Effects of Uninephrectomy and Mercuric Chloride on Renal Glutathione Homeostasis

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ABSTRACT
The effects of uninephrectomy and i.v. injections of inorganic Hg on renal glutathione (GSH) homeostasis were studied in rats. Compensatory renal growth occurred in all uninephrectomized (NPX) rats 12 days after surgery. The weights of, and the amounts of total protein in, the remnant left kidneys from the NPX rats were significantly greater than those of the corresponding left kidneys from sham-operated (SHAM) rats. The concentration of GSH in samples of whole kidney, cortex and outer stripe of the outer medulla increased after uninephrectomy, with the most striking changes occurring in the outer stripe of the outer medulla. The concentration of GSH in all samples from the SHAM and NPX rats was greater after the administration of a low, nontoxic dose of HgCl₂ (0.5-μmol/kg). As with uninephrectomy alone, the increases due to inorganic Hg in both SHAM and NPX rats were greatest in the outer stripe of the outer medulla. The concentration of GSH increased further with a higher, toxic dose of HgCl₂ (2.0-μmol/kg). Increasing the dose of HgCl₂ to 3.0-μmol/kg resulted in more severe damage to the kidneys of all rats and in decreased concentration of renal GSH. The concentration of Hg under the same conditions as above was also measured, and closely paralleled that of GSH, with the greatest differences occurring in the outer stripe of the outer medulla. To explain these results, we hypothesize that both during compensatory renal growth and after administration of low, nontoxic to mildly toxic doses of Hg, GSH synthesis is induced. At higher, more toxic doses of Hg, GSH depletion becomes more prominent.

Reduction in renal mass, due either to surgery or to renal disease, produces an adaptive response known as compensatory renal growth. The simplest experimental method for studying this process is uninephrectomy (removal of one kidney). Decreasing the number of nephrons produces changes in renal homeostasis that allow the remaining nephrons to increase their functional capacity (Harris et al., 1986). In this adaptive response, a number of processes increase in proportion to the increase in renal mass. Certain reactions and processes, however, increase disproportionately to the increase in renal mass, suggesting a special importance in the maintenance of renal function (Harris et al., 1986). Additionally, various regions of the nephron respond differently so that there is a specific pattern of change. The cellular hypertrophy that occurs in compensatory renal growth, for example, is most prominent in the proximal segments of the nephron (Arrizurieta de Muchnik et al., 1969; Harris et al., 1986). There may be toxicological implications of compensatory renal growth because of the various functional and metabolic changes that occur in association with this process. These changes may alter the susceptibility of the remaining renal tissue to chemical-induced toxicity.

An example of uninephrectomy and compensatory renal growth altering the susceptibility to, and the pattern of, injury may be found in the case of the nephropathy induced by inorganic Hg (Houser and Berndt, 1986, 1988; Zalups and Diamond, 1987; Zalups et al., 1988a). Accumulation of Hg in the renal outer medulla of the rat after administration of a relatively nontoxic dose of HgCl₂ increases after uninephrectomy (Zalups and Diamond, 1987; Zalups et al., 1987). Moreover, injury to the tubular epithelium in the renal outer medulla, after low toxic doses of HgCl₂, is greater in rats that have undergone unilateral nephrectomy and compensatory renal growth than in SHAM rats (Zalups and Diamond, 1987), suggesting a correlation between the accumulation and toxicity of Hg. This correlation may not hold at higher, more toxic doses of HgCl₂ (Houser and Berndt, 1986).

Although little is known about the mechanism of renal accumulation of Hg, a recent study (Zalups and Veltman, 1988) suggests a possible hypothesis to explain these observations. Preliminary evidence indicates that the concentration of reduced GSH may increase in the renal outer medulla of the rat after a reduction in renal mass. Because the mercuric ion has

ABBREVIATIONS: SHAM, sham-operated rats; GSH, glutathione; NPX, unilateral nephrectomized rats; GSSG, glutathione disulfide.
a high affinity for thiol groups, one of the mechanisms by which the concentration of Hg increases in the outer medulla of the rat after uninephrectomy may be by the binding of mercuric ions to the increased number of available thiol groups (in particular, nonprotein thiols such as GSH) that are present within the tubular epithelial cells. Several previous studies (Johnson, 1982; Berndt et al., 1985; Baggett and Berndt, 1986; Fukino et al., 1984, 1986; Siegers et al., 1987) found a relationship between the cellular concentration of GSH and that of Hg.

In the present work we investigated renal GSH homeostasis after uninephrectomy and a period of compensatory renal growth to confirm and study in greater detail the finding of increased concentration of GSH in the outer medulla. We also studied the effects of nontoxic and toxic doses of HgCl₂ on renal GSH homeostasis in NPX and SHAM rats. Although the concentration of GSH and Hg was assessed in samples of the whole kidney and cortex, we focussed primarily on the outer stripe of the outer medulla. This region of the kidney contains the pars recta segment of the proximal tubule. This portion of the proximal tubule is the most sensitive segment of the nephron to the nephrotoxic effects of HgCl₂ (Ganote and Reimer, 1974; Gritzka and Trump, 1968; Zalups and Diamond, 1987).

**Materials and Methods**

**Surgical procedures.** Eighty-four male Sprague-Dawley rats (175–200 g; Harlan Sprague-Dawley, Indianapolis, IN) were used in the present study. The rats were divided into two surgical groups of equal size. One group underwent unilateral nephrectomy and the other underwent sham operation. Animals were first anesthetized with sodium pentobarbital (50 mg/kg i.p.) before surgery. Unilateral nephrectomies were performed by making a 2.5-cm flank incision on the right side of the body with a No. 11 scalpel blade, beginning at erector spinas muscles and continuing along the angle of the 12th rib. The incision was made through the skin and underlying fascia. The abdominal muscles were then incised along the same plane as the skin was cut to expose the retroperitoneal region where the right kidney is situated. With blunt dissection, the right kidney was exteriorized from the animal and the renal blood vessels and ureter were ligated with sterile 2.0 silk suture. The right kidney was then excised distal to the ligature. The abdominal muscles were sewn together with 4.0 sterile silk suture and the opposite ends of the incised skin and fascia were brought back together with sterile 9-mm wound clips. For the sham operations, the same operative procedures were performed, except that the right renal blood vessels and ureter were not ligated and the right kidney was not excised.

**Treatment and processing of samples.** The NPX and SHAM rats were further subdivided into groups that were to receive a 0.5-, 0.5- or 3.0-mmol/kg dose of HgCl₂ i.v. Twelve NPX and 12 SHAM rats were not given any HgCl₂ and 10 NPX and 10 SHAM rats were placed in the groups to receive one of the three doses of HgCl₂. All the animals were given 12 days to recover from the surgery. This time period also allowed for the completion of the rapid phase of compensatory renal growth in the NPX rats. In the NPX and SHAM rats that received a dose of HgCl₂, the dose was administered on the morning of the 11th day after surgery. All doses of HgCl₂ were prepared in solutions containing 0.9% (w/v) sodium chloride (2.0 ml/kg) and were administered by injection into the femoral vein after light anesthesia was induced with ether. Four NPX and four SHAM rats that received the 0.5-, 2.0- or 3.0-mmol/kg dose of HgCl₂ also received 4 μCi/kg of ³²HgCl₂. These animals were used to study the renal accumulation and intrarenal distribution of Hg. The specific activity of the radiolabeled Hg was 1.7 mCi/mg. On the morning of the 12th day, all the animals were anesthetized with an overdose of sodium pentobarbital (100 mg/kg), the left kidney was removed and the animals were sacrificed by exsanguination.

After removing the left kidneys from the NPX and SHAM rats, they were weighed and dissected. A half kidney and samples of renal cortex and outer stripe of the outer medulla were obtained from each left kidney. All the samples of renal tissue from the rats that did not receive ³²HgCl₂ were placed immediately into a cold (4°C) solution containing 1 mM bathophenanthroline disulfonic acid in 10% (v/v) perchloric acid. Bathophenanthroline was added to inhibit GSH autoxidation during processing of the tissue samples. The samples of half-kidney were placed in 10 ml of the solution and the samples of cortex and outer stripe of the outer medulla were placed in 1.5 ml of the solution. All the tissues were homogenized in a glass/Teflon homogenizer with a variable speed motor. Homogenates were then centrifuged at 4°C at 1250 × g for 10 min. The supernatants (perchloric acid extracts) were used for all subsequent analyses. Kidneys and samples of kidney were placed in ice-cold acid within 30 sec of their removal from the animals in order to minimize any postmortem artifact. The samples of renal tissue obtained from the animals that received ³²HgCl₂ were placed in plastic syringes for gamma counting.

**Assays.** Concentrations of GSH and GSSG were measured as the S-carboxymethyl, N-dinitrophenyl derivatives and concentrations of GSSG were measured as the N,N-bis-dinitrophenyl derivative by the high-pressure liquid chromatographic method of Faris and Reed (1987). Separations were achieved with a µBondapak amine 10-µm cartridge (8 mm × 10 cm) (Waters Associates, Milford, MA) with a Waters model 600E multisolve delivery system and used a methanol-acetate mobile phase and gradient elution. Derivatives were detected at 365 nm on a Waters model 490 detector and were quantitated with respect to standards using a Waters model 745 data module.

Concentrations of Hg were measured with radiolabeled ³²HgCl₂ (New England Nuclear Research Products, Boston, MA) in a gamma counter as described previously (Zalups et al., 1987).

Protein concentrations were measured by the method of Lowry et al. (1951) with bovine serum albumin as a standard. Perchloric acid-insoluble pellets (0.2 ml) were re-suspended in 1 ml of 0.1 M NaOH for assay.

**Histological analyses.** A 4-mm cross-sectional slice of the left kidney from each NPX and SHAM rat was taken for histological examination. The tissue slices were fixed for 48 hr in 10% neutral buffered formalin. They were then dehydrated in a series of graded ethanol, cleared in xylene and embedded in paraffin wax. From the blocks of paraffin-embedded tissue, 5-µm sections were cut. The sections were stained in hematoxylin and eosin and were viewed under a light microscope. As part of the histological examination of renal tissue, HgCl₂-induced cellular and tubular necrosis in the pars recta segment (mainly SI3) of proximal tubules in both the cortex and outer stripe of the outer medulla was assessed on a qualitative basis and a quantitative basis. Quantitatively, necrosis was ranked on a scale of 0 to 4 in severity. The definition of each rank score used in this study is: 0, no necrosis; 1, less than 25% of the tubules displaying signs of cellular necrosis; 2, 26 to 50% of the tubules displaying signs of cellular necrosis; 3, greater than 50% of the tubules displaying signs of cellular necrosis; and 4, greater than 50% of the tubules displaying signs of complete tubular necrosis. A rank score was assigned to each animal after counting the number of proximal tubules affected by necrosis in at least four randomly selected low power (200×) fields of the cortex and outer stripe of the outer medulla. Epithelial cells that had undergone necrosis were identified by their bright eosinophilic cytoplasm and dark pyknotic nuclei. Tubular necrosis was characterized by the death and shedding of all epithelial cells in an affected tubule.

**Statistics.** All values are expressed as mean ± S.E. Differences between means for any set of data for the NPX and SHAM rats at the four doses of HgCl₂ were evaluated statistically by performing a 2 × 4 two-way analysis of variance. When F values were found to be statistically significant at the 95% confidence interval, comparisons between combinations of two means were carried out to determine at which level the significance for the analysis was arising. The statistical
comparisons between means were performed by using the "protected t test" designed for the analysis of variance. Differences in severity of cellular and tubular necrosis in the para cortex segments of proximal tubules between NPX and SHAM rats given the same dose of HgCl₂ were evaluated statistically using the two-tailed Mann-Whitney rank sum test (Zar, 1974).

Results

In the present study we examined first the effects of uninephrectomy and compensatory renal growth, and then the effects of HgCl₂, on renal GSH homeostasis. This design enabled us to assess how Hg alters renal GSH status and how uninephrectomy and compensatory renal growth in turn alter the response to Hg. In addition to looking for effects in samples of the whole kidney, samples of cortex and outer stripe of the outer medulla were studied to look for regional differences in responses.

The phenomenon of compensatory renal growth was confirmed and occurred in all groups of NPX rats 12 days after uninephrectomy. Evidence for this is that the weights of the remnant left kidneys from the NPX rats were significantly greater than the weights of the corresponding left kidneys in the SHAM rats (table 1). Weights of the left kidney in both SHAM and NPX rats also increased with increasing concentrations of Hg. No significant differences were seen in body weights between SHAM and NPX rats but, in both groups, body weights decreased with increasing concentrations of Hg, presumably due to Hg intoxication.

Although new protein was synthesized during compensatory renal growth, the concentration of protein in whole kidney, cortex or outer stripe of the outer medulla was not significantly different from that in SHAM rats at the two lowest (0 or 0.5 μmol/kg) or at the highest dose (3.0 μmol/kg) of Hg (table 2). Only at the 2.0-μmol/kg dose of Hg was the concentration of protein in NPX rats lower than that in SHAM rats. This was probably due to an enhanced sensitivity to Hg-induced nephrotoxicity after uninephrectomy at this particular dose of Hg (see below).

The concentrations of GSH in samples of whole kidney, cortex and outer stripe of the outer medulla from the NPX rats that did not receive any Hg were all significantly greater (P < .05) than those in the corresponding samples from SHAM rats

### TABLE 1

Effects of uninephrectomy and dose of HgCl₂ on body and kidney weights in the rat

Values are mean ± S.E. for 12 animals (0.0 μmol of HgCl₂/kg) or 6 animals (all other doses).

<table>
<thead>
<tr>
<th>Surgical Group</th>
<th>Dose of HgCl₂ (μmol/kg)</th>
<th>Body WT</th>
<th>Left Kidney WT</th>
<th>Total Renal Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM</td>
<td>0.0</td>
<td>286 ± 6</td>
<td>1.12 ± 0.03</td>
<td>2.22 ± 0.06</td>
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<tr>
<td></td>
<td>0.5</td>
<td>263 ± 4</td>
<td>1.06 ± 0.05</td>
<td>2.10 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>254 ± 4</td>
<td>1.21 ± 0.04</td>
<td>2.49 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>257 ± 4</td>
<td>1.34 ± 0.04</td>
<td>2.66 ± 0.05</td>
</tr>
<tr>
<td>NPX</td>
<td>0.0</td>
<td>279 ± 5</td>
<td>1.48 ± 0.04</td>
<td>1.48 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>264 ± 2</td>
<td>1.41 ± 0.03</td>
<td>1.41 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>254 ± 4</td>
<td>1.69 ± 0.04</td>
<td>1.69 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>252 ± 4</td>
<td>1.60 ± 0.06</td>
<td>1.60 ± 0.06</td>
</tr>
</tbody>
</table>

* Significantly different (P < .05) from the mean for the corresponding SHAM animals given the same dose of HgCl₂; ** significantly different (P < .05) from the mean for animals in the same series (SHAM or NPX) given 0.0 μmol of HgCl₂ per kg; † significantly different (P < .05) from the mean for animals in the same series (SHAM or NPX) given 0.5 μmol of HgCl₂ per kg; ‡ significantly different (P < .05) from the mean for animals in the same series (SHAM or NPX) given 2.0 μmol of HgCl₂ per kg.

### TABLE 2

Effects of uninephrectomy and dose of HgCl₂ on renal protein concentrations in the rat

Values are mean ± S.E. for 12 animals (0.0 μmol of HgCl₂/kg) or 6 animals (all other doses).

<table>
<thead>
<tr>
<th>Surgical Group</th>
<th>Dose of HgCl₂ (μmol/kg)</th>
<th>Protein Conc. (mg/g tissue)</th>
<th>Whole Kidney</th>
<th>Cortex</th>
<th>Outer stripe of outer medulla</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM</td>
<td>0.0</td>
<td>193 ± 11</td>
<td>213 ± 14</td>
<td>229 ± 16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>216 ± 11</td>
<td>240 ± 13</td>
<td>220 ± 16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>215 ± 7</td>
<td>215 ± 7</td>
<td>229 ± 16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>145 ± 7* II</td>
<td>183 ± 5* III</td>
<td>157 ± 7* II</td>
<td></td>
</tr>
<tr>
<td>NPX</td>
<td>0.0</td>
<td>185 ± 11</td>
<td>193 ± 13</td>
<td>229 ± 20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>183 ± 3</td>
<td>221 ± 19</td>
<td>225 ± 15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>185 ± 10</td>
<td>179 ± 8</td>
<td>197 ± 9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>152 ± 5*</td>
<td>181 ± 5</td>
<td>164 ± 16</td>
<td></td>
</tr>
</tbody>
</table>

* Significantly different (P < .05) from the mean for the corresponding SHAM animals given the same dose of HgCl₂.

12 days after surgery (fig. 1). The most striking changes in the concentration of GSH occurred in the outer stripe of the outer medulla (fig. 1C), where the concentration of GSH increased 36% after uninephrectomy and compensatory renal growth (2.33 ± 0.08 μmol/kg of tissue in NPX rats vs. 1.71 ± 0.12 μmol/g of tissue in SHAM rats). In contrast, the concentration of GSH in the cortex (fig. 1B) increased by only 18% after uninephrectomy and compensatory renal growth (3.53 ± 0.13 μmol/g of tissue in NPX rats vs. 3.00 μmol/g of tissue in SHAM rats).

In NPX and SHAM rats that received the relatively nontoxic (0.5 μmol/kg) dose of HgCl₂, the concentration of GSH in all regions of the kidney that were examined was statistically greater (P < .05) than that in the corresponding NPX and SHAM rats that did not receive any Hg (fig. 1). Moreover, the concentration of GSH in samples of the whole kidney and outer stripe of the outer medulla from the NPX rats given the 0.5-μmol/kg dose of HgCl₂ was significantly greater than that from the SHAM rats given the same dose of HgCl₂, indicating that this low dose of Hg did not alter the response to uninephrectomy. As in the case of the NPX and SHAM rats that did not receive any Hg, the most striking change was detected in the concentration of GSH in the outer stripe of the outer medulla. In this region, the concentration of GSH in NPX rats was 47% higher than that in SHAM rats (fig. 1C).

Administration of a toxic dose of Hg (2.0 μmol/kg) also produced statistically significant increases in the concentration of GSH in samples of cortex, outer stripe of the outer medulla and whole kidney in both SHAM and NPX rats when compared with that in corresponding samples from the SHAM and NPX rats given no Hg (fig. 1). Although the concentration of GSH in samples of renal tissue from SHAM and NPX rats given the 2.0-μmol/kg dose was slightly higher than that in the corresponding SHAM and NPX rats given the 0.5-μmol/kg dose, the differences were not statistically significant. Only in the outer stripe of the outer medulla of kidneys from the NPX rats given the 2.0-μmol/kg dose of Hg was the mean concentration of GSH significantly greater than that in the outer stripe of the outer medulla of the left kidneys from the corresponding SHAM rats given the same dose of Hg. Once again, it is the
of GSH in samples of whole kidney and outer stripe of the outer medulla from SHAM rats given the 3.0-μmol/kg dose was significantly different from that of the corresponding samples from SHAM rats given the 0.5-μmol/kg dose and was similar to that of the corresponding samples from SHAM rats not given any Hg. The concentration of GSH in the renal cortex of SHAM rats given the 3.0-μmol/kg dose, however, was similar to that of SHAM rats given the 0.5-μmol/kg dose and was significantly greater than that of SHAM rats not given any Hg. Differences between SHAM and NPX rats given the 3.0-μmol/kg dose, with respect to the concentration of GSH in all three samples of renal tissue, were statistically significant.

The differences in the status of renal GSH due to uninephrectomy and Hg are more pronounced when total amounts of GSH in the left kidney and total renal mass are examined (data not shown). The same general pattern of increased GSH with increased dose of Hg up to 2.0-μmol/kg and decreased GSH at 3.0 μmol/kg was observed in both SHAM and NPX rats. Uninephrectomy alone increased the total amount of GSH in the left kidney by 50% as compared with the amount in the left kidney of SHAM rats. Even at the highest dose of Hg, the total amount of GSH in the left kidney of NPX rats was 79% higher than that of SHAM rats. Examination of total renal GSH at the various doses of Hg shows that the NPX animals were able to compensate to a large extent and had greater than 70% of the total renal GSH as that in the corresponding SHAM rats.

Another indicator of the status of renal GSH that we examined was the concentration of cysteine after uninephrectomy and HgCl₂ (table 3). Uninephrectomy alone had no effect on the concentration of cysteine in either whole kidney, cortex or outer stripe of the outer medulla. The concentration of cysteine in samples of whole kidney, cortex and outer stripe of the outer medulla for both SHAM and NPX rats increased after administration of the low, nontoxic dose of HgCl₂. Administration of higher, toxic doses of HgCl₂ caused decreases in cysteine concentration in all samples. When the total amounts of cysteine in the left kidney and in the total renal mass in SHAM and NPX rats were examined, the same pattern with increasing dose of Hg as described above was observed (data not shown).

### TABLE 3

<table>
<thead>
<tr>
<th>Surgical Group</th>
<th>Dose of HgCl₂ (μmol/kg)</th>
<th>Cysteine Conc.</th>
<th>Whole kidney</th>
<th>Cortex</th>
<th>Outer stripe of outer medulla</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmol/kg</td>
<td>μmol/g tissue</td>
<td>μmol/kg</td>
<td>μmol/g tissue</td>
<td>μmol/kg</td>
</tr>
<tr>
<td>SHAM</td>
<td>0.0</td>
<td>0.69 ± 0.08</td>
<td>0.68 ± 0.07</td>
<td>1.77 ± 0.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>1.08 ± 0.07*</td>
<td>1.08 ± 0.16</td>
<td>2.25 ± 0.13</td>
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</tr>
<tr>
<td></td>
<td>2.0</td>
<td>0.61 ± 0.11*</td>
<td>1.10 ± 0.13</td>
<td>2.17 ± 0.34</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>0.41 ± 0.03*</td>
<td>0.65 ± 0.04*</td>
<td>0.56 ± 0.11**</td>
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</tr>
<tr>
<td>NPX</td>
<td>0.0</td>
<td>0.70 ± 0.03</td>
<td>0.84 ± 0.05</td>
<td>1.61 ± 0.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.87 ± 0.04*</td>
<td>1.09 ± 0.06</td>
<td>2.31 ± 0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>0.37 ± 0.04*</td>
<td>0.81 ± 0.08</td>
<td>1.38 ± 0.29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>0.65 ± 0.11</td>
<td>0.95 ± 0.06</td>
<td>1.47 ± 0.18*</td>
<td></td>
</tr>
</tbody>
</table>

* Significantly different (P < .05) from the mean for the corresponding SHAM animals given the same dose of HgCl₂. ** Significantly different (P < .05) from the mean for animals in the same series (SHAM or NPX) given 0.5-μmol of HgCl₂/kg. 

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date not shown)
Many nephrotoxic chemicals that interact with GSH also alter GSH redox status (Lash et al., 1988). We therefore also measured concentrations of GSSG in SHAM and NPX rats in the various tissue samples (table 4). Uninephrectomy alone had no effect on the concentration of GSSG in the cortex but increased the concentration of GSSG in the outer stripe of the outer medulla (50 ± 7 nmol/g of tissue for SHAM rats vs. 80 ± 7 nmol/g of tissue for NPX rats). With increasing doses of Hg, the concentration of GSSG in the outer stripe of the outer medulla increased in NPX rats relative to that in SHAM rats, but only at the 3.0-μmol/kg dose of Hg was the difference between SHAM and NPX rats statistically significant. Calculation of GSH/GSSG ratios (data not shown) demonstrated that the increases observed in concentrations of GSSG were in samples that had increased concentrations of GSH, so that few significant changes in GSH redox status occurred.

The concentration of Hg in samples of whole kidney, cortex and outer stripe of the outer medulla was also measured in SHAM and NPX rats given the same three doses of HgCl₂ (fig. 2). In samples of whole kidney and cortex from both SHAM and NPX rats, the concentration of Hg increased with increasing dose, although the change between the 2.0- and the 3.0-μmol/kg doses was not significant. The concentration of Hg in the whole kidney of the NPX rats at the 0.5-μmol/kg dose was significantly greater than that in the SHAM rats. As the dose of HgCl₂ was increased from 0.5- to 2.0-μmol/kg, the concentration of Hg in the outer stripe of the outer medulla increased in both SHAM and NPX rats. The concentration of Hg in the outer stripe of the NPX rats given the 3.0-μmol/kg dose of Hg was not significantly different from that in the outer stripe of the NPX rats given the 2.0-μmol/kg dose of Hg. In the SHAM rats, however, the concentration of Hg in the outer stripe was significantly lower at the 3.0-μmol/kg dose than at the 2.0-μmol/kg dose. At all three doses of HgCl₂, the concentration of Hg in the outer stripe was significantly greater in the NPX rats than in the SHAM rats. Expression of tissue Hg concentration as percentage of dose per gram of tissue rather than as nanomoles per gram of tissue to correct for changes in body weight gave qualitatively, very similar results.

To assess the relationship between tissue concentrations of GSH and Hg, data in figures 1 and 2 were replotted to compare directly these two parameters under the same experimental conditions (i.e., SHAM or NPX rats and the various doses of HgCl₂) (fig. 3). In samples of whole kidney, renal cortex and outer stripe of the outer medulla, increasing tissue concentrations of Hg were associated with increasing tissue concentrations of GSH. This relationship held best at HgCl₂ doses of 0- to 2-μmol/kg. At the highest dose of HgCl₂ that was administered (3-μmol/kg), tissue GSH concentrations decreased sharply whereas tissue Hg concentrations increased or only slightly decreased.

Finally, we sought to correlate the observed changes in the status of renal GSH and renal Hg with the known nephrotoxicity of Hg. Histological analysis of SHAM and NPX rats

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**TABLE 4**

**Effects of uninephrectomy and dose of HgCl₂ on renal GSSG status in the rat**

Values are mean ± S.E. for 12 animals (0.0 μmol of HgCl₂ per kg) or 6 animals (all other doses).

<table>
<thead>
<tr>
<th>Surgical Group</th>
<th>Dose of HgCl₂ (μmol/kg)</th>
<th>GSSG Conc.</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole kidney</td>
<td>Cortex</td>
<td>Outer stripe of outer medulla</td>
<td></td>
</tr>
<tr>
<td>SHAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>124 ± 13</td>
<td>122 ± 7</td>
<td>50 ± 7</td>
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<td>0.5</td>
<td>176 ± 14</td>
<td>179 ± 12&quot;</td>
<td>77 ± 9</td>
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<tr>
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<td>133 ± 9</td>
<td>185 ± 28</td>
<td>72 ± 9</td>
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<tr>
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<td>142 ± 19</td>
<td>172 ± 16&quot;</td>
<td>#3 ± 3</td>
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<tr>
<td>NPX</td>
<td>149 ± 16</td>
<td>144 ± 13&quot;</td>
<td>80 ± 7</td>
<td></td>
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<td>221 ± 6&quot;</td>
<td>152 ± 22</td>
<td>88 ± 5</td>
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<tr>
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<td>207 ± 6&quot;</td>
<td>177 ± 17</td>
<td>93 ± 7&quot;</td>
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</table>

* Significantly different (P < .05) from the mean for the corresponding SHAM animals given the same dose of HgCl₂. ** significantly different (P < .05) from the mean for animals in the same series (SHAM or NPX) given 0.0 μmol of HgCl₂ per kg.

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**Fig. 2.** Effects of uninephrectomy and dose of HgCl₂ on renal accumulation of Hg. Concentrations of Hg (expressed as nanomoles per gram of tissue) were determined with radiolabeled HgCl₂ and counting of radioactivity in a gamma counter. Values are mean ± S.E. for three animals (NPX: 2.0-μmol/kg dose) or 4 animals (all other cases). For both SHAM and NPX in whole kidney, cortex and outer stripe of the outer medulla, Hg concentrations in animals given either the 2.0-μmol or the 3.0-μmol HgCl₂/kg dose were significantly greater (P < .05) than those in animals given the 0.5-μmol HgCl₂/kg dose. *Significantly different (P < .05) from the mean for the corresponding SHAM animals given the same dose of HgCl₂. **Significantly different (P < .05) from the mean for animals in the same series (SHAM or NPX) given 2.0-μmol of HgCl₂/kg.
treated with the four doses of HgCl$_2$ (0–3-μmol/kg) were thus performed and cellular and tubular necrosis in the pars recta segment of the proximal tubules was quantitated by a ranking system (table 5). No signs of injury were found in any sections of kidneys from the SHAM and NPX rats not given any Hg or from the SHAM and NPX rats that received the 0.5-μmol/kg dose of HgCl$_2$. In general, the renal tissue from these animals appeared to be normal.

Renal cellular and tubular necrosis was prominent in both the SHAM and NPX rats given the 2.0-μmol/kg dose of Hg. Damage was localized exclusively to the pars recta segments of the proximal tubules, primarily those situated in the outer stripe of the outer medulla. Qualitatively, the damage appeared to be more severe in the kidneys of the NPX rats. This was confirmed by the semiquantitative rank analysis. The mean rank score for damage to the pars recta segment of the proximal tubules in the cortex and the outer stripe calculated for the NPX rats was 3.25, which was significantly greater than the mean rank score of 1.83 that was calculated for the SHAM rats.

Damage was very severe in the kidneys of both the SHAM and NPX rats given the 3.0-μmol/kg dose of Hg. Qualitatively, it was not possible to discern any differences between SHAM and NPX rats with respect to the severity of damage to the pars recta segment of the proximal tubules in the cortex and the outer stripe of the outer medulla. Damage to the pars recta segment was so extensive in both groups that a broad band of necrosis could be visualized easily on sections of unfixed, freshly cut renal tissue with the unaided eye. By using the semiquantitative analysis, the rank scores for proximal tubular injury were similar for both groups of rats.

**Discussion**

The findings in the present study demonstrate that uninephrectomy in the rat causes the concentration of GSH to increase in the remnant kidney 12 days after surgery. This increase is most pronounced in the outer stripe of the outer medulla. A significant increase in the concentration of GSH also occurs in the cortex, although the increase is not as great as that in the outer stripe of the outer medulla. Both the increase in the concentration of GSH in the cortex and outer stripe of the outer medulla are sufficient enough to cause an increase in the concentration of GSH at the level of the whole kidney. Our findings are consistent with those of a recent preliminary study (Zalups and Veltman, 1988) in which the investigators observed significant increases in the concentration of GSH in the renal outer medulla of the rat 12 days after uninephrectomy. In that study, however, they did not observe significantly higher concentrations of GSH in the renal cortex or in the whole kidney. This difference between the two studies may be explained by the different strains of rat that were used. Furthermore, fewer animals were used in the previous study, which may have masked any statistical differences in the concentration of GSH in the renal cortex or whole kidney between SHAM and NPX rats.

The reason for the increase in the concentration of renal GSH after uninephrectomy and compensatory renal growth is not known. We can hypothesize, however, that because GSH...
plays an important role in protecting cells from various forms of chemical and pathological injury (Lash et al., 1988), the increase in the concentration of GSH in the remnant kidney may be an adaptive response that is related to some protective mechanism and to the ability of the remnant kidney to increase its functional capacity to compensate for the reduction in renal mass. In agreement with this, prior depletion of tissue GSH decreased accumulation of Hg in the kidney and led to increased Hg-induced nephrotoxicity (Johnson, 1982; Berndt et al., 1985; Baggett and Berndt, 1986; Fukino et al., 1986).

Several observations, however, cast doubt on this view and indicate that increased tissue concentrations of GSH may increase susceptibility to certain types of nephrotoxic agents. For example, the findings from three recent studies (Houser and Berndt, 1986; Zalups and Diamond, 1987; Zalups et al., 1988a) showed that NPX rats develop a more severe form of the nephropathy induced by Hg than SHAM rats. An important finding of the present study is that the greatest increase in the concentration of GSH occurred in the outer stripe of the outer medulla. The pars recta segment of the proximal tubule is found principally in this region of the kidney and it is this segment of the nephron that is primarily affected by the toxic effects of HgCl\(_2\) (Ganote et al., 1974; Gritzka and Trump, 1965; Zalups and Diamond, 1987; Zalups et al., 1988a). Additionally, many other nephrotoxicants, such as uranyl fluoride (Zalups et al., 1988b) and cysteine S-conjugates of halogenated hydrocarbons that are bioactivated by the cysteine conjugate \(\beta\)-lyase pathway (Jones et al., 1988; MacFarlane et al., 1989; Nash et al., 1984), and pathological conditions such as hypoxia and ischemia (Shanley et al., 1986a-c), produce selective nephropathy in the pars recta segment of the proximal tubule. This indicates that the effects observed in the outer stripe of the outer medulla may be attributed primarily to increases in intracellular concentrations of GSH in the epithelial cells of the pars recta of the proximal tubules. Besides being the target site in the nephron for Hg-induced nephrotoxicity, the pars recta segment of the proximal tubule is also the site with the highest concentration of GSH and the highest contents of \(\gamma\)-globulin GSH synthetase and GSH synthetase (Brehe et al., 1976; Guder and Ross, 1984; Mohandas et al., 1984). Under these experimental conditions, the increased concentration of GSH does not play a protective role but may function to enhance access of Hg to target sites within the cell.

To extend the observations on compensatory renal growth and GSH homeostasis, we also examined the effects of HgCl\(_2\) on the status of renal GSH in SHAM and NPX rats. Two recent studies (Zalups et al., 1987; Zalups and Diamond, 1987) showed that after giving a nontoxic dose of HgCl\(_2\) (0.5-\(\mu\)mol/kg), accumulation of Hg in the renal outer medulla of NPX rats was higher than that in SHAM rats. Houser and Berndt (1986) compared the toxicity of HgCl\(_2\) at a dose of 2 mg/kg 2 days after uninephrectomy, and found increased Hg toxicity in the NPX rats. In their case, however, they found no association between toxicity and renal cortical Hg content. Due to tissue necrosis, measurement of tissue Hg concentration may not accurately reflect the concentration of Hg to which the kidney was exposed during the development of injury and eventual cell death.

Although several compensatory changes occur in the remnant kidney, many of which may contribute to altered accumulation of Hg, the high affinity of Hg for sulfhydryl groups indicates a probable role for changes in the status of sulfhydryl groups and, in particular, the status of GSH, in the accumulation of Hg. Indeed, we found in the present study a good correlation, at least at nontoxic to moderately toxic doses of Hg, between tissue concentrations of Hg and those of GSH (fig. 3). At a highly toxic dose of HgCl\(_2\) (3 \(\mu\)mol/kg), the correlation did not hold well, indicating that other factors are important in Hg-induced nephrotoxicity.

Significant changes in the status of GSH were observed after the administration of Hg in both SHAM and NPX rats. This finding is in agreement with previous findings by others (Fukino et al., 1984, 1986; Siegers et al., 1987). The concentration of GSH was higher in both the cortex and the outer stripe of the outer medulla at both a low, nontoxic dose of HgCl\(_2\) (0.5-\(\mu\)mol/kg), and at a toxic dose of HgCl\(_2\) (2.0-\(\mu\)mol/kg), when compared with that found with no Hg. At the highest dose of Hg that was administered (3.0-\(\mu\)mol/kg), the concentration of GSH decreased in both cortex and outer stripe of the outer medulla. As was seen with uninephrectomy alone, the largest increases in the concentration of GSH occurred in the outer stripe of the outer medulla. This increase is probably due to the increase in the concentration of GSH in the epithelial cells of the pars recta segment of the proximal tubule. It is possible, however, that some of the increase is due to changes in the metabolism of GSH in the thick ascending limb or in the collecting ducts. This seems less likely, however, because of the low activities of enzymes involved in GSH metabolism in these segments of the nephron (Guder and Ross, 1984; Mohandas et al., 1984).

The increases in the concentration of GSH due to uninephrectomy and compensatory renal growth and those due to Hg exhibited the same pattern in the nephron in that they were highest in the outer stripe of the outer medulla and, when combined (e.g., NPX, 0.5-\(\mu\)mol of Hg per kg vs. SHAM, 0.0-\(\mu\)mol of Hg per kg), they were additive. This indicates that both compensatory renal growth and Hg increase the concentration of GSH by the same mechanism. Minimal effects were observed on the ratio of GSH/GSSG, indicating that the GSH redox status was not significantly altered by these procedures. This is important because GSH oxidation coupled with Hg could enhance toxicity. The biological importance of the small changes in the ratio of GSH/GSSG is unclear in that these changes did not follow the same pattern as the changes in the concentration of GSSG. This indicates further that oxidative stress, which can alter renal GSH redox status (McCoy et al., 1988), is probably not important in the mechanism of Hg-induced nephropathy.

The importance of distinguishing phenomena associated with low, nontoxic to mildly toxic doses of Hg and those associated with higher, very toxic doses of Hg should be emphasized. This is because the overall cellular response changes dramatically as the dose of Hg increases. At lower doses, little toxicity occurs and other processes that are undoubtedly involved in the cell's compensatory mechanisms predominate. At higher doses, toxicity predominates. This point would explain many of the differences with respect to effects of Hg on GSH status between this study and previous studies (Addya et al., 1984; Fukino et al., 1986; Siegers et al., 1987), which found decreased or only slightly increased concentrations of GSH after administration of high doses (i.e., 5-\(\mu\)mol/kg) of HgCl\(_2\). In this study, at the highest dose of HgCl\(_2\) administered (i.e., 3-\(\mu\)mol/kg), the concentration of GSH decreased, indicating that two separate processes occurred. At low, nontoxic to mildly toxic doses of
Hg, the predominant effect is an increase in the concentration of GSH. At higher doses of Hg, the predominant effect is a depletion of GSH. This occurs probably because inorganic Hg is both nephrotoxic and reacts with free sulfhydryl groups.

Intracellular concentrations of GSH are tightly regulated in mammalian cells. The rate of GSH synthesis is controlled by the activity of γ-glutamylcysteine synthetase, which in turn is regulated by feedback inhibition (Meister, 1989). This mode of regulation prevents intracellular concentrations of GSH from exceeding a preset limit. A logical hypothesis for the mechanism by which both compensatory renal growth and low doses of Hg increase concentrations of GSH is that they induce γ-glutamylcysteine synthetase. By promoting synthesis of more enzyme, the preset concentration of GSH is increased. The proposed competing effects of Hg, induction of GSH synthesis and depletion of GSH require confirmation and further study to determine what role they play in the nephropathy induced by Hg. Although the increased accumulation of Hg at low doses may be related to the increased concentrations of GSH, it is not uniformly accompanied by increased Hg-induced renal damage, as evidenced by the lack of cellular or tubular necrosis at the 0.5-μmol/kg dose.

In addition to the changes in GSH status, we observed increases in tissue cysteine concentration in samples of whole kidney, renal cortex and renal outer stripe of the outer medulla from both SHAM and NPX rats (table 3). Cysteine concentration was higher after administration of 0.5-μmol of HgCl₂ per kg as compared with animals that received no Hg. At higher doses of HgCl₂, tissue cysteine concentration decreased. One possible explanation for this is that Hg-induced changes in metallothionein synthesis occurred and that this in turn produced changes in tissue cysteine concentration.

Exposure of rats to elemental Hg vapor selectively increases renal tissue Hg concentration and selectively induces renal metallothionein (Cherian and Clarkson, 1976). Administration of HgCl₂ also increases renal metallothionein content several-fold within 24 hr (R. K. Zalups and G. Cherian, unpublished data). Greater induction of metallothionein by the administration of Hg also occurs after uninephrectomy (R. K. Zalups and G. Cherian, unpublished data). These findings provide a possible additional mechanism, besides the changes in GSH status reported in this study, to explain the enhanced accumulation of Hg and the greater nephrotoxicity of Hg after uninephrectomy and compensatory renal growth. Work by Fukino et al. (1984, 1986), showing that pretreatment of rats with zinc to induce metallothionein increased tissue GSH concentration and Hg accumulation, support a role for metallothionein in this process. Houser and Berndt (1988) showed that Hg distributed rapidly into “metallothionein-like” proteins. They concluded, however, that metallothionein was rapidly saturated with Hg, suggesting that this protein could not account for the enhanced nephrotoxicity of Hg after uninephrectomy. Furthermore, uninephrectomy by itself does not alter tissue metallothionein content (R. K. Zalups and G Cherian, unpublished data), indicating that metallothionein may not be involved in the NPX or Hg-induced changes in renal GSH status. Further study will be required to clarify the role of metallothionein.

In conclusion, this study has confirmed that compensatory renal growth occurs after uninephrectomy and that this is accompanied by increases in the concentration of renal GSH, which occurs predominantly in the outer stripe of the outer medulla. Administration of low, nontoxic and mildly toxic doses of HgCl₂ to both SHAM and NPX rats produced large increases in the concentration of GSH, which also occurred predominantly in the outer stripe of the outer medulla. At higher doses of Hg, renal damage was more prominent and the concentration of GSH decreased. We hypothesize that these results are explained by induction of the enzymes of GSH synthesis by both compensatory renal growth associated with uninephrectomy and treatment with Hg. This induction may in turn influence renal susceptibility to injury induced by Hg as well as other nephrotoxins.

References


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