

# Reductions in Renal Mass and the Nephropathy Induced by Mercury<sup>1</sup>

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The severity of renal injury induced by several graded doses of mercuric chloride and the disposition of mercury were evaluated and compared in control, uninephrectomized (50% NPX), and 75% nephrectomized (75% NPX) rats in an attempt to determine the effect of increased reductions of renal mass on the nephropathy induced by inorganic mercury. Consistent with previously published findings, proximal tubular necrosis (as assessed histopathologically and by the urinary excretion of lactate dehydrogenase (LDH) and total protein) was significantly more severe in 50% NPX rats than in control rats 24 hr after the administration of any of three lowest (1.0, 1.5, or 1.75  $\mu\text{mol/kg}$ ) doses of mercuric chloride used in the study. Interestingly, the severity of proximal tubular necrosis in the 75% NPX rats was not greater than that in control rats at these same doses. The reason for this appeared to be due to decreased renal accumulation of mercury, particularly in the renal cortex and outer stripe of the outer medulla. At the highest (8.0  $\mu\text{mol/kg}$ ) dose of mercuric chloride used, renal tubular injury was very extensive in all three groups of rats, with the level of injury being greatest in the 50% NPX rats. The injury was so severe in all three groups that acute renal failure was induced within the first 24 hr after the injection of mercury. An important finding that was made at this dose was that the level of blood urea nitrogen (BUN) was significantly greater in the 75% NPX rats than in either the 50% NPX or control rats, which indicates that 75% NPX rats may have entered into acute renal failure sooner than the 50% NPX or control rats. Overall, the findings from the present study indicate that as renal mass is reduced to a level at which renal function is not significantly impaired (50% NPX), the severity of the nephropathy induced by mercury is increased. By contrast, when the reduction of renal mass progresses to a level at which renal function begins to become impaired, the level of proximal tubular injury is not greatly different from that of animals with two kidneys, especially at low nephrotoxic doses of inorganic mercury. In addition, low nephrotoxic doses of inorganic mercury do not appear to affect significantly the reduced glomerular filtration rate in 75% NPX rats. However, it does appear that

**75% NPX rats may be at greater risk of entering into acute renal failure at higher toxic doses of inorganic mercury than 50% NPX or control rats.** © 1997 Academic Press

Beginning in the late 1970s, a series of animal studies were undertaken in an attempt to begin providing relevant data on the effects of varied degrees of reduced renal mass on the renal handling, disposition, and toxicity of mercuric compounds (Ramos-Frendo *et al.*, 1979; Houser and Berndt, 1986; Zalups, 1991a,b, 1993; Lash and Zalups, 1992; Zalups and Cherian, 1992a,b; Zalups and Diamond, 1987; Zalups and Lash, 1990, 1994; Zalups *et al.*, 1987, 1988, 1992). The rationale for some of these studies comes from the fact that there are populations of individuals who have reduced renal mass and that they may be at greater risk of becoming intoxicated by nephrotoxic compounds such as mercury. One of the key findings from these previous studies is that the accumulation of mercury in the renal outer stripe of the outer medulla, specifically along the pars recta segments of proximal tubules, increases significantly (on a per gram tissue basis) after uninephrectomy (Houser and Berndt, 1986; Zalups, 1991a,b, 1993; Zalups and Cherian, 1992a,b; Zalups and Lash, 1990; Zalups *et al.*, 1987, 1992). Interestingly, additional findings from toxicity studies indicate that rats that have undergone uninephrectomy develop a much more severe form of the nephropathy induced by low toxic doses of inorganic mercury than rats with two kidneys (Ramos-Frendo *et al.*, 1979; Houser and Berndt, 1986; Lash and Zalups, 1992; Zalups and Diamond, 1987; Zalups and Lash, 1990; Zalups *et al.*, 1988). The enhanced severity of the nephropathy appears to be linked mechanistically to the enhanced accumulation of mercury that occurs along the pars recta segment of proximal tubules, which is the most vulnerable segment of the nephron to the nephrotoxic effects of mercury (Zalups and Lash, 1994). The findings obtained from uninephrectomized animals have proven to be extremely valuable in understanding the effects of reductions of renal mass that do not compromise renal function significantly on the renal disposition and toxicity of mercury. However, despite these previous findings, very little is currently known about the renal handling and toxicity of mercury after renal mass has been reduced to a level that results in impaired

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renal function, comparable with that found in the early stages of chronic renal failure.

Only one very recent study provides some preliminary findings regarding the disposition of a nontoxic dose of mercury in rats that have undergone essentially a 75% nephrectomy (Zalups, 1995a), which does cause a significant impairment in renal function (Zalups, 1989, 1995a; Zalups *et al.*, 1985). The findings from the study indicate that there is somewhat of a biphasic response with the respect to the renal uptake and accumulation of mercury as renal mass is progressively decreased from a level where renal function is not compromised to one where it comprised significantly. Unlike after uninephrectomy, the renal accumulation of mercury in 75% nephrectomized rats, particularly in the outer stripe of the outer medulla, remains similar to, or becomes less than, that found in control animals with two kidneys. A major point that remains unclear, however, is whether there is an altered risk of intoxication associated with the dispositional findings obtained in this model of chronic renal failure.

In the present study, the disposition of mercury and the severity of the nephropathy induced by inorganic mercury following the intravenous administration of several nephrotoxic doses of mercuric chloride were evaluated in normal, uninephrectomized, and 75% nephrectomized rats. The principal aim of the present study was to determine if a 75% reduction of renal mass alters the susceptibility to the nephropathy induced by mildly nephrotoxic to highly nephrotoxic doses of inorganic mercury to the same or different extent as does a 50% reduction of renal mass.

## 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). After arriving from the vendor, they were allowed to acclimate for several days prior to experimentation. During this acclimation period the animals were allowed food and water *ad libitum*.

**Groups.** Three groups of five or six rats were used for each portion of the present study. The groups consisted of the following: one group of control rats with two kidneys, one group of rats that had undergone a uninephrectomy (right-sided), and a third group of rats that had undergone an approximately 75% reduction of renal mass. The disposition and renal toxicity of five different nephrotoxic doses of mercuric chloride (ranging from a very mildly nephrotoxic dose to a dose that induces acute renal failure) were evaluated and compared in each of the three groups 24 hr after intravenous administration.

**Surgery.** Prior to any surgery, animals were always anesthetized with a 50 mg/kg intraperitoneal dose of sodium pentobarbital. The protocols used to perform uninephrectomy (Lash and Zalups, 1992, 1994; Zalups, 1991a,b, 1993, 1995a; Zalups and Cherian, 1992a,b; Zalups and Diamond, 1987; Zalups *et al.*, 1987, 1992) and 75% nephrectomy (Zalups, 1989, 1995a; Zalups *et al.*, 1985) were the same as those used previously in this laboratory.

**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 (50% NPX) rats and 75%

nephrectomized (75% NPX) rats. During this time, the animals were handled in the same manner established previously (Zalups, 1989, 1995a; Zalups *et al.*, 1985).

**Evaluation of renal function after surgery and compensatory renal growth.** On the morning of the 12th day after surgery, each of five corresponding sets of five or six control, 50% NPX, and 75% NPX rats were placed in plastic metabolic cages for a control 24-hr period to evaluate renal functional parameters. This control period was used to obtain a baseline for the renal clearance of creatinine, which was used to estimate the glomerular filtration rate (GFR), and the urinary excretion of lactate dehydrogenase (LDH) and total protein, which were used as indicators for renal cellular injury (Zalups and Diamond, 1987; Zalups *et al.*, 1988). Control levels for plasma creatinine and blood urea nitrogen (BUN) were also obtained.

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 to determine the concentration of creatinine and urea nitrogen in plasma. At the end of the 24-hr control period, the volume of urine excreted by each animal was determined gravimetrically and a 1-ml sample of urine was obtained for analysis.

**Estimation of Glomerular Filtration Rate (GFR).** As mentioned above, the renal clearance of creatinine ( $C_{Cr}$ ) was used as an estimator of GFR and overall renal function using the following equation,

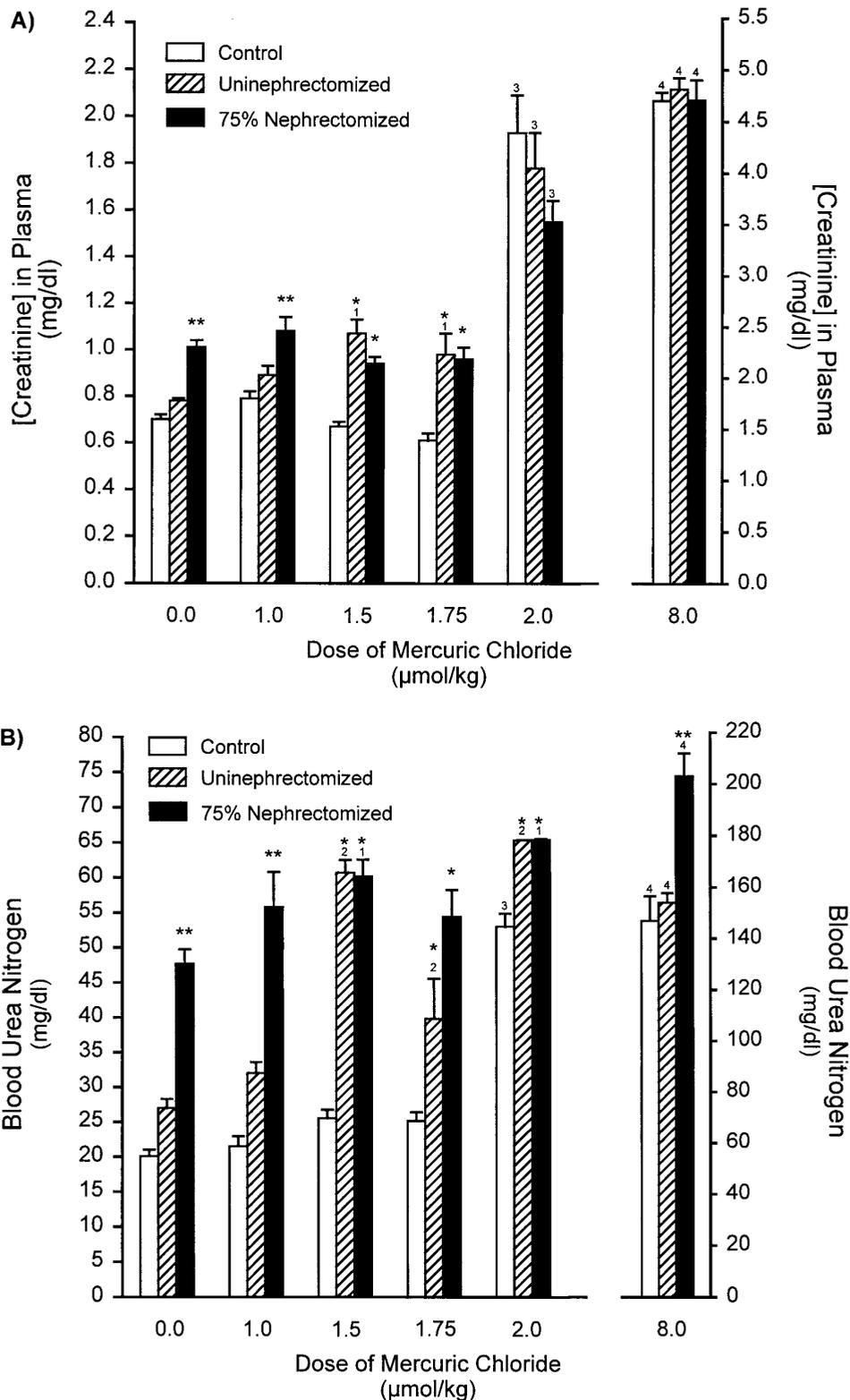
$$C_{Cr}(GFR) = (U_{Cr} \div P_{Cr}) \times V$$

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.

**Injection of inorganic mercury.** On the morning of the 13th day after surgery, the corresponding groups of control rats, 50% NPX rats, and 75% NPX rats received a single intravenous 1.0, 1.5, 1.75, 2.0, or 8.0  $\mu\text{mol/kg}$  dose of mercuric chloride containing  $^{203}\text{Hg}^{2+}$ . These doses represent a range from one inducing very mild cellular injury along the pars recta of proximal tubules to one that induces acute renal failure. Each dose was administered in the right femoral vein while the animal was anesthetized lightly with ether. The injection solution contained nonradioactive inorganic mercury (Aldrich Chemical, Milwaukee, WI), radioactive inorganic mercury (2.0  $\mu\text{Ci}/\mu\text{g}$ ; 2.0  $\mu\text{Ci}/\text{ml}$ ; Buffalo Materials Corporation, Buffalo, NY), and sodium chloride (9 mg/ml). The animals received 2.0 ml of the injection solution per kilogram of body weight.

**Experimental period after injection of inorganic mercury.** After the injection of mercury, each set of control, 50% NPX, and 75% NPX rats was placed back into the plastic metabolic cages and urine and feces were collected from all animals for 24 hr. At the end of the 24-hr experimental period, the volume of urine excreted and the amount of feces excreted by each animal were determined gravimetrically. A 1-ml sample of urine was obtained from each collection and was placed in a sealed 12  $\times$  75-mm polystyrene gamma-counting tube for the determination of the amount of mercury excreted in the urine. An additional 1-ml sample of urine was obtained for the determination of the concentration of creatinine in urine and the amount of LDH and protein excreted. The entire amount of feces excreted in a 24-hr period was placed in a 16  $\times$  100-mm polypropylene gamma-counting tube for determination of the amount of mercury excreted in the feces. Subsequently, the animals were anesthetized with an overdose (100 mg/kg) of sodium pentobarbital and tissues and organs were obtained to determine the disposition of mercury.

**Acquisition and handling of tissues.** Once anesthesia was induced, samples of blood were 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



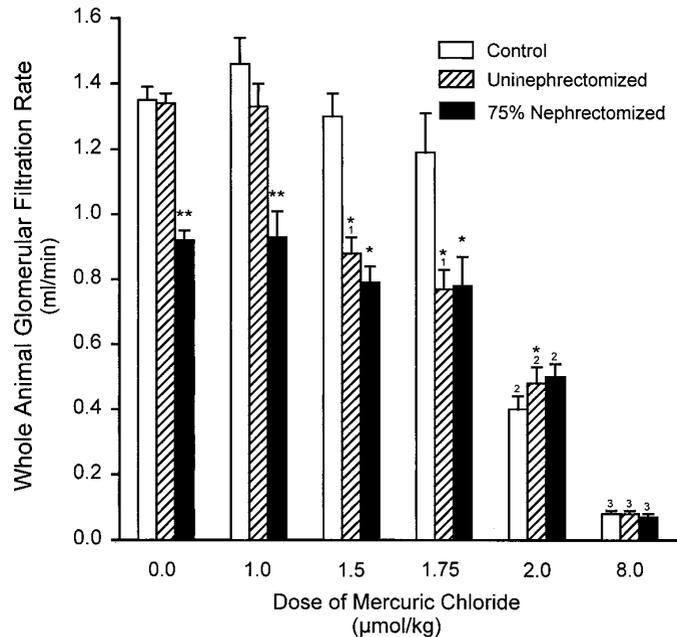
**FIG. 1.** Concentration of creatinine (A) and urea nitrogen (B) in plasma of control, uninephrectomized, and 75% nephrectomized rats, 24 hr prior to (Time 0.0) and the 24 hr after the intravenous injection of a 1.0, 1.5, 1.75, 2.0, or 8.0  $\mu\text{mol/kg}$  dose of mercuric chloride. Each value, except for at Time 0.0, represents a mean  $\pm$  SE for 5 or 6 rats. Each value for Time 0.0 represents a pooled mean  $\pm$  SE for 35 or 36 rats. \*Significantly different ( $p < 0.05$ ) from the mean for the corresponding group of control rats. \*\*Significantly different ( $p < 0.05$ ) from the means for the corresponding groups of uninephrectomized and control rats. <sup>1</sup>Significantly different ( $p < 0.05$ ) from the mean obtained during the control period (Time 0.0) from the rats of the same surgical group. <sup>2</sup>Significantly different ( $p < 0.05$ ) from the mean obtained during the control period (Time 0.0) from the rats of the same

medulla, 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. The plasma and cellular fractions of one of the samples of blood were placed and sealed in 12 × 75-mm polystyrene gamma-counting tubes for the determination of the content of mercury in each fraction. In addition, a 3-mm mid-transverse slice of the left kidney was obtained for histological analysis.

**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  $^{203}\text{Hg}^{2+}$ . 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 the 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 a 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 the percentage of the administered dose excreted per 24 hr.

**Biochemical determination of plasma and urine used to evaluate levels of renal injury and function.** The concentration of creatinine in samples of plasma and urine were determined spectrophotometrically using the Lancer Creatinine II Rapid Stat Diagnostic Kit (Lancer Division of Sherwood Medical, St. Louis, MO). Concentrations of BUN were determined spectrophotometrically using endpoint quantitative enzymatic spectrophotometric procedure No. 66-UV (Sigma Chemical Co., St. Louis, MO). Concentrations of both creatinine and urea nitrogen increase when renal function becomes impaired significantly. Determinations for concentrations of LDH and total protein in samples of urine were also accomplished spectrophotometrically using a procedure provided by Sigma Chemical Co. The concentration of LDH was determined using the kinetic LDH optimized lactate dehydrogenase EC 1.1.1.27 UV test and the concentration of total protein was determined with endpoint microprotein-PR procedure No. 611. Both enzymuria and proteinuria are hallmarks of renal cellular injury induced by nephrotoxicants. Moreover, the urinary excretion of LDH (factored by renal mass) has proven to be one of the more sensitive and accurate measures of the level of proximal tubular necrosis following the acute exposure to inorganic forms of mercury that correlates well with the level of necrosis that is demonstrable histologically (Zalups and Diamond, 1987).

**Histopathological analysis.** The slice of left kidney obtained for histopathological analysis was fixed for 48 hr at 4°C in a solution containing 4% formaldehyde (v/v) and 1% glutaraldehyde (v/v). The fixative was buffered with 11.6 g/liter of sodium dihydrogen phosphate ( $\text{NaH}_2\text{PO}_4$ ) and 2.7 g/liter of sodium hydroxide (NaOH). The pH of the fixative was adjusted to 7.35. The total osmolality of the fixative was 1120 mOsmol. After the slices of kidney were fixed, they were dehydrated in an ascending graded series of ethanols, cleared in xylene, and embedded in paraffin wax. Sections (3–5  $\mu\text{m}$ ) of each embedded slice of left kidney were obtained with a standard microtome and were mounted on glass slides. The sections were later stained with hematoxylin and eosin for histological analysis.



**FIG. 2.** Estimated glomerular filtration rate (GFR) in control, uninephrectomized, and 75% nephrectomized rats during the 24 hr prior to (Time 0.0) and the 24 hr after the intravenous injection of a 1.0, 1.5, 1.75, 2.0, or 8.0  $\mu\text{mol/kg}$  dose of mercuric chloride. \*Significantly different ( $p < 0.05$ ) from the mean for the corresponding group of control rats. \*\*Significantly different ( $p < 0.05$ ) from the means for the corresponding groups of uninephrectomized and control rats. <sup>1</sup>Significantly different ( $p < 0.05$ ) from the mean obtained during the control period (Time 0.0) from the rats of the same surgical group and the mean from the corresponding group of rats treated the same way surgically and injected with the 1.0  $\mu\text{mol/kg}$  dose of mercuric chloride. <sup>2</sup>Significantly different ( $p < 0.05$ ) from the mean obtained during the control period (Time 0.0) from the rats of the same surgical group and the means from the corresponding groups of rats treated the same way surgically and injected with the 1.0, 1.5, or 1.75  $\mu\text{mol/kg}$  dose of mercuric chloride. <sup>3</sup>Significantly different ( $p < 0.05$ ) from the mean obtained during the control period (Time 0.0) from the rats of the same surgical group and the means from the corresponding groups of rats treated the same way surgically and injected with the 1.0, 1.5, 1.75, or 2.0  $\mu\text{mol/kg}$  dose of mercuric chloride.

Qualitative and semiquantitative assessments of the level of necrosis to the pars recta segments of proximal tubules and other pathological changes in the sections of left kidney were made using a light microscope equipped with standard bright-field optics. These assessments were performed in a blind manner. Subsequently, relative comparisons of the type and degree of histopathology were made among the control, 50% NPX, and 75% NPX rats. The level or severity of necrosis in proximal tubules was ranked for each animal on a scale of 0–12, where 0 represents no cellular necrosis

surgical group and the mean from the corresponding group of rats treated the same way surgically and injected with the 1.0  $\mu\text{mol/kg}$  dose of mercuric chloride. <sup>3</sup>Significantly different ( $p < 0.05$ ) from the mean obtained during the control period (Time 0.0) from the rats of the same surgical group and the means from the corresponding groups of rats treated the same way surgically and injected with the 1.0, 1.5, or 1.75  $\mu\text{mol/kg}$  dose of mercuric chloride. <sup>4</sup>Significantly different ( $p < 0.05$ ) from the mean obtained during the control period (Time 0.0) from the rats of the same surgical group and the means from the corresponding groups of rats treated the same way surgically and injected with the 1.0, 1.5, 1.75, or 2.0  $\mu\text{mol/kg}$  dose of mercuric chloride.

**TABLE 1**  
**Metabolic Data**

Dose of HgCl <sub>2</sub> (μmol/kg)	Group	Animal body weight (g)	Weight of left kidney (g)	Weight of liver (g)	Volume of urine excreted prior to treatment with HgCl <sub>2</sub> (ml/24 hr)	Volume of urine excreted after treatment with HgCl <sub>2</sub> (ml/24 hr)
1.0	Control (n = 6)	263 ± 3	0.96 ± 0.04	10.3 ± 0.24	12.0 ± 0.58	16.1 ± 0.75
1.0	NPX (n = 6)	278 ± 7	1.56 ± 0.08*	10.4 ± 0.55	15.7 ± 1.62	22.6 ± 2.27*
1.0	75% NPX (n = 6)	253 ± 4	1.64 ± 0.09*	9.85 ± 0.63	33.8 ± 3.17*	37.6 ± 3.12**
1.5	Control (n = 6)	230 ± 5	0.91 ± 0.03	9.22 ± 0.30	14.8 ± 1.12	23.1 ± 3.63
1.5	NPX (n = 6)	236 ± 2	1.45 ± 0.05*	10.4 ± 0.48	22.5 ± 2.13*	28.1 ± 2.00*
1.5	75% NPX (n = 5)	226 ± 8	1.25 ± 0.07*	9.60 ± 0.26	35.0 ± 1.85**	31.1 ± 1.63*
1.75	Control (n = 6)	232 ± 13	0.96 ± 0.04	9.49 ± 0.41	19.3 ± 1.33	19.3 ± 4.45
1.75	NPX (n = 6)	251 ± 7	1.34 ± 0.06*	9.19 ± 0.30	30.2 ± 1.24*	30.1 ± 2.47*
1.75	75% NPX (n = 6)	241 ± 13	1.57 ± 0.10*	9.79 ± 0.52	51.3 ± 3.89**	39.8 ± 3.63**
2.0	Control (n = 6)	238 ± 4	0.92 ± 0.04	7.35 ± 0.42	12.2 ± 0.90	41.1 ± 5.17
2.0	NPX (n = 6)	258 ± 7	1.44 ± 0.09*	9.11 ± 0.48	21.8 ± 1.38*	39.0 ± 3.00
2.0	75% NPX (n = 6)	234 ± 5	1.33 ± 0.08*	8.89 ± 0.35	43.5 ± 2.75**	39.0 ± 2.74
8.0	Control (n = 6)	253 ± 1	1.13 ± 0.03	9.37 ± 0.11	16.4 ± 1.21	22.3 ± 2.68
8.0	NPX (n = 6)	276 ± 3	1.51 ± 0.07*	9.09 ± 0.17	23.7 ± 1.26*	29.6 ± 2.42
8.0	75% NPX (n = 6)	255 ± 2	1.51 ± 0.07*	9.25 ± 0.17	38.8 ± 2.86**	19.5 ± 1.05

Note. Mercuric chloride 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. Body weights were measured just prior to the injection of inorganic mercury. The volume of urine excreted represents the amount of urine excreted during the 24 hr prior to the injection of inorganic mercury.

\* 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 and NPX rats.

detected and 12 represents a level of necrosis that involves virtually every proximal tubular segment visible in the plane of section.

**Statistics.** All values for experimental data, except for the morphological data, are expressed as mean ± SE for  $n = 5$  or 6 animals per group and the values for the control data acquired prior to the administration of mercury are expressed as a mean ± SE for  $n = 29$  or 30 animals per surgical group (i.e., control, 50% NPX, or 75% NPX).

Since the morphological data are ranked data, they are expressed as median, 25th–75th percentile, and 5th–95th percentile. Differences in the level of necrosis in proximal tubules between corresponding sets of control, 50% NPX, and 75% NPX rats were assessed nonparametrically using Friedman's analysis of variance on ranks followed by Dunn's multiple comparison test.

Differences between means for parameters other than morphological ones were evaluated by first by performing either a  $3 \times 6$  or  $3 \times 5$  two-way analysis of variance (ANOVA). When statistically significant  $F$  values were obtained with the ANOVA, the Newman-Keuls post hoc comparison 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 two-way ANOVA was run only when the conditions for performing parametric statistical analyses were met.

All data expressed as a percentage were first normalized by using the arcsine transformation, which takes the arcsine of the square root of the decimal fraction of the percentage score.

The level for  $\alpha$  for each statistical analysis was chosen a priori to be 0.05.

## RESULTS

### Control Metabolic Data

**Renal clearance data.** The concentrations of creatinine and urea nitrogen in the plasma were significantly greater

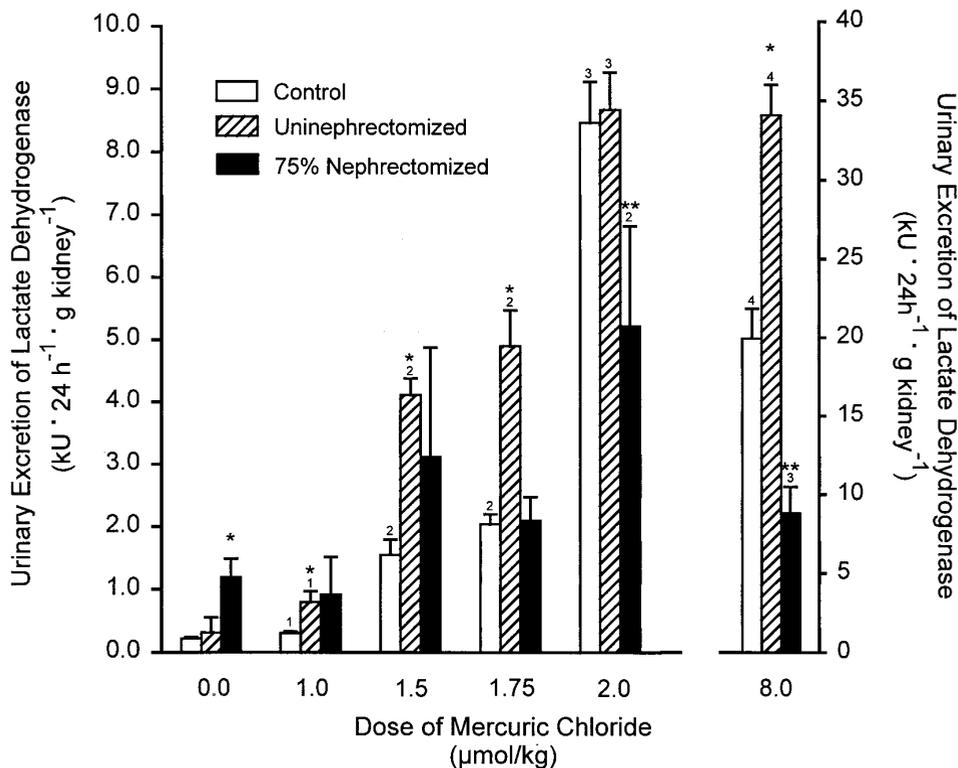
in the 75% NPX rats than in the control rats or 50% NPX rats 12 days after surgery (prior to the injection of mercury; Figs. 1A and 1B). There was, however, no significant difference in the concentration of creatinine or urea nitrogen in the plasma between the control rats and 50% NPX rats.

In association with the enhanced level of creatinine in the plasma, the estimated whole animal GFR was significantly decreased in the 75% NPX rats, relative to that in the control rats or 50% NPX rats (Fig. 2). GFR was similar in the control rats and 50% 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).

These findings confirm that the 75% nephrectomy was sufficient to induce changes consistent with the early stages of chronic renal failure, in which renal function begins to become compromised. Moreover, these findings confirm that uninephrectomy does not result in a significant compromise in renal function.

**Body, kidney, and liver weights.** Body weights between the corresponding groups of control rats, 50% NPX rats, and 75% NPX rats on the day the rats were administered their respective dose of mercuric chloride were similar (Table 1).

At all times studied after the injection of mercuric chloride, the left kidney in the 50% 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 1), which indicates that compensatory



**FIG. 3.** Urinary excretion of lactate dehydrogenase (LDH) in control, uninephrectomized, and 75% nephrectomized rats during the 24 hr prior to (Time 0.0) and the 24 hr after the intravenous injection of a 1.0, 1.5, 1.75, 2.0, or 8.0  $\mu\text{mol/kg}$  dose of mercuric chloride. Each value, except for at Time 0.0, represents a mean  $\pm$  SE for 5 or 6 rats. Each value for Time 0.0 represents a pooled mean  $\pm$  SE for 35 or 36 rats. \*Significantly different ( $p < 0.05$ ) from the mean for the corresponding group of control rats. \*\*Significantly different ( $p < 0.05$ ) from the means for the corresponding groups of uninephrectomized and control rats. <sup>1</sup>Significantly different ( $p < 0.05$ ) from the mean obtained during the control period (Time 0.0) from the rats of the same surgical group. <sup>2</sup>Significantly different ( $p < 0.05$ ) from the mean obtained during the control period (Time 0.0) from the rats of the same surgical group and the mean from the corresponding group of rats treated the same way surgically and injected with the 1.0  $\mu\text{mol/kg}$  dose of mercuric chloride. <sup>3</sup>Significantly different ( $p < 0.05$ ) from the mean obtained during the control period (Time 0.0) from the rats of the same surgical group and the means from the corresponding groups of rats treated the same way surgically and injected with the 1.0, 1.5, or 1.75  $\mu\text{mol/kg}$  dose of mercuric chloride. <sup>4</sup>Significantly different ( $p < 0.05$ ) from the mean obtained during the control period (Time 0.0) from the rats of the same surgical group and the means from the corresponding groups of rats treated the same way surgically and injected with the 1.0, 1.5, 1.75, or 2.0  $\mu\text{mol/kg}$  dose of mercuric chloride.

renal growth had occurred in the 50% NPX and 75% NPX rats.

#### Indicators of Renal Function

**[Creatinine] in plasma.** Twenty-four hours after the administration of any of the three lowest doses of mercuric chloride, the concentration of creatinine in plasma was significantly greater in the 75% NPX rats than in the corresponding control rats (Fig. 1A). The concentration of creatinine in the plasma in the 50% NPX rats was also significantly greater in the 50% NPX rats than in the control rats 24 hr after the 1.5 or 1.75  $\mu\text{mol/kg}$  dose.

Plasma creatinine increased very markedly, above the levels induced by lower doses of mercuric chloride, in all three surgical groups of rats administered the 2.0 or 8.0  $\mu\text{mol/kg}$  dose of mercuric chloride. In fact, in the animals given the 8.0  $\mu\text{mol/kg}$  dose, the concentration of creatinine in plasma

increased by more than fourfold relative to corresponding control levels. No significant differences in the level of plasma creatinine were detected between corresponding groups of control, 50% NPX, and 75% NPX rats.

**BUN.** BUN was significantly greater in the 75% NPX rats than in the control rats 24 hr after each dose of mercuric chloride (Fig. 1B). It was also significantly greater in the 75% NPX rats than in the 50% NPX rats 24 hr after the 1.0 or 8.0  $\mu\text{mol/kg}$  dose.

In the control rats, the concentration of urea nitrogen in plasma did not increase significantly above control levels until the 2.0  $\mu\text{mol/kg}$  dose of mercuric chloride had been administered. By contrast, BUN increased significantly above control levels in the 50% NPX rats after the administration of the 1.5  $\mu\text{mol/kg}$  dose.

A tremendous increase in BUN, above control levels, occurred 24 hr after the administration of the 8.0  $\mu\text{mol/kg}$  dose

of mercuric chloride in all three groups of rats. An interesting finding that has significant implications is that BUN was significantly greater in the 75% NPX rats than in the corresponding 50% NPX or control rats.

**GFR.** After the injection of 1.0  $\mu\text{mol/kg}$  of mercuric chloride, GFR remained unchanged from control levels in all three groups of rats (Fig. 2). In fact, no changes in GFR in the control and the 75% NPX rats were detected until after the injection of the 2.0  $\mu\text{mol/kg}$  dose of mercuric chloride. By contrast, a significant reduction of GFR was detected in the 50% NPX rats after the injection of the 1.5  $\mu\text{mol/kg}$  dose of mercuric chloride. GFR in these animals decreased to a level not statistically different from that in the corresponding 75% NPX rats. During the 24 hr after the injection of the 8.0  $\mu\text{mol/kg}$  dose of mercuric chloride, GFR in all three groups of rats fell to levels consistent with acute renal failure.

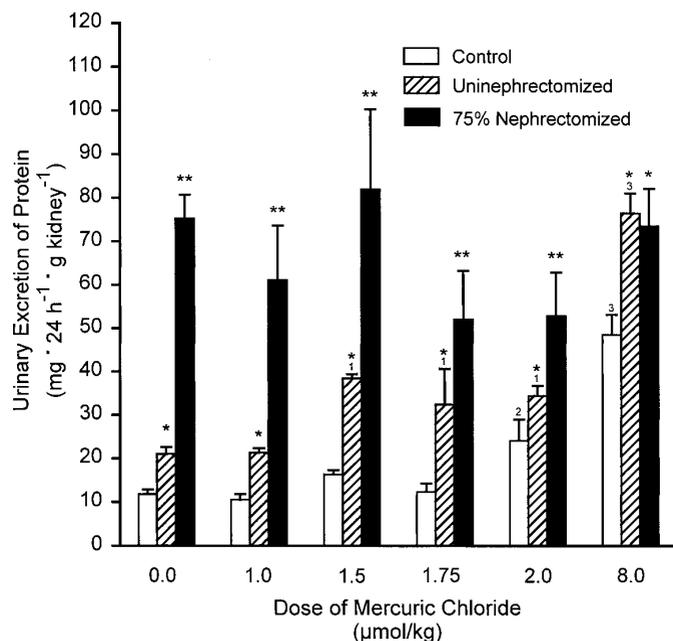
### Biochemical Indices of Renal Tubular Injury

**Urinary excretion of LDH.** During the 24-hr control period prior to the injection of mercuric chloride, the 75% NPX rats excreted significantly more LDH per gram kidney than either the control or 50% NPX rats (Fig. 3).

Dose-dependent changes in the urinary excretion of LDH ( $\text{kilounits} \cdot 24 \text{ hr}^{-1} \cdot \text{g kidney}^{-1}$ ) were generally detected in the three surgical groups of rats during the 24-hr experimental period, but particularly in the control and 50% NPX rats at the lower doses of mercuric chloride (Fig. 3). Some of the more salient findings obtained during the experimental period are described below.

After the injection of the 1.0, 1.5, or 1.75  $\mu\text{mol/kg}$  dose of mercuric chloride, the urinary excretion of LDH was significantly greater in the 50% NPX rats than in the corresponding control rats. The urinary excretion of LDH in the corresponding groups of 75% NPX rats given these three doses of mercuric chloride varied greatly enough that no significant differences were detected between the 75% NPX rats and the control or 50% NPX rats, or among the three groups of 75% NPX given one of these doses of mercuric chloride. In fact, no significant increase in the urinary excretion of LDH, above the level measured during the control period, was detected in the 75% NPX rats until the 2.0  $\mu\text{mol/kg}$  dose of mercuric chloride had been administered. Also after the injection of 2.0  $\mu\text{mol/kg}$  dose of mercuric chloride, the urinary excretion of LDH was significantly lower in the group of 75% NPX rats than in the control or 50% NPX rats.

The urinary excretion of LDH increased tremendously, above control levels, in all three groups of rats administered the 8.0  $\mu\text{mol/kg}$  dose of mercuric chloride. Interestingly, the urinary excretion of LDH was significantly greater in the 50% NPX rats than in either the control or 75% NPX rats. In addition, the urinary excretion of LDH in the 75% NPX

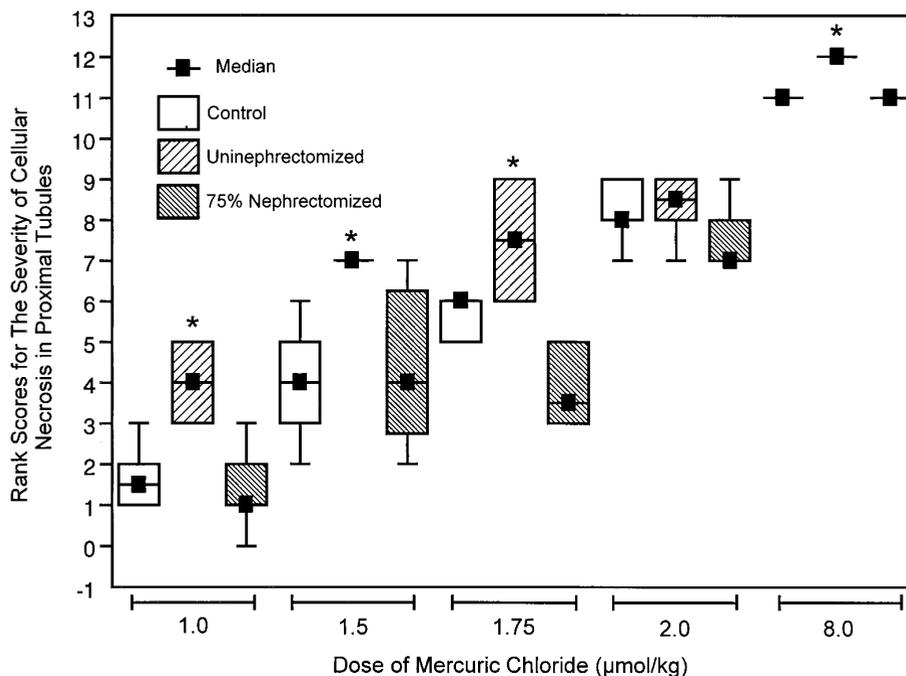


**FIG. 4.** Urinary excretion of total protein in control, uninephrectomized, and 75% nephrectomized rats during the 24 hr prior to (time 0.0) and the 24 hr after the intravenous injection of a 1.0, 1.5, 1.75, 2.0, or 8.0  $\mu\text{mol/kg}$  dose of mercuric chloride. Each value, except for at Time 0.0, represents a mean  $\pm$  SE for 5 or 6 rats. Each value for Time 0.0 represents a pooled mean  $\pm$  SE for 35 or 36 rats. \*Significantly different ( $p < 0.05$ ) from the mean for the corresponding group of control rats. \*\*Significantly different ( $p < 0.05$ ) from the means for the corresponding groups of uninephrectomized and control rats. <sup>1</sup>Significantly different ( $p < 0.05$ ) from the mean obtained during the control period (Time 0.0) from the rats of the same surgical group and the mean from the corresponding group of rats treated the same way surgically and injected with the 1.0  $\mu\text{mol/kg}$  dose of mercuric chloride. <sup>2</sup>Significantly different ( $p < 0.05$ ) from the mean obtained during the control period (Time 0.0) from the rats of the same surgical group and the means from the corresponding groups of rats treated the same way surgically and injected with the 1.0, 1.5, or 1.75  $\mu\text{mol/kg}$  dose of mercuric chloride. <sup>3</sup>Significantly different ( $p < 0.05$ ) from the mean obtained during the control period (Time 0.0) from the rats of the same surgical group and the means from the corresponding groups of rats treated the same way surgically and injected with the 1.0, 1.5, 1.75, or 2.0  $\mu\text{mol/kg}$  dose of mercuric chloride.

rats was significantly lower than that in the control or 50% NPX rats, which may have been due, in part, to a greater level of diminished GFR.

**Urinary excretion of protein.** Urinary excretion of protein ( $\text{mg} \cdot 24 \text{ hr}^{-1} \cdot \text{g kidney}^{-1}$ ) in the 75% NPX rats during the control 24-hr period was on average more than 7-fold greater than that in the control rats and more than 3.5-fold greater than that in the 50% NPX rats (Fig. 4). The 50% NPX rats also excreted significantly more protein in the urine than the control rats during the control period.

The urinary excretion of protein increased significantly above control levels in the 50% NPX rats after the injection of the 1.5  $\mu\text{mol/kg}$  dose of mercuric chloride and in the control



**FIG. 5.** Rank score analysis of the severity of cellular necrosis in proximal tubules of control, uninephrectomized, and 75% nephrectomized rats 24 hr after the intravenous injection of a 1.0, 1.5, 1.75, 2.0, or 8.0  $\mu\text{mol/kg}$  dose of mercuric chloride. Each value represents a mean  $\pm$  SE for 5 or 6 rats. \*Significantly different ( $p < 0.05$ ) from the mean for the corresponding group of control rats.

rats only after the 2.0  $\mu\text{mol/kg}$  dose. By contrast, the urinary excretion of protein in the 75% NPX rats did not increase significantly above control levels after the injection of any of the five doses of mercuric chloride. This finding may be related to the fact that the variability in the excretion of protein within any one group of 75% NPX rats was very high. At all doses, except for the highest dose, of mercuric chloride, the urinary excretion of protein in the 75% NPX rats was significantly greater than that in the 50% NPX rats, which was significantly greater than that in the corresponding control rats. After the injection of the 8.0  $\mu\text{mol/kg}$  dose of mercuric chloride, the urinary excretion of protein was similar in both the 50% NPX and 75% NPX rats. In the corresponding control rats, the urinary excretion of protein was significantly less than that in the 50% NPX or 75% NPX rats.

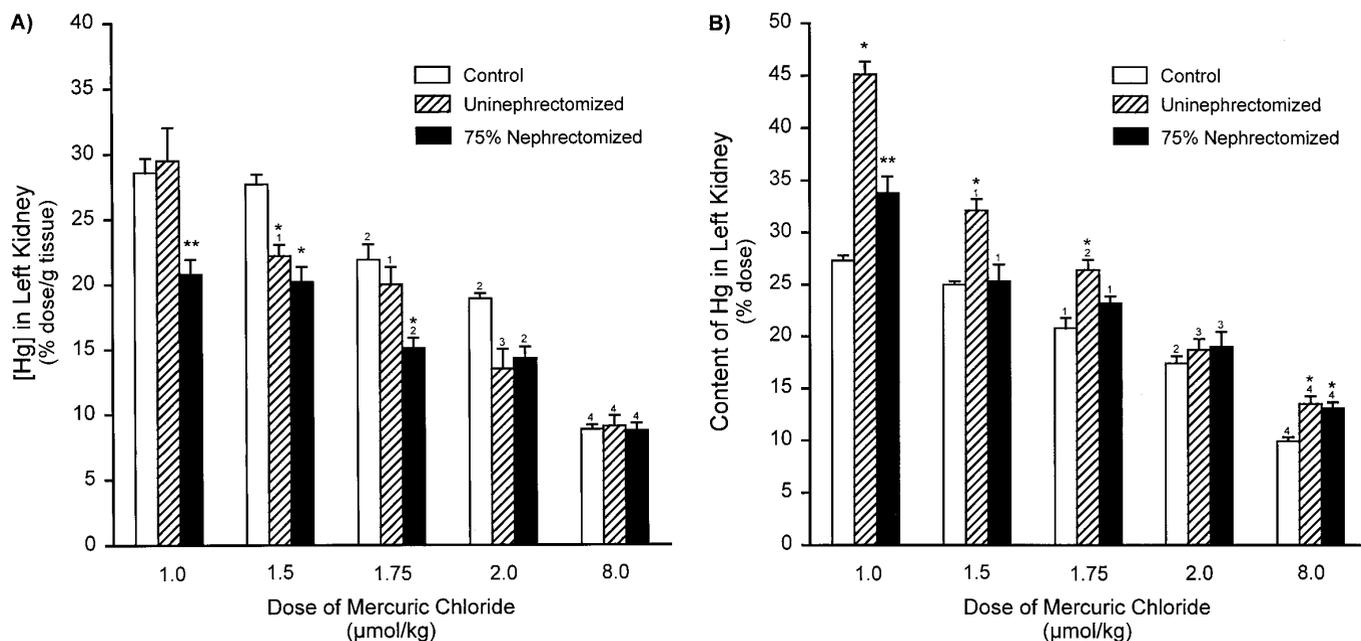
### Histopathological Findings

**Qualitative analysis.** On a qualitative level, cellular necrosis along the pars recta of proximal tubules in the outer stripe of the outer medulla and inner cortex, particularly at the cortico-medullary junction, was the major histopathological finding induced by the administration of the 1.0, 1.5, 1.75, and 2.0  $\mu\text{mol/kg}$  doses of mercuric chloride. At the lowest dose of mercuric chloride, a very mild level of cellular necrosis was detected in a few isolated areas in the kidneys of the control rats. Moderate levels of proximal tubular necrosis were detected in the control rats administered either the 1.5 or 1.75  $\mu\text{mol/}$

kg dose of mercuric chloride, while severe cellular necrosis involving primarily the pars recta of proximal tubules was detected in the control rats given the 2.0  $\mu\text{mol/kg}$  dose. Extensive necrosis along both pars recta and pars convoluta of proximal tubules was induced in the kidneys of all the rats, especially in the 50% NPX rats, injected with the 8.0  $\mu\text{mol/kg}$  dose of mercuric chloride. In fact, in the 50% NPX rats administered this dose, it appeared that every segment of proximal tubule in the cortex and outer stripe of the outer medulla, visible in the plane of section, was affected.

It should be mentioned that additional pathological features were detected in the kidneys of some of the 75% NPX rats. These features were found outside the area where complete tubular necrosis occurred after the cessation of blood induced by the ligation of the posterior branch of the renal artery, but did appear to be due to surgery rather than the injection of inorganic mercury. Some of these changes included varying degrees of interstitial fibrosis, tubular and glomerular dilatation, glomerular sclerosis, and evidence of epithelial regeneration.

**Quantitative analysis.** The relative magnitude and degree of necrosis in proximal tubules, based on semiquantitative rank-scoring in the three surgical groups of rats at each dose of mercuric chloride, is summarized in Fig. 5. After the administration of the 1.0, 1.5, 1.75, and 8.0, but not the 2.0,  $\mu\text{mol/kg}$  doses of mercuric chloride, the severity of proximal tubular necrosis was significantly greater in the



**FIG. 6.** Concentration of mercury (A) and total content of mercury (B) in the left kidney of control, uninephrectomized, and 75% nephrectomized rats 24 hr after the intravenous injection of a 1.0, 1.5, 1.75, 2.0, or 8.0  $\mu\text{mol/kg}$  dose of mercuric chloride. Each value represents a mean  $\pm$  SE for 5 or 6 rats. \*Significantly different ( $p < 0.05$ ) from the mean for the corresponding group of control rats. \*\*Significantly different ( $p < 0.05$ ) from the means for the corresponding groups of uninephrectomized and control rats. <sup>1</sup>Significantly different ( $p < 0.05$ ) from the mean for the corresponding group of rats treated the same way surgically and injected with the 1.0  $\mu\text{mol/kg}$  dose of mercuric chloride. <sup>2</sup>Significantly different ( $p < 0.05$ ) from the mean for the corresponding group of rats treated the same way surgically and injected with the 1.0 or 1.5  $\mu\text{mol/kg}$  dose of mercuric chloride. <sup>3</sup>Significantly different ( $p < 0.05$ ) from the mean for the corresponding group of rats treated the same way surgically and injected with the 1.0, 1.5, or 1.75  $\mu\text{mol/kg}$  dose of mercuric chloride. <sup>4</sup>Significantly different ( $p < 0.05$ ) from the mean for the corresponding group of rats treated the same way surgically and injected with the 1.0, 1.5, 1.75, or 2.0  $\mu\text{mol/kg}$  dose of mercuric chloride.

50% NPX rats than in the corresponding control rats. The severity of proximal tubular necrosis in the 75% NPX rats given the 1.0 or 1.75  $\mu\text{mol/kg}$  dose of mercuric chloride was significantly less than that in the corresponding 50% NPX rats, and not significantly different from that in corresponding control rats.

### Disposition of Mercury

**Renal disposition of mercury.** As the dose of mercuric chloride was increased, the percentage of the dose of mercury that accumulated in each gram of the kidney decreased in all three groups of rats. Despite these findings, it must be kept in mind that the actual concentration of mercury in terms of nanomoles per gram kidney increased in all three groups of animals as the dose of mercuric chloride was increased (Fig. 6A).

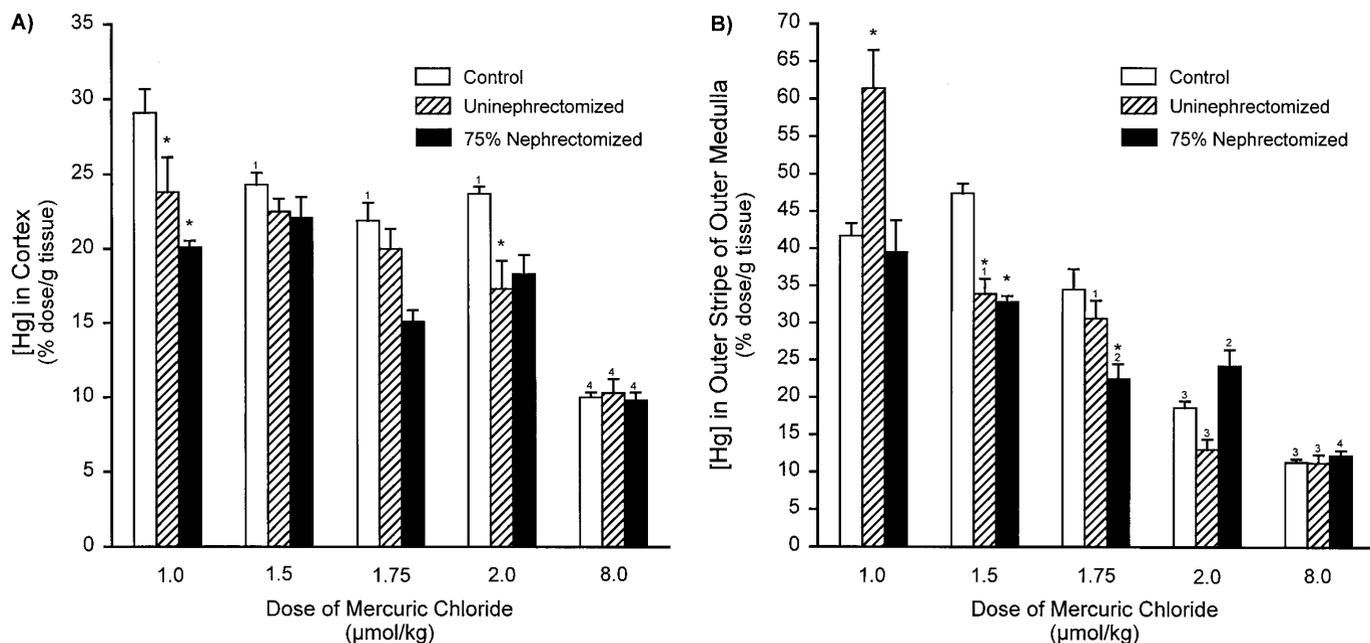
Differences in the concentration of mercury in the left kidney (in terms of percentage of the administered dose per gram kidney) were detected between the three groups of rats at the 1.0, 1.5, or 1.75  $\mu\text{mol/kg}$  doses. At all three of these doses, the concentration of mercury in the left kidney was significantly lower in the 75% NPX rats than in the control rats. The concentration of mercury in the 50% NPX rats given the 1.5

$\mu\text{mol/kg}$  dose of mercuric chloride was also significantly lower than that in the corresponding control rats.

No significant differences in the concentration of mercury were detected between the three groups of rats administered the 2.0 or 8.0  $\mu\text{mol/kg}$  dose of mercuric chloride.

At the level of the whole left kidney, the percentage of the administered dose of mercury present 24 hr after the injection of the 1.0  $\mu\text{mol/kg}$  dose of mercuric chloride was significantly greater in the 50% NPX rats than in 75% NPX rats, which was significantly greater than that in the control rats (Fig. 6B). The content of mercury in the left kidney of the 50% NPX rats was also significantly greater than that in the control rats 24 hr after the administration of the 1.5 or 1.75  $\mu\text{mol/kg}$  dose of mercuric chloride. No differences in the content of mercury in the left kidney were detected between the three groups of rats given the 2.0 or 8.0  $\mu\text{mol/kg}$  dose. As reflected by the concentration of mercury, the percentage of the administered dose of mercury in the left kidney decrease in all three groups of rats as the dose of mercuric chloride was increased.

**Intrarenal distribution of mercury.** In the renal cortex, the percentage of the administered dose per gram tissue, 24 hr after the administration of mercuric chloride, became



**FIG. 7.** Concentration of mercury in the renal cortex (A) and outer stripe of the outer medulla (B) of control, uninephrectomized, and 75% nephrectomized rats 24 hr after the intravenous injection of a 1.0, 1.5, 1.75, 2.0, or 8.0  $\mu\text{mol/kg}$  dose of mercuric chloride. Each value represents a mean  $\pm$  SE for 5 or 6 rats. \*Significantly different ( $p < 0.05$ ) from the mean for the corresponding group of control rats. \*\*Significantly different ( $p < 0.05$ ) from the means for the corresponding groups of uninephrectomized and control rats. †Significantly different ( $p < 0.05$ ) from the mean for the corresponding group of rats surgically and injected with the 1.0  $\mu\text{mol/kg}$  dose of mercuric chloride. ‡Significantly different ( $p < 0.05$ ) from the mean for the corresponding group of rats treated the same way surgically and injected with the 1.0 or 1.5  $\mu\text{mol/kg}$  dose of mercuric chloride. §Significantly different ( $p < 0.05$ ) from the mean for the corresponding group of rats treated the same way surgically and injected with the 1.0, 1.5, 1.75, or 2.0  $\mu\text{mol/kg}$  dose of mercuric chloride.

significantly lower as the dose of mercuric chloride was increased, particularly at the 8.0  $\mu\text{mol/kg}$  dose (Fig. 7A).

Meaningful differences in the renal cortical concentration of mercury between corresponding control, 50% NPX, and 75% NPX rats were detected mainly after the 1.0  $\mu\text{mol/kg}$  dose of mercuric chloride. After the administration of this dose, the relative concentration of mercury in the cortex of both the 50% NPX and 75% NPX rats was significantly lower than that in the control rats.

Some interesting changes in the accumulation of mercury in the outer stripe of the outer medulla were detected between the three groups of rats as the dose of mercuric chloride was increased (Fig. 7B). At the 1.0  $\mu\text{mol/kg}$  dose, the concentration of mercury (% dose/g tissue) in the outer stripe of the 50% NPX was significantly greater than that in outer stripe of the control or 75% NPX rats. However, after the administration of any of the higher doses of mercuric chloride the concentration of mercury in the outer stripe was less than, or similar to, that in the control or 75% NPX rats. After administration of the 2.0 or 8.0  $\mu\text{mol/kg}$  dose of mercuric chloride, the concentration of mercury in the outer stripe of the outer medulla was similar in all three groups of rats studied.

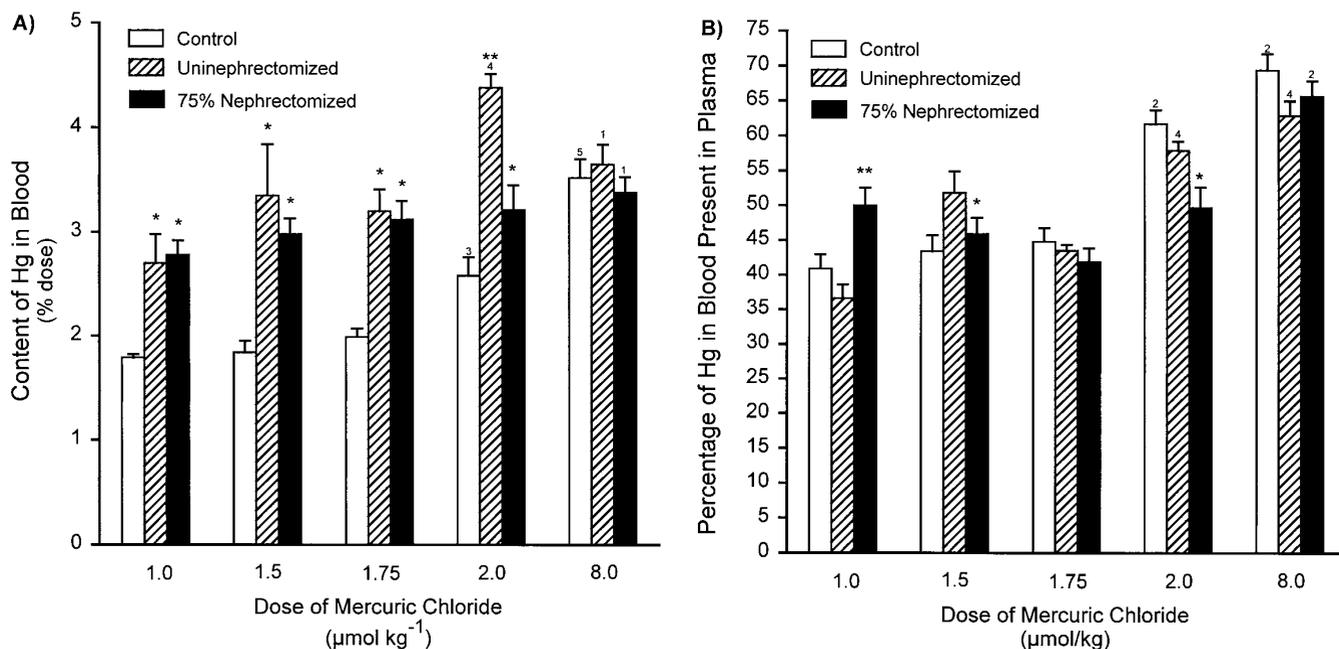
**Disposition of mercury in the blood.** The content of mercury (% dose) in the blood 24 hr after the administration of

mercury tended to increase in all three groups of rats as the dose of mercury was increased, particularly in the control animals (Fig. 8A).

By the end of 24 hr after the administration of the 1.0, 1.5, 1.75, or 2.0  $\mu\text{mol/kg}$  dose of mercuric chloride, total content of mercury in the blood was significantly greater in the 50% NPX and 75% NPX rats than in the control rats. No significant differences in the content of mercury in blood were detected between the 50% NPX and 75% NPX rats, except after the injection of the 2.0  $\mu\text{mol/kg}$  dose, at which point the content of mercury was significantly greater in the 50% NPX rats. No differences in the content of mercury in the blood were detected among the three groups of rats administered the 8.0  $\mu\text{mol/kg}$  dose of mercuric chloride.

Of the total mercury in blood, the fraction present in the plasma also tended to increase as the dose of mercury was increased, especially in the control rats (Fig. 8B). After the administration of the lowest dose of mercuric chloride, more mercury was in the plasma of the 75% NPX rats than in the plasma of the control or 50% NPX.

**Content of mercury in the liver.** The content of mercury (% dose) in the liver increased significantly in all three group of rats as the dose of mercury was increased, particularly after the 8.0  $\mu\text{mol/kg}$  dose of mercuric chloride (Fig. 9).



**FIG. 8.** Content of mercury in the estimated total volume of blood (A) and the percentage of the mercury in blood present in the plasma (B) of control, uninephrectomized, and 75% nephrectomized rats 24 hr after the intravenous injection of a 1.0, 1.5, 1.75, 2.0, or 8.0  $\mu\text{mol/kg}$  dose of mercuric chloride. Each value represents a mean  $\pm$  SE for 5 or 6 rats. \*Significantly different ( $p < 0.05$ ) from the mean for the corresponding group of control rats. \*\*Significantly different ( $p < 0.05$ ) from the means for the corresponding groups of uninephrectomized and control rats. <sup>1</sup>Significantly different ( $p < 0.05$ ) from the mean for the corresponding group of rats treated the same way surgically and injected with the 1.0  $\mu\text{mol/kg}$  dose of mercuric chloride. <sup>2</sup>Significantly different ( $p < 0.05$ ) from the mean for the corresponding group of rats treated the same way surgically and injected with the 1.0, 1.5, or 1.75  $\mu\text{mol/kg}$  dose of mercuric chloride. <sup>3</sup>Significantly different ( $p < 0.05$ ) from the mean for the corresponding group of rats treated the same way surgically and injected with the 1.0, 1.5, 1.75, or 2.0  $\mu\text{mol/kg}$  dose of mercuric chloride. <sup>4</sup>Significantly different ( $p < 0.05$ ) from the mean for the corresponding group of rats treated the same way surgically and injected with the 1.0 or 1.75  $\mu\text{mol/kg}$  dose of mercuric chloride.

Twenty-four hours after the administration of the of the 1.0  $\mu\text{mol/kg}$  dose of mercuric chloride, the content of mercury in the liver was significantly greater in the 75% NPX rats than in the 50% NPX or control rats. After the administration of either the 1.5 or 1.75  $\mu\text{mol/kg}$  dose, the content of mercury in the liver of the 75% NPX was significantly greater than that in only the control rats. No significant differences were detected among the three groups of rats following the administration of the 2.0 or 8.0  $\mu\text{mol/kg}$  dose of mercuric chloride.

**Urinary and fecal excretion of mercury.** Differences in the 24-hr urinary excretion of mercury among the control, 50% NPX, and 75% NPX rats were detected only after the injection of the 1.0, 1.5, or 2.0  $\mu\text{mol/kg}$  dose of mercuric chloride. In all of these cases, the urinary excretion of mercury in both 50% NPX and 75% NPX rats was greater than that in corresponding control rats (Fig. 10A).

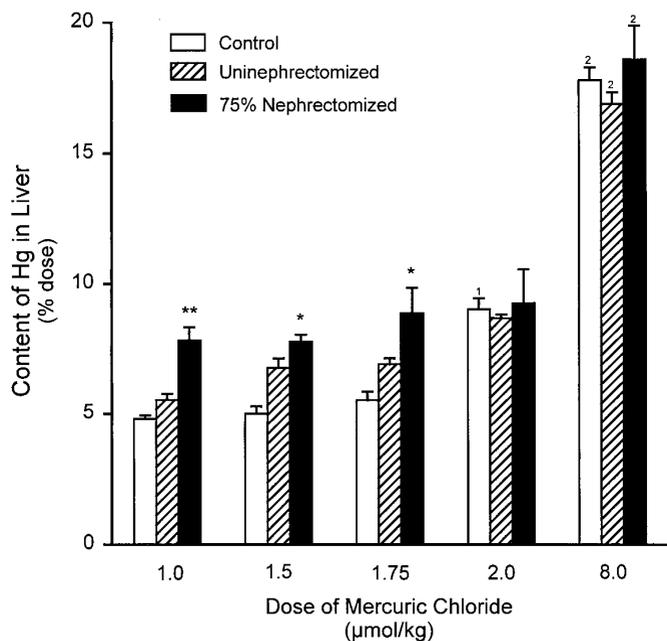
The amount of the administered dose excreted in the urine increased as the dose was increased to the middle doses, and then came down at the highest dose. Despite this, the actual mole quantity of mercury excreted in the urine increased as the dose of mercury was increased.

Fecal excretion of mercury (in terms of % of the administered dose) during the initial 24 hr after the injection of

mercury tended to be greater in the 50% NPX rats and 75% NPX rats than in the control rats after the administration of the 1.0, 1.5, 1.75, or 2.0- $\mu\text{mol/kg}$  dose of mercuric chloride (Fig. 10B). Only after the 8.0  $\mu\text{mol/kg}$  dose of mercuric chloride were no significant differences detected among the three groups of rats. In terms of the actual number of moles of mercury excreted in the feces, the fecal excretion of mercury increased in all groups of rats as the dose was increased, although the percentage of the dose excreted may have been less than that detected at the lower doses, such as that detected after the 8.0  $\mu\text{mol/kg}$  dose of mercuric chloride.

## DISCUSSION

On the basis of histopathological analysis of kidneys and evaluation of the urinary excretion of LDH and total protein, the level of cellular injury and necrosis along pars recta segments of proximal tubules was significantly greater in 50% NPX rats than in control rats 24 hr after the administration of any of the three lowest doses of mercuric chloride used in this study. These findings are consistent with data obtained from previous studies (Ramos-Frendo *et al.*, 1979; Houser and Berndt, 1986; Zalups and Diamond, 1987; Zal-



**FIG. 9.** Content of mercury in the liver of control, uninephrectomized, and 75% nephrectomized rats 24 hr after the intravenous injection of a 1.0, 1.5, 1.75, 2.0, or 8.0  $\mu\text{mol/kg}$  dose of mercuric chloride. Each value represents a mean  $\pm$  SE for 5 or 6 rats. \*Significantly different ( $p < 0.05$ ) from the mean for the corresponding group of control rats. \*\*Significantly different ( $p < 0.05$ ) from the means for the corresponding groups of uninephrectomized and control rats. †Significantly different ( $p < 0.05$ ) from the mean for the corresponding group of rats treated the same way surgically and injected with the 1.0, 1.5, or 1.75  $\mu\text{mol/kg}$  dose of mercuric chloride. ‡Significantly different ( $p < 0.05$ ) from the mean for the corresponding group of rats treated the same way surgically and injected with the 1.0, 1.5, 1.75, or 2.0  $\mu\text{mol/kg}$  dose of mercuric chloride.

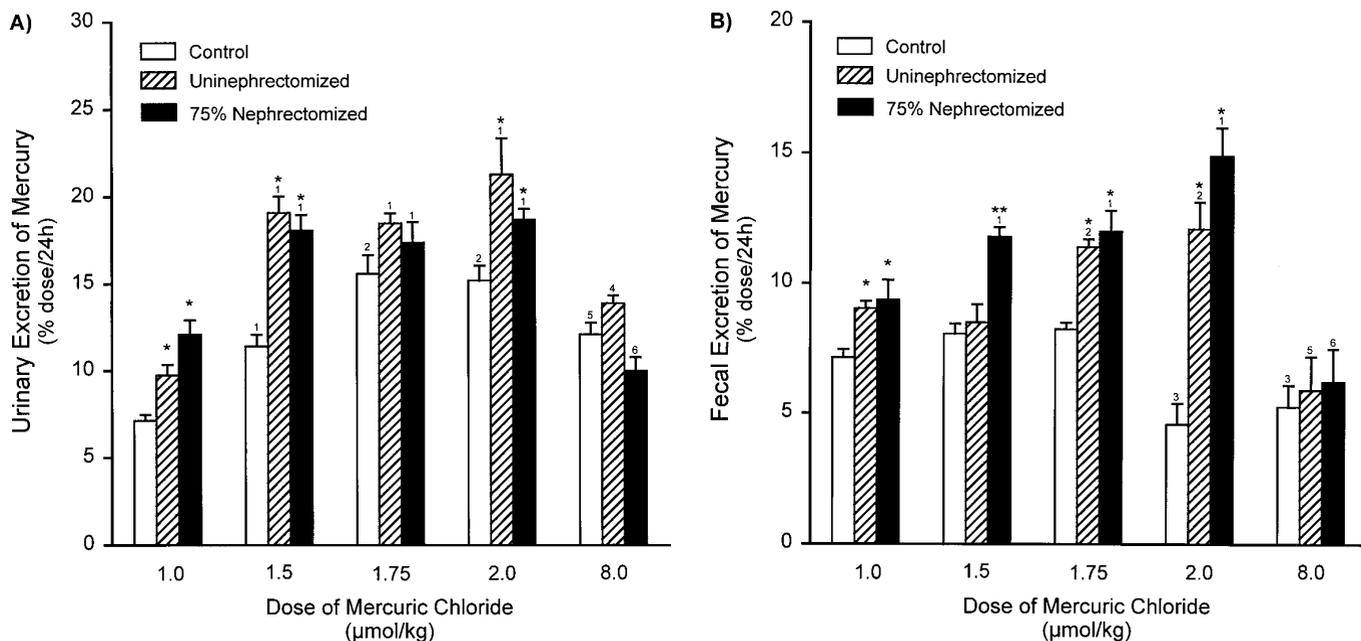
ups and Lash, 1990; Zalups *et al.*, 1988) and support the hypothesis that 50% NPX rats (in which compensatory renal growth has occurred) develop a more severe form of the nephropathy induced by low nephrotoxic doses of inorganic mercury than do normal rats with two kidneys. The nephropathy induced by the 8.0  $\mu\text{mol/kg}$  dose of mercuric chloride was also significantly greater in the 50% NPX rats than the control rats. Taking these findings together, it appears that as the number of functioning nephrons is reduced significantly to a level where overall renal function is not compromised significantly, there is an increased risk of renal injury or an increased risk of developing a more severe form of the nephropathy induced by inorganic mercury, particularly at lower doses. Data obtained from the 75% NPX rats, however, suggest that once renal function becomes compromised significantly, the risk of proximal tubular injury induced by low doses of inorganic may not be much greater than in control animals.

According to the dispositional data, the relatively decreased levels of proximal tubular injury in the 75% NPX rats treated with the lower doses of mercuric chloride was

apparently related or due to decreased concentrations of mercury in the remnant kidney, particularly in the cortex and outer stripe of the outer medulla. Some of this decrease may have been related directly to decreased whole animal GFR. Despite decreased GFR, the urinary excretion of mercury was either similar to, or greater than, the levels in corresponding control rats. This could have been due, in part, to a decreased capacity to reabsorb filtered mercury, which could be an additional mechanism responsible for the decreased renal concentration of mercury. It should be mentioned that interpretation of the urinary excretion of mercury is somewhat deceptive in animals given nephrotoxic doses of mercuric chloride, since a substantial fraction of mercury excreted is due to mercury being released into the luminal compartment from necrotic epithelial cells (Zalups and Diamond, 1987; Zalups *et al.*, 1988). However, diminished renal uptake of mercury has been detected recently in 75% NPX rats administered a nonnephrotoxic dose of inorganic mercury (Zalups, 1995a), which tends to strengthen the argument that significant alterations in renal hemodynamics, such as decreases in GFR, have significant effects on the renal disposition of mercury. Further support for this hypothesis comes from a couple of recent studies, in which it was shown that renal uptake of mercury (which occurs almost exclusively along the proximal tubule) is due to both luminal and basolateral mechanisms (Zalups, 1995b; Zalups and Minor, 1995). In one of the studies, induction of stop-flow conditions by ureteral ligation was used to demonstrate that filtration and subsequent reabsorption of mercury were involved as mechanisms in the proximal tubular uptake of mercury (Zalups and Minor, 1995). The luminal mechanism was shown in the other study to involve the activity of the brush-border enzyme  $\gamma$ -glutamyltranspeptidase (Zalups, 1995b). Both previous studies also demonstrated that a basolateral mechanism dependent on the activity of the organic anion transport system is also involved, to a substantial degree, in the proximal tubular uptake of mercury (Zalups, 1995b; Zalups and Minor, 1995).

In all three groups of rats, the 8.0  $\mu\text{mol/kg}$  dose of mercuric chloride caused such severe tubular injury that acute renal failure ensued. GFR fell from control levels of approximately 1.35 ml/min in the control and 50% NPX rats and 0.92 ml/min in the 75% NPX rats to less than 0.1 ml/min in all three groups of rats within 24 hr. Interestingly, BUN was significantly greater in the 75% NPX rats than in either of the other two groups of rats. This finding seems to indicate that the 75% NPX rats may have gone into acute renal failure much sooner than either of the other two groups. If so, this would indicate that at highly nephrotoxic doses of inorganic mercury, 75% NPX rats may be more prone to acute renal failure than normal or 50% NPX rats.

A 75% reduction of renal mass not only caused significant changes in the renal disposition of mercury, but also caused



**FIG. 10.** Urinary (A) and fecal excretion of mercury in control, uninephrectomized, and 75% nephrectomized rats during the initial 24 hr after the intravenous injection of a 1.0, 1.5, 1.75, 2.0, or 8.0  $\mu\text{mol/kg}$  dose of mercuric chloride. Each value represents a mean  $\pm$  SE for 5 or 6 rats. \*Significantly different ( $p < 0.05$ ) from the mean for the corresponding group of control rats. \*\*Significantly different ( $p < 0.05$ ) from the means for the corresponding groups of uninephrectomized and control rats. <sup>1</sup>Significantly different ( $p < 0.05$ ) from the mean for the corresponding group of rats treated the same way surgically and injected with the 1.0  $\mu\text{mol/kg}$  dose of mercuric chloride. <sup>2</sup>Significantly different ( $p < 0.05$ ) from the mean for the corresponding group of rats treated the same way surgically and injected with the 1.0 or 1.5  $\mu\text{mol/kg}$  dose of mercuric chloride. <sup>3</sup>Significantly different ( $p < 0.05$ ) from the mean for the corresponding group of rats treated the same way surgically and injected with the 1.0, 1.5, or 1.75  $\mu\text{mol/kg}$  dose of mercuric chloride. <sup>4</sup>Significantly different ( $p < 0.05$ ) from the mean for the corresponding group of rats treated the same way surgically and injected with the 1.0, 1.5, 1.75, or 2.0  $\mu\text{mol/kg}$  dose of mercuric chloride. <sup>5</sup>Significantly different ( $p < 0.05$ ) from the mean for the corresponding group of rats treated the same way surgically and injected with the 1.0, 1.75, or 2.0  $\mu\text{mol/kg}$  dose of mercuric chloride. <sup>6</sup>Significantly different ( $p < 0.05$ ) from the mean for the corresponding group of rats treated the same way surgically and injected with the 1.5, 1.75, or 2.0  $\mu\text{mol/kg}$  dose of mercuric chloride.

significant changes in the hepatic disposition of mercury and fecal excretion of mercury, mainly following the administration of the lowest three doses of mercuric chloride. Twenty-four hours after the administration of any of these three doses of inorganic mercury, the hepatic content of mercury and the fecal excretion of mercury were significantly greater in the 75% NPX rats than in the control rats. Hepatic content of mercury also tended to be greater in the 50% NPX rats than in the control rats. Similar findings have also been documented in 75% NPX and 50% NPX rats administered a nontoxic dose of mercuric chloride (Zalups, 1995a). Increased hepatic content of mercury was most likely due to increased concentration of mercury in the blood caused by reduced renal mass. Although inorganic mercury can have hepatotoxic effects, no obvious signs of hepatocellular necrosis were evident in this study. Since decreased renal clearance of toxicants, like inorganic mercury, most likely occurs as a result of a significant reduction of renal mass, increased hepatic handling and fecal excretion may be important compensatory mechanisms involved in the metabolism and excretion of these toxicants. These mechanisms may also prove to be deleterious to the health of the organism should the

hepatic load of a toxicant overwhelm the ability of the hepatocytes to metabolize and excrete it through the bile. This in turn could lead to hepatocellular injury and necrosis. The increased fecal excretion of mercury in the 75% NPX rats given any of the three lowest doses of mercuric chloride may be due to increased hepatobiliary excretion of mercury and intestinal secretion of mercury. Very recent data indicate that the intestines are capable of secreting a significant quantity of inorganic mercury into the lumen (Zalups and Barfuss, 1996).

In summary, there appears to be a varied response to the level of cellular necrosis induced by mercury following a reduction of renal mass. The principal factors that seem to affect the degree or severity of proximal tubular necrosis are the magnitude of the reduction of renal mass and the dose of inorganic mercury. Obviously much more research is required to better understand the effects of reduced renal mass on the risk of rats becoming intoxicated with inorganic or organic mercury-containing compounds.

Due to the fact that hypothesis that reduced renal mass alters the susceptibility to the nephrotoxic effects of mercury (or other nephrotoxicants) would be very difficult, if not

impossible, to test in humans, extrapolation from animal studies has been, and remains, the only reasonable means by which this issue can begin to be addressed. One of the major reasons that it is difficult to test the above-mentioned hypothesis in humans is the difficulty in identifying many individuals who have reduced renal mass. This is due mainly to the fact that certain individuals who have reduced renal mass do not become aware of their problem until chronic renal failure occurs, at which time they are in need of hemodialysis and a transplant.

As a final note, although the model 75% nephrectomy used in this study is a well accepted model for the induction of early stages of chronic renal failure, it represents only one means by which renal mass can be reduced by 75%, and therefore the data obtained using this model need to be viewed with this in mind.

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