significantly greater than that in the corresponding control rats 1 day after injection. However, no significant differences were found in the concentration of mercury in the renal outer stripe of the outer medulla between the 75% NPX rats and the control rats at the other two times studied. The concentration of mercury in the renal outer stripe of the

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**FIG. 1.** Concentration of mercury ([Hg]) in the left kidney (a) and the content of mercury (Hg) in the total renal mass (b) of control rats, uninephrectomized (NPX) rats, and 75% nephrectomized (75% NPX) rats 1, 2, and 7 days after the intravenous injection of a nontoxic 0.5 μmol/kg dose of mercuric chloride. The injection of mercuric chloride was administered 12 days after surgery, which was allowed for recovery and for the completion of the rapid phase of compensatory renal growth. All values are mean ± SE for n = 4 rats. *Significantly different (p < 0.05) from the mean for the corresponding group of control rats. **Significantly different (p < 0.05) from the mean for the corresponding group of control rats and the mean for the corresponding group of NPX rats. †Significantly different (p < 0.05) from the mean for the corresponding group of NPX rats.
FIG. 2. Concentration of mercury (\(\text{Hg} \)) in the renal cortex (a) and renal outer stripe of the outer medulla (b) of control rats, uninephrectomized (NPX) rats, and 75% nephrectomized (75% NPX) rats 1, 2, and 7 days after the intravenous injection of a nontoxic 0.5 \(\mu\)mol/kg dose of mercuric chloride. The injection of mercuric chloride was administered 12 days after surgery, which was allowed for recovery and for the completion of the rapid phase of compensatory renal growth. All values are mean ± SE for \(n = 4\) rats. *Significantly different (\(p < 0.05\)) from the mean for the corresponding group of control rats. **Significantly different (\(p < 0.05\)) from the mean for the corresponding group of control rats and the mean for the corresponding group of NPX rats. †Significantly different (\(p < 0.05\)) from the mean for the corresponding group of NPX rats.

outer medulla of both control rats and NPX rats tended to increase between the 1st and 7th day after injection.

No real significant changes were detected in the concentration of mercury in the combined renal inner stripe of the outer medulla and papilla between the three corresponding groups of rats studied at the three times after injection of mercuric chloride. The concentration of mercury in these combined zones never exceeded 5% of the dose per gram of tissue.

Disposition of Mercury in the Liver

By the end of the 1st day after injection of mercuric chloride, approximately 6–7% of the administered dose of inorganic mercury was present in the liver of the control rats and NPX rats (Fig. 3). The total content of mercury in the liver of the 75% NPX rats was significantly greater. By the close of the 2nd and 7th days after injection of mercuric chloride, the total content of mercury in the liver was also significantly greater in the 75% NPX rats than in the corresponding control rats or NPX rats. There was also a tendency for the hepatic content of mercury to be greater in the NPX rats than in the corresponding control rats by the close of the 1st and 2nd days after injection. However, the differences were statistically different only following the 2nd day after injection. The hepatic content of mercury decreased in all three groups of rats between the 1st and 7th day after treatment with inorganic mercury.

Disposition of Mercury in the Blood

The total content of mercury (% dose) in the blood was significantly greater in the 75% NPX rats than in control rats 1, 2, and 7 days after injection of the 0.5 \(\mu\)mol/kg dose of mercuric chloride (Fig. 4a). In the portion of the study designed to evaluate the disposition of inorganic mercury 7 days after injection of mercuric chloride, the content of mercury in the blood of the 75% NPX rats was also significantly greater than that in the NPX rats. As in the liver, the

FIG. 3. Content of mercury (\(\text{Hg} \)) in the liver of control rats, uninephrectomized (NPX) rats, and 75% nephrectomized (75% NPX) rats 1, 2, and 7 days after the intravenous injection of a nontoxic 0.5 \(\mu\)mol/kg dose of mercuric chloride. The injection of mercuric chloride was administered 12 days after surgery, which was allowed for recovery and for the completion of the rapid phase of compensatory renal growth. All values are mean ± SE for \(n = 4\) rats. *Significantly different (\(p < 0.05\)) from the mean for the corresponding group of control rats. **Significantly different (\(p < 0.05\)) from the mean for the corresponding group of control rats and the mean for the corresponding group of NPX rats.
FIG. 4. Content of mercury (Hg) in the blood (a) and the percentage of Hg in blood present in the plasma (b) of control rats, uninephrectomized (NPX) rats, and 75% nephrectomized (75% NPX) rats 1, 2, and 7 days after the intravenous injection of a nontoxic 0.5 μmol/kg dose of mercuric chloride. The injection of mercuric chloride was administered 12 days after surgery, which was allowed for recovery and for the completion of the rapid phase of compensatory renal growth. All values are mean ± SE for n = 4 rats. *Significantly different (p < 0.05) from the mean for the corresponding group of control rats. **Significantly different (p < 0.01) from the mean for the corresponding group of control rats and the mean for the corresponding group of NPX rats.

The total content of mercury in the blood decreased in all three groups of rats between the 1st and 7th day after injection. Of the total mercury in blood, the fraction present in the plasma was about 40–45% in all three groups of rats 1 day after injection of mercuric chloride (Fig. 4b). By the end of 2nd day following the injection of mercuric chloride, the fraction of mercury present in the plasma in the control rats remained around 40%. However, the fraction of mercury in the plasma of the NPX rats and the 75% NPX rats was significantly greater than that in the control rats. No significant differences in the fraction of mercury in the plasma were detected between the three groups of rats studied 7 days after injection of mercuric chloride. The fraction of mercury in the plasma at this time was around 46–48%.

Urinary and Fecal Excretion of Mercury

Profiles for the cumulative urinary excretion of mercury in the control rats, NPX rats, and 75% NPX rats are presented in Fig. 5a. During each day of the 7 days in which the urinary excretion of mercury was measured, the 75% NPX rats excreted more mercury in the urine (% dose) on a cumulative basis than the control rats or NPX rats. By the end of the 7th day after injection of the nontoxic dose of mercuric chloride, the 75% NPX rats had excreted greater than 25% of the administered dose of mercury in the urine. There were no significant differences in the cumulative urinary excretion of mercury between the NPX rats and the control rats during the 7 days that the urinary excretion of mercury was measured. Both groups of rats excreted approximately 15% of the administered dose of mercury by the end of 7 days following the injection of mercuric chloride.

Differences were also detected in the profiles for the cumulative fecal excretion of mercury between the three groups of rats. As with the urinary excretion of mercury, the cumulative fecal excretion of mercury was greater in the 75% NPX rats than in the corresponding control rats or NPX rats at all times studied (Fig. 5b). However, unlike the cumulative urinary excretion of mercury, the cumulative fecal excretion of mercury was also significantly greater in the NPX rats than in the control rats. By the end of the 7th day following the injection of mercuric chloride, the 75% NPX rats had excreted about 28% of the dose, the NPX rats had excreted about 23% of the dose, and the control rats had excreted about 18% of the dose of administered inorganic mercury in the feces.

DISCUSSION

On the basis of the clearance data, it appears that 75% nephrectomy (produced by the combination of uninephrectomy plus ligation of the posterior branch of the contralateral renal artery) caused functional changes to occur in rats in the present study that were consistent with the early changes associated with chronic renal failure. Most notably, there was a significant increase in the concentration of creatinine in the plasma and a decrease in whole animal GFR in the 75% NPX rats relative to that in the control rats or NPX rats. A significant decrease in GFR following 75% nephrectomy is a consistent finding in rats that has been documented in previous studies (Kunau and
Whinnery, 1978; Zalups, 1989; Zalups and Henderson, 1992; Zalups et al., 1985). Other functional changes that occurred in the 75% NPX rats that are consistent with previous findings are increases in the fractional excretion of sodium and the fractional excretion of potassium in the absence of any change in the concentration of sodium or potassium in the plasma (Zalups, 1989; Zalups and Henderson, 1992; Zalups et al., 1985). These findings indicate that the remnant kidney in 75% NPX rats is functionally capable of maintaining normal homeostasis of sodium and potassium by increasing the fractional excretion of these two ions. The mechanism for the increased fractional excretion of potassium following 75% nephrectomy appears to be linked to a hypertrophic response in the cortical collecting duct that involves a significant increase in the surface density of the basolateral membrane of principal cells (Zalups, 1989; Zalups et al., 1985). It is believed that the amplification of the basolateral membrane in these cells is due to the insertion of newly synthesized membrane-containing sodium pumps, which appears to be regulated by some factor(s) governing and regulating the homeostasis of potassium (Zalups, 1989).

In contrast to 75% nephrectomy, uninephrectomy did not have a statistically significant effect on whole animal GFR, which indicates that renal blood flow and single nephron glomerular filtration rate were able to increase sufficiently in the contralateral kidney of the NPX rats to maintain apparently normal levels of GFR. Since whole animal GFR did not change significantly in the NPX rats, one would not have expected significant changes in the concentration of sodium or potassium in the plasma or in the fractional excretion of sodium or potassium, which was the case in the NPX rats.

Enhanced intrarenal accumulation of mercury was detected in the NPX rats at all times studied after the administration of the 0.5 μmol/kg dose of mercuric chloride. The most consistent finding was a specific increase in the accumulation and/or retention of mercury in the outer stripe of the outer medulla. Increased accumulation and/or retention of mercury in this zone of the kidney is an established response found in NPX rats treated with nontoxic doses of inorganic mercury (Zalups, 1991a,b, 1993; Zalups and Cherian, 1992a,b; Zalups and Lash, 1990).

Notwithstanding the fact that there was a significant increase in the concentration of mercury in the outer stripe of the outer medulla of the NPX rats at all times studied after injection of mercuric chloride, the concentration of mercury, at the level of the whole left kidney, was significantly elevated only during the first day following injection. One reason that the renal concentration of mercury in the NPX rats was elevated significantly only after the first day of injection may be related to the fact that the renal cortical concentration of mercury was also elevated significantly only during the same time. Subsequently, the intrarenal distribution of mercury changed in the NPX rats. Between the first and seventh days after injection, the concentration of mercury in the renal outer stripe of the outer medulla continued to increase, while the concentration of mercury in the renal cortex tended to decrease. The difference in the increase in concentration of mercury in the outer stripe of the outer medulla and the decrease in the concentration of mercury in the renal cortex, plus whatever mercury was
excreted in the urine, may be the reason why a significant increase in the concentration of mercury at the level of the whole kidney was not detected at the end of the second day and seventh day after the injection of mercuric chloride. Thus, measuring concentrations of mercury in the various zones of the kidney is important when studying the renal disposition of mercury, since changes in the intrarenal distribution of mercury can occur in the absence of detectable changes in the concentration of mercury at the level of the whole organ.

Although it is not clear what specific mechanism(s) is responsible for the enhanced accumulation and/or retention of mercury in the outer stripe of the outer medulla in NPX rats, it is most likely due to some factors associated with compensatory renal growth. It is well documented that certain renal transport and metabolic processes are increased and that renal blood flow and single nephron glomerular filtration rate are increased when renal mass is reduced significantly (Meyer et al., 1991). Some possible mechanisms associated with compensatory renal growth that could influence the uptake and retention of mercury in the outer stripe of the outer medulla (most likely in pars recta of proximal tubules; Zalups, 1991a) include enhanced cellular transport of mercury, and/or increased concentration of intracellular thiols, and/or increased delivery of mercury via the blood and/or luminal fluid.

Recent findings indicate that the concentration of the intracellular thiols glutathione and metallothionein increase significantly in the renal cortex and outer stripe of the outer medulla of rats after uninephrectomy and compensatory renal growth (Zalups and Lash, 1990; Fraser et al., 1994). Increased intracellular concentrations of these two thiols in the outer stripe of the outer medulla may be an important mechanism involved in the increased concentration of mercury that occurs in this renal zone of NPX rats. Since there is a high affinity between inorganic mercury and reduced sulfhydryl groups, increased intracellular concentrations of glutathione and/or metallothionein would presumably increase the probability of a mercuric ion that has entered into a renal epithelial cell being retained in the cytosol as a result of binding to one of the molecules of glutathione or metallothionein.

A 75% reduction in renal mass induced different changes in the renal accumulation and intrarenal distribution of mercury than did uninephrectomy. Unlike in the NPX rats, there was no difference in the overall renal concentration of mercury relative to that in the control rats 1 day after injection of mercuric chloride. In this case, the concentration of mercury in the renal cortex was significantly lower, and the concentration of mercury in the outer stripe of the outer medulla was significantly greater in the 75% NPX rats than in the control rats. The renal cortical concentration of mercury remained significantly lower in the 75% NPX rats, relative to that in the control rats or NPX rats, throughout the remainder of the study. In the outer stripe of the outer medulla, the concentration of mercury was not different from that in control rats during the period from the end of the second day to the end of the seventh day after injection of the nontoxic dose of mercuric chloride. As a result of the changes in the intrarenal distribution of mercury, the overall renal concentration of mercury in the remnant left kidney of the 75% NPX rats was significantly lower than that in the control rats or NPX rats.

It is not exactly clear at this time why the renal accumulation of mercury, particularly in the outer stripe of the outer medulla, in the 75% NPX rats was either similar to or less than that in the control rats, or was always less than that in the NPX rats. Since compensatory renal growth had occurred in both the NPX rats and 75% NPX rats, one might have expected that the intrarenal patterns of accumulation of mercury between the two groups of rats would have been similar. The question then is what factor(s) is responsible for the differences in the intrarenal accumulation and distribution of mercury between the NPX and 75% NPX rats. One important functional difference between the two groups of rats that might explain the disparity in the renal accumulation of mercury is that GFR was significantly decreased in the 75% NPX rats and not in the NPX rats. In fact, the present findings appear to indicate that as renal mass is reduced to the point where whole animal GFR is decreased significantly, the renal accumulation of mercury, specifically in the cortex and outer stripe of the outer medulla, does not reach levels attained in rats that have undergone a reduction of renal mass that does not have a significant effect on GFR. This is an important concept, since it indicates that filtration of mercury at the glomerulus and the subsequent reabsorption of mercury by segments of the proximal tubule may be the primary mechanisms involved in the renal tubular uptake and accumulation of mercury.

On the basis of the intrarenal accumulation and distribution of mercury in the 75% NPX rats, it is not clear at present how 75% nephrectomy would affect the nephropathy induced by toxic doses of inorganic mercury. One can speculate, however, that since the overall population of nephrons is reduced to the point where renal function is significantly impaired, and since the epithelial cells in some segments of the nephron are metabolically hyperactive as a result of compensatory hypertrophy (Meyer et al., 1991), the remaining functional renal mass in the remnant kidney may be more sensitive to inorganic mercury than the renal mass of normal rats or NPX rats. Further studies are clearly needed to evaluate how 75% nephrectomy affects the nephropathy induced by toxic doses of mercuric chloride.

Although secretion of mercury was not evaluated, one can assume that some secretory pathways were intact in the remnant kidneys of the 75% NPX rats inasmuch as the secretion of potassium by the cortical collecting duct was sufficient to maintain normal plasma levels of potassium.
Thus, if the uptake of mercury across the basolateral membrane was an important mechanism in the renal tubular uptake of mercury, one would not have expected the data obtained from the 75% NPX rats. Rather, one might have expected to find a pattern for the renal accumulation of mercury that was similar to that found in NPX rats.

The findings from the present study also indicate that a 75% reduction in renal mass causes the concentration of mercury to remain higher in the blood than in control or normal animals. This effect is probably due to decreased renal clearance of mercury resulting from the reduction in the number of functioning glomeruli.

It is interesting that while there was an apparent decrease in the renal clearance of mercury after 75% nephrectomy, the hepatic uptake and concentration of mercury were increased and there was an increase in the biliary excretion of mercury, as reflected by a significant increase in the cumulative fecal excretion of mercury. Thus, it appears that when the renal clearance of mercury is decreased significantly, the hepatic uptake and the hepatic (and biliary) clearance of mercury are increased. This effect could be deleterious to hepatocytes since increased intracellular concentrations of mercury may result in cellular injury and or necrosis.

The cumulative fecal excretion of mercury was also significantly increased in the NPX rats relative to that in the control rats, despite the fact that no statistically significant changes in GFR could be detected in the NPX rats. Enhanced fecal excretion of mercury in NPX rats relative to that in control rats has also been documented in a couple of recent studies (Zalups, 1993; Zalups et al., 1987). Despite the inability to detect significant changes in GFR in the NPX rats, data from this and previous studies (Zalups, 1993; Zalups et al., 1987) indicate that the renal clearance of mercury is decreased slightly (as reflected by small increases in the level of mercury in the blood) following uninephrectomy and compensatory renal growth. Further studies are needed to clarify this issue.

In addition to the greater cumulative fecal excretion of mercury in the 75% NPX rats than in the control or NPX rats, the cumulative urinary excretion of mercury was significantly greater in the 75% NPX rats. Although the exact mechanisms for the increased urinary excretion of mercury in the 75% NPX rats are not known at present, it is possible that the polyuria detected in the rats is a factor involved the increased urinary excretion of mercury. It has been detected in this laboratory that 75% NPX rats are far more proteinuric than normal rats or NPX rats, and this too may be a factor that contributes to the enhanced urinary excretion of mercury.

In summary, the present findings in rats show that 75% nephrectomy does not cause the same changes in the renal accumulation of mercury that are caused by uninephrectomy. The decreased renal accumulation of mercury that occurs in 75% NPX rats, relative to that in NPX rats, appears to be related to a significant decrease in the renal clearance of mercury, caused by a significant decrease in GFR. The present findings also show that 75% nephrectomy causes content of mercury in the blood and liver to increase significantly, and that it causes the urinary and fecal excretion of mercury to increase significantly.

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REFERENCES


