

## Quantitative Evaluation of Urinary Porphyrins as a Measure of Kidney Mercury Content and Mercury Body Burden during Prolonged Methylmercury Exposure in Rats

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Changes in urinary porphyrin excretion patterns (porphyrin profiles) during prolonged mercury exposure are attributable to mercury accumulation in the kidney and to consequent effects of  $Hg^{2+}$  on renal porphyrin metabolism. In the present study, we evaluated the quantitative relationship of urinary porphyrin concentrations to mobilizable renal mercury content, using the metal chelator 2,3-dimercapto-1-propanesulfonic acid (DMPS) to modulate kidney mercury levels. Rats exposed to methylmercury hydroxide (MMH) at 10 ppm in drinking water for 6 weeks were treated with up to 3 consecutive doses of DMPS (100mg/kg, ip) at 72-h intervals. Consistent with previous findings, the concentrations of pentacarboxyl- (5-) and copro- (4-) porphyrins and of an atypical porphyrin specific to mercury exposure, precoproporphyrin, were significantly elevated in urine of MMH-exposed rats, compared with that of rats exposed to distilled water ( $dH_2O$ ) for the same period. Consecutive DMPS treatments of MMH-exposed rats significantly decreased kidney concentrations of total, as well as  $Hg^{2+}$  and  $CH_3Hg^+$  species, and promoted increased urinary mercury excretion. Concomitantly, DMPS treatment decreased both kidney and urinary porphyrin concentrations, consistent with depletion of renal mercury levels. Regression analyses demonstrated a high correlation ( $r \sim 0.9$ ) between prechelation urinary porphyrins and postchelation urinary mercury levels and also between prechelation urinary porphyrins and prechelation kidney mercury concentrations. These findings demonstrate that urinary porphyrin concentrations relate quantitatively to DMPS-mobilizable mercury in the kidney and, therefore, serve as a biochemical measure of renal mercury content.

**Key Words:** 2,3-dimercapto-1-propanesulfonate (DMPS); methylmercury; mercury; porphyrins; porphyrin profiles; kidney; biomarker.

Porphyrins are formed in mammalian tissues as intermediates in the biosynthesis of heme (Fig. 1). In most tissues, porphyrins with 8, 7, 6, 5, and 4 carboxyl group side-chains are produced in excess of that required for heme biosynthesis and are excreted in the urine in a well-established pattern (Woods

*et al.*, 1991). Previous studies from this laboratory have described metal-specific changes in the urinary porphyrin excretion pattern (porphyrin profile) associated with prolonged exposure of animals and humans to low levels of mercury, arsenic, lead, and other metals (reviewed in Woods, 1995). The etiology of these changes involves both metal-directed impairment of specific heme biosynthetic pathway enzymes in target tissues as well as metal-facilitated oxidation of reduced porphyrins (porphyrinogens) that accumulate in tissue cells because of impaired porphyrin metabolism (Lund *et al.*, 1993; Miller and Woods, 1993; Woods *et al.*, 1990a,b; Woods and Southern, 1989). These observations have supported the view that urinary porphyrin profiles may serve as surrogate measures (biomarkers) of metal exposures and their effects in human subjects (Fowler *et al.*, 1987; Marks, 1985; Woods, 1995).

Of particular interest to the investigation of urinary porphyrin profile changes as biomarkers of metal exposure and toxicity are findings from studies of methylmercury-exposed rats that have demonstrated highly specific changes in the urinary porphyrin excretion pattern caused by mercury-induced alterations in heme biosynthesis in the kidney (Bowers *et al.*, 1992; Woods *et al.*, 1984, 1991). These changes are characterized by significantly elevated urinary concentrations of pentacarboxyl- (5-) and copro- (4-) porphyrins as well as by the appearance of an atypical porphyrin (precoproporphyrin), tentatively identified as keto-isocoproporphyrin (Woods, 1995), that appears to be specific to mercury exposure. Comparable findings have been described in human subjects with occupational exposure to mercury vapor ( $Hg^0$ ; Gonzalez-Ramirez *et al.*, 1995; Woods *et al.*, 1993).

The kidney is a principal target organ of mercury compounds and the major tissue depot of mercury in the body (Clarkson *et al.*, 1986). In previous studies (Woods *et al.*, 1991) we have demonstrated that methylmercury rapidly dissociates to  $Hg^{2+}$  and  $CH_3Hg^+$  in kidney cortex during prolonged exposure to methylmercury hydroxide in drinking water. Moreover, an equilibrium between  $Hg^{2+}$  and  $CH_3Hg^+$  is established and maintained by the kidney over a wide range of total mercury concentrations. Additionally, we have shown in

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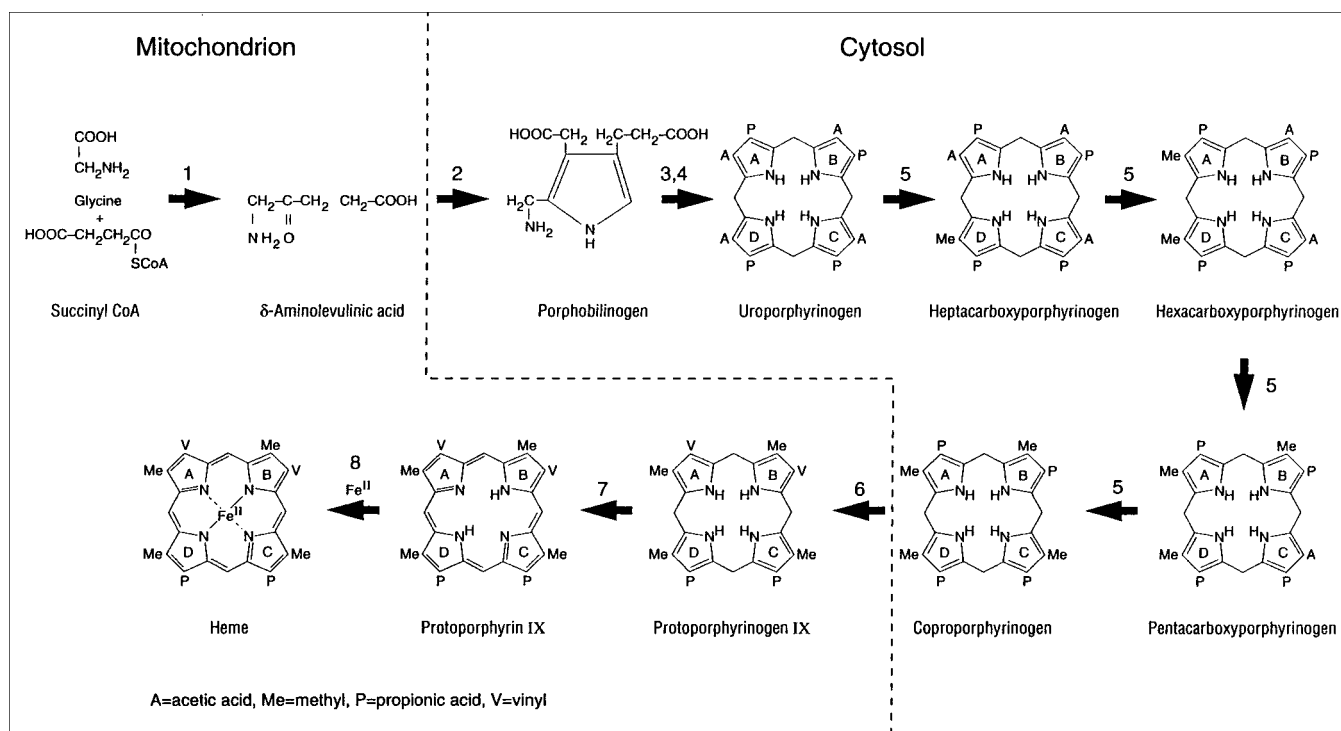


FIG. 1. Heme biosynthetic pathway. Steps are catalyzed by (1)  $\delta$ -aminolevulinic acid (ALA) synthetase, (2) ALA dehydratase, (3) uroporphyrinogen I synthetase, (4) uroporphyrinogen III cosynthetase, (5) uroporphyrinogen decarboxylase, (6) coproporphyrinogen oxidase, (7) protoporphyrinogen oxidase, and (8) ferrochelatase.

mass balance studies (Pingree *et al.*, 2001) that the chelating agent, 2,3-dimercapto-1-propanesulfonic acid (DMPS), readily depletes both  $Hg^{2+}$  and  $CH_3Hg^+$  in equimolar fashion from kidney tissue. Hence, the amount of mercury excreted in the urine following DMPS treatment corresponds quantitatively to that removed from the kidney.

In the present study, we evaluated quantitatively the relationship between the renal mercury concentration (as an approximate measure of mercury body burden) and the concentration of porphyrins excreted in the urine, using DMPS to modulate renal mercury content during prolonged methylmercury exposure. The findings demonstrate that urinary porphyrin concentrations are highly correlated with mercury in the kidney over a wide range of total mercury concentrations and, therefore, constitute a biochemical measure of mercury accumulation in kidney cells.

## MATERIALS AND METHODS

**Materials.** Male Fischer 344 rats (200–225 g) were purchased from Simonsen Labs (Gilroy, CA). Methylmercury (II) hydroxide ( $CH_3HgOH$ , MMH) was obtained from Alfa Aesar (Ward Hill, MA). DMPS was acquired from Sigma Chemical Co. (St. Louis, MO). 10 ml disposable C-18 Bond Elute columns were purchased from Varian Associates (Harbor City, CA). Porphyrin acid chromatographic marker kits were obtained from Porphyrin Products (Logan, UT). HPLC-grade methanol was acquired from J. T. Baker. All other chemicals were reagent grade and were purchased from standard commercial

sources. Distilled deionized water was used for preparation of all aqueous solutions.

**Animal treatment.** Animal handling and treatment protocols have been described in the accompanying paper (Pingree *et al.*, 2001).

**Study design.** Thirty rats were placed on a continuous regimen of drinking water containing 10 ppm MMH for 6 weeks. This exposure period was selected on the basis of previous observations (Pingree *et al.*, 2001) demonstrating that prechelation concentrations of both  $Hg^{2+}$  and  $CH_3Hg^+$  in kidney cortex following 6 weeks of MMH exposure are sufficient to permit evaluation the efficacy of DMPS chelation in clearing both organic and inorganic mercury species from the kidney. A control group of 16 rats received distilled water only.

To determine the effects of multiple DMPS treatments on urinary porphyrin levels as a function of renal mercury concentration, animals were given up to 3 consecutive DMPS injections over a period of 3–7 days prior to sacrifice. For this assessment, the 30 MMH-exposed rats were divided into 2 groups of 18 and 12 animals, respectively. The first group of 18 rats received a single ip injection of 100 mg/kg DMPS, whereas the remaining 12 were given a saline injection, as prechelation controls. All 30 animals were then transferred to individual metabolism cages for 24-h urine collections. Rats were denied food but were provided  $dH_2O$  drinking water *ad libitum* during the urine collection period. Following urine collections, 6 animals from the DMPS-treatment group and 4 rats from the saline-treated group were sacrificed, and kidneys were retrieved for mercury and porphyrin analyses. Seventy-two hours after the first injection, the remaining 12 DMPS-treated rats were given a second 100-mg/kg DMPS injection, while the remaining 8 saline-treated rats were given a second saline injection. After 24 hours, 6 of the DMPS-treated rats and 4 of the saline-treated rats were sacrificed and tissues collected. Seventy-two hours after the second injection the remaining animals were given a third DMPS or saline injection. Twenty-four hours after the third injection all remaining rats

were sacrificed and kidneys collected. A comparable DMPS treatment and sacrifice regimen was followed with respect to control rats receiving distilled water for 6 weeks.

Between DMPS treatments, animals were held in metabolism cages without food but with dH<sub>2</sub>O for 24-h urine collections and then returned to their hanging cages and permitted food and water *ad libitum* for 48 h. No animals were deprived of food for more than 24 h during the treatment period. In all studies, animals were anesthetized by carbon dioxide (CO<sub>2</sub>) and then sacrificed by decapitation. Kidneys were harvested surgically immediately following sacrifice and were preserved at -80°C until mercury and porphyrin analyses.

**Collection of urine samples.** Animals were placed in hanging metabolism cages for 24 h with free access to drinking water but without food. The metabolism cages were fitted with metal funnels attached to the bottom with a plastic ping-pong ball placed at the hole of the funnel to allow urine but not feces to pass through. Aluminum foil-covered, polypropylene 125-ml volumetric flasks were placed under the funnels to collect the urine without allowing evaporation. At the end of 24 h the urine volume was measured and the sample then divided into 2 portions for mercury or porphyrin analysis, respectively. The portion obtained for mercury analysis was acidified with a drop of 6 N HCl. Both fractions were then frozen at -20°C until mercury or porphyrin analysis.

**Urine and kidney porphyrin analysis.** Porphyrin concentrations in urine samples were measured using high-pressure liquid chromatography (HPLC) separation and spectrofluorometric quantitation techniques, as previously described (Bowers *et al.*, 1992). This procedure permits quantitation of urinary porphyrins with a detection sensitivity of 0.5 pmol. Porphyrin concentrations in kidney cortical tissue were determined as previously described (Woods and Miller, 1993) with modification of the porphyrin extraction procedure as follows: A 0.1–0.2 g tissue sample was homogenized in 5 ml of a solution of 50:50 1 N HCl and concentrated acetonitrile in a 15-ml glass centrifuge tube. The mixture was centrifuged at 3,000 × g for 15 min at 7°C, and the supernatant was then transferred to a 100-ml glass beaker in ice. Six ml of the HCl:acetonitrile mixture was added to the pellet, and the tube was vortexed until the pellet was fully suspended. The tissue mixture was again centrifuged at 3,000 × g at 7°C for 5 min. The supernatant was removed and added to that in the 100-ml beaker, and pellet was discarded. Double distilled water was added to the combined supernatants to a final volume of 70 ml. For each tissue sample a C-18 column was pre-conditioned with 10 ml acetonitrile followed by 15 ml H<sub>2</sub>O. The diluted supernatant solution was then allowed to gravity drip through the pre-conditioned C-18 column. A slight vacuum was added if there was no detectable movement of solution through the column over a 15-min period. The concentrated porphyrins were eluted from the column into a round-bottom glass tube by 10 ml acetonitrile, which was allowed to gravity drip through the column. The tube containing the tissue porphyrin solution was placed in a heating block at 32°C, and the fluid was evaporated to dryness aided by a stream of nitrogen gas. Tissue porphyrins were then reconstituted in 500 μl 1 N HCl. The final porphyrin solution was filtered through a 0.45 μm syringe filter and stored at -20°C for up to 1 week before analysis by HPLC, as previously described (Woods and Miller, 1993).

**Mercury determination.** Urinary mercury was measured using a modified version of the digestion method by Corns *et al.* (1994), as described in the accompanying paper (Pingree *et al.*, 2001). The total and inorganic mercury concentrations in kidney tissue were analyzed by CVAFS following digestion and preparation of tissues as described by Atallah and Kalman (1993). The organic mercury content of tissue sample was determined by the difference in the total and inorganic mercury concentrations.

**Other assays.** Tissue protein concentrations were determined by the method of Smith *et al.*, (1985) using the bicinchoninic acid (BCA) protein assay reagent (Pierce Chemical Co., Rockford, IL). Urinary creatinine levels were determined using a standard colorimetric method (Sigma Chemical Co., St. Louis, MO).

**Statistical analyses.** Data are presented as means ± standard error of the mean (SEM). Statistical analyses were conducted using Student's *t*-test with

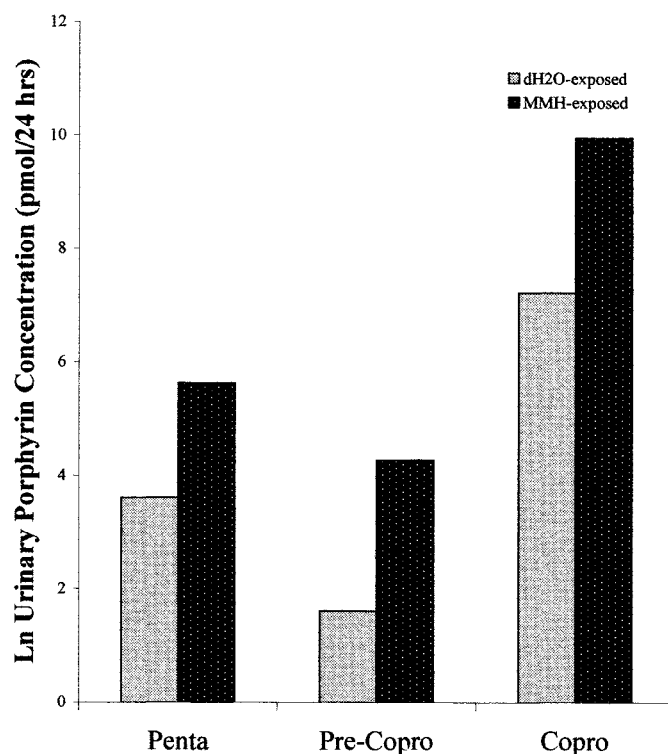


FIG. 2. Urinary porphyrin concentrations from unexposed (dH<sub>2</sub>O-exposed) rats or from rats exposed to 10 ppm methylmercury hydroxide (MMH) in drinking water for 6 weeks. Data are presented as natural log (Ln) of the mean porphyrin concentrations (pmol/24 h) in 24-h urine samples of 6 MMH-exposed or 4 H<sub>2</sub>O-exposed animals. Porphyrin concentrations were determined as described under Materials and Methods. Actual values are presented in the text.

one-tailed distribution; *p* values less than 0.05 were considered significant. Linear regression analysis was performed using the Excel function (Microsoft, Redland, WA).

## RESULTS

### *Mercury Exposure Elicits Characteristic Changes in the Urinary Porphyrin Excretion Pattern*

Initial studies were conducted to measure the changes in urinary porphyrin levels in response to prolonged MMH exposure. As depicted in Figure 2, the concentrations of pentacarboxyl- (5-), precopro-, and copro- (4-) porphyrins were substantially elevated in 24-h urine samples of rats exposed to 10 ppm MMH in drinking water for 6 weeks as compared with those in urine of dH<sub>2</sub>O-exposed controls. Pentacarboxyl porphyrin was elevated 7.5-fold from  $37 \pm 6$  to  $279 \pm 72$  pmol/24 h, whereas coproporphyrin (4-carboxyl porphyrin) was elevated to 15.3 times control levels from  $1,373 \pm 153$  to  $21,049 \pm 1,432$  pmol/24 h. The atypical porphyrin, precopro-porphyrin, was also abundant in urine of MMH-exposed rats. In contrast, urinary concentrations of 8-, 7-, and 6-carboxyl porphyrins were not significantly elevated as a consequence of

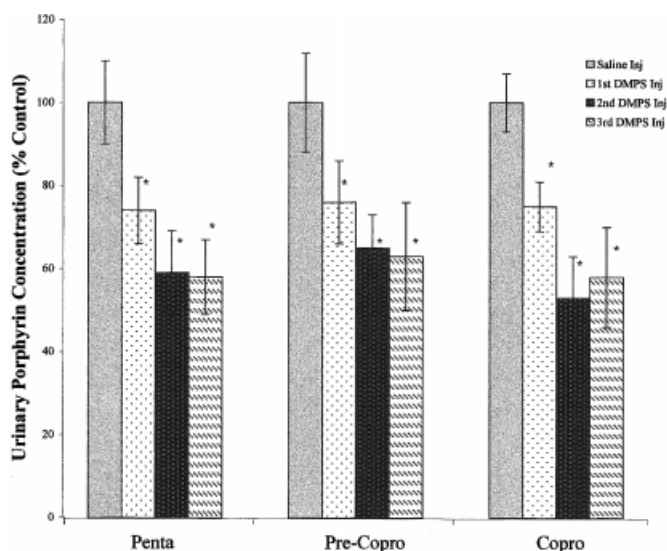


FIG. 3. Effects of consecutive DMPS injections on urinary porphyrin concentrations in MMH-exposed rats. DMPS (100 mg/kg) or saline was administered by ip injection at 72-h intervals according to the regimen described under Materials and Methods. Porphyrin concentrations are presented as a percent of prechelation (saline-injected) values ( $\pm$  SD) during the 24-h periods immediately following the first, second, or third consecutive DMPS injections. Prechelation (saline-injected) concentrations of 5- and 4-carboxyl porphyrins and of precoproporphyrin are presented in the text. \*Indicates significantly different ( $p < 0.05$ ) from saline-injected (prechelation) value.

MMH exposure (not shown), consistent with previous findings (Woods *et al.*, 1991).

#### Urinary Porphyrin Levels in MMH-Exposed Rats Were Highly Correlated with Kidney Mercury Levels and Declined with Consecutive DMPS Treatments

As described in the accompanying paper (Pingree *et al.*, 2001), the mean concentrations of  $\text{Hg}^{2+}$  and  $\text{CH}_3\text{Hg}^+$  were 43.0 and 32.6  $\mu\text{g/g}$  of renal cortex, respectively, in rats exposed to 10 ppm MMH in drinking water for 6 weeks, compared with barely detectable levels (0.03 and 0.02  $\mu\text{g/g}$ , respectively) in kidneys of  $\text{dH}_2\text{O}$ -exposed animals. A single DMPS injection (100 mg/kg, ip) produced a decrease in total kidney mercury content to approximately 73% (55.2  $\mu\text{g/g}$ ) of that found in kidneys of saline-injected MMH-exposed rats, indicative of prechelation mercury values. Successive DMPS treatments at 72-h intervals produced additional reductions in total kidney mercury content to 59 and 55%, respectively, of mercury levels found in kidneys of saline-injected MMH-exposed rats, with comparable decreases in organic and inorganic mercury constituents. Concomitantly, the urinary concentrations of 5- and 4-carboxyl and precopro- porphyrins declined significantly after DMPS treatment. As shown in Figure 3, the urinary levels of all 3 porphyrins decreased to approximately 75% of those seen in urine of saline-injected rats during the 24-h period immediately following a single

DMPS injection. Additional DMPS treatments administered at subsequent 72-h intervals produced further reductions in urinary porphyrin concentrations, consistent with the additional decrease observed in renal mercury content.

#### Prechelation Urinary Porphyrin Levels Are Highly Correlated with Postchelation Urinary Mercury Concentrations

Regression analyses were performed to assess the strength of the association between prechelation urinary porphyrin concentrations and postchelation urinary mercury levels in rats with prolonged MMH exposure. As shown in Table 1, a high correlation ( $r \sim 0.9$ ) was found for the association of prechelation urinary porphyrin levels with the postchelation urinary mercury concentrations when assessed as inorganic, organic, or total mercury content during the 24-h period following a single DMPS injection.

#### Prechelation Urinary Porphyrin Levels Are Highly Correlated with Renal Mercury Content

Additional analyses were performed to assess the strength of the association between prechelation urinary porphyrin levels and prechelation concentrations of organic, inorganic and total mercury concentrations in the kidney. Table 2 presents correlation coefficients for the linear association of urinary porphyrin levels with renal mercury concentrations of saline-injected MMH-exposed rats. Prechelation urinary porphyrin concentrations in MMH-exposed animals were compared to the mercury levels in the kidneys of the same animals that received the saline injection, representing prechelation kidney mercury burden. High correlation coefficients ( $r \sim 0.9$ ) were again observed for each regression, indicating a strong association between prechelation urinary porphyrin concentrations and renal mercury content.

TABLE 1

#### Linear Relationship of Prechelation Urinary Porphyrin Concentrations with Urinary Mercury Concentrations Following a Single DMPS Injection

Porphyrin	Correlation coefficient ( $r$ )		
	Total Hg	$\text{Hg}^{2+}$	$\text{CH}_3\text{Hg}^+$
Penta	0.927	0.984	0.876
Precopro	0.961	0.884	0.987
Copro	0.998	0.989	0.984

Note. Correlation coefficients ( $r$ ) are presented for the linear associations of postchelation total, inorganic, and organic urinary mercury concentrations with prechelation coproporphyrin, precoproporphyrin, and pentacarboxyporphyrin excreted in the urine following a single DMPS injection (100 mg/kg, ip). Urinary porphyrin and mercury concentrations were determined as described in Materials and Methods. Linear regression analysis was performed using the EXCEL general-purpose biostatistics package.

TABLE 2

Linear Relationship of Prechelation Urinary Porphyrin Concentrations with Kidney Mercury Concentrations Following MMH Exposure for 6 Weeks

Porphyrin	Correlation coefficient ( <i>r</i> )		
	Total Hg	Hg <sup>2+</sup>	CH <sub>3</sub> Hg <sup>+</sup>
Penta	0.967	0.996	0.879
Precopro	0.895	0.987	0.762
Copro	0.989	0.908	0.984

Note. Correlation coefficients (*r*) are presented for the linear associations of prechelation total, inorganic, and organic kidney mercury concentrations with prechelation coproporphyrin, pre-coproporphyrin, and pentacarboxyporphyrin excreted in the urine following 6 weeks of MMH exposure. Kidney mercury concentrations were determined as described in Materials and Methods. Linear regression analysis was performed using the EXCEL general-purpose biostatistics package.

To assess the strength of the association of prechelation urinary porphyrins over a wider range of renal mercury levels, additional regression analyses were performed, using combined renal mercury levels following first, second, and third consecutive DMPS injections. The correlation coefficients for the relationship of urinary porphyrin levels with the broader range of kidney mercury concentrations affected by 3 consecutive DMPS injections are presented in Table 3. While the correlation coefficients are smaller than those presented in Table 2, a strong positive correlation, nonetheless, remains between prechelation urinary porphyrin concentrations and kidney mercury content. In contrast, very low correlations (*r* < 0.2) were found for the association of prechelation urinary porphyrins to prechelation urinary mercury levels (data not shown).

TABLE 3

Linear Relationship of Urinary Porphyrin Concentrations with Kidney Mercury Concentrations over a Wide Range of Kidney Mercury Levels

Porphyrin	Correlation coefficient ( <i>r</i> )		
	Total Hg	Hg <sup>2+</sup>	CH <sub>3</sub> Hg <sup>+</sup>
Penta	0.726	0.518	0.814
Precopro	0.714	0.566	0.740
Copro	0.772	0.586	0.829

Note. Correlation coefficients (*r*) are presented for the linear associations of total, inorganic, and organic kidney mercury concentrations with urinary coproporphyrin, precoproporphyrin, and pentacarboxyl porphyrin concentrations measured following 1, 2, or 3 consecutive DMPS or saline injections of rats exposed to MMH (100 ppm) in drinking water for up to 6 weeks. Kidney total mercury concentrations ranged from 46.8–84.6 μg/g. Urinary porphyrin and kidney mercury concentrations were determined as described in Materials and Methods. Linear regression analysis was performed using the EXCEL general-purpose biostatistics package.

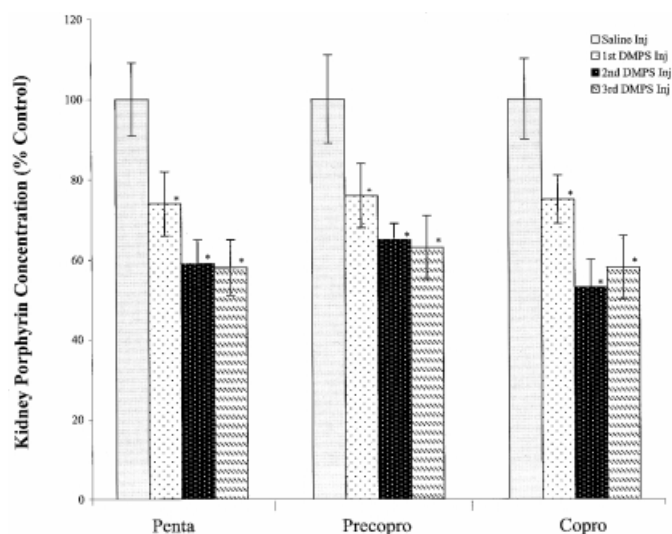


FIG. 4. Effects of consecutive DMPS injections on kidney porphyrin concentrations in MMH-exposed rats. DMPS (100 mg/kg) or saline was administered by ip injection at 72-h intervals according to the regimen described under Materials and Methods. Porphyrin concentrations are presented as a percent of prechelation (saline-injected) values ( $\pm$  SD) rat kidney obtained 24 h after the first, second, or third consecutive DMPS injections. Prechelation (saline-injected) concentrations of 5- and 4-carboxyl porphyrins and of precoproporphyrin are presented in the text. Kidney porphyrin concentrations were determined as described under Materials and Methods. \*Indicates significantly different ( $p < 0.05$ ) from saline-injected (prechelation) value.

Finally, studies were conducted to demonstrate that urinary porphyrins are derived largely from porphyrins that accumulate in the kidney during mercury exposure. For this assessment, renal porphyrin content was measured in relation to mercury removed from the kidney by successive DMPS injections of MMH-exposed rats. Consistent with previous findings (Woods and Miller, 1993), a 4.8-fold increase in total porphyrin content was observed in kidneys of saline-injected MMH-exposed rats, as compared with those of dH<sub>2</sub>O-exposed controls. This increase was attributable principally to increases in the concentrations of 5- and 4-carboxyl and precopro-porphyrins, without significant changes in levels of porphyrins with 8, 7, or 6 carboxyl groups. Pentacarboxyl porphyrin was increased nearly 9-fold from  $5.8 \pm 2.3$  to  $52.1 \pm 6.2$  pmol/g, whereas coproporphyrin was elevated approximately 4 times control levels from  $78.3 \pm 4.7$  to  $319.6 \pm 58.2$  pmol/g. Precoproporphyrin was not found in kidneys of dH<sub>2</sub>O-exposed rats but was readily detectable in kidneys of MMH-exposed animals at concentrations similar to that of pentacarboxyl porphyrin.

#### Kidney Porphyrin Content of MMH-Exposed Rats Declined following DMPS Treatment

As shown in Figure 4, the concentrations of each of 5- and 4-carboxyl and precopro-porphyrins declined by approximately 25% after the first DMPS injection, consistent with the decrease in renal mercury content. Subsequent DMPS injec-

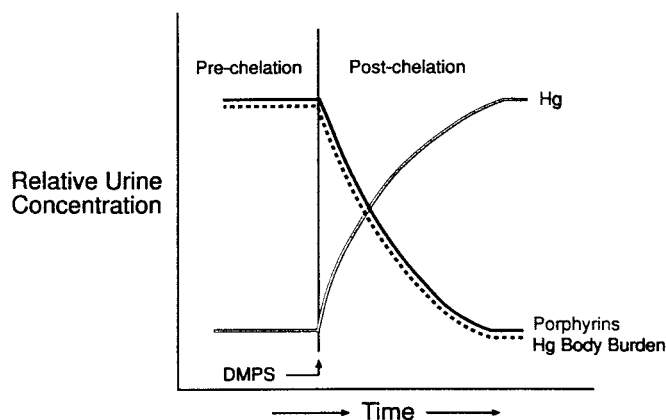


FIG. 5. Relationship of urinary porphyrins to mercury body burden. Urinary porphyrin concentrations are shown to vary quantitatively with pre- and postchelation mercury body burden and to have an inverse correlation with urinary mercury content.

tions at 72-h intervals produced further decreases in renal porphyrin concentrations, although proportionately less than that observed following the first injection. Nevertheless, the decline in renal porphyrin concentrations corresponded to the overall decrease in renal mercury content. In contrast, DMPS treatment did not significantly alter the porphyrin concentrations in kidneys of rats exposed to  $\text{dH}_2\text{O}$  alone (not shown). These findings support the view that mercury accumulation in the kidney contributes to the observed elevation of kidney porphyrin levels and consequent increased excretion of porphyrins observed in the urine during mercury exposure.

## DISCUSSION

Previous studies have shown a strong correlation between changes in urinary porphyrin concentrations and mercury body burden associated with prolonged mercury exposure in both animals and human subjects (Gonzalez-Ramirez *et al.*, 1995; Woods *et al.*, 1993). The present studies provide additional evidence of this relationship in demonstrating that urinary porphyrins are highly correlated with mercury body burden measured both as DMPS-mobilizable kidney mercury content and postchelation urinary mercury concentrations. The hypothetical relationship of urinary porphyrin levels to mercury body burden suggested by these findings is schematically depicted in Figure 5. Notably, prechelation urinary mercury excretion does not change dramatically with mercury exposure (Pingree *et al.*, 2001), whereas prechelation urinary porphyrin levels increase in proportion with DMPS-mobilizable mercury body burden during mercury exposure. In contrast, prechelation urinary porphyrin levels and prechelation urinary mercury concentrations in MMH-exposed rats were only weakly correlated ( $r < 0.2$ ), suggesting that prechelation urinary mercury levels are a relatively weaker measure of cumulative mercury body burden.

The sensitivity of urinary porphyrins as a biochemical measure of renal mercury content and body burden is supported by the present findings of a strong association of prechelation porphyrin levels with DMPS-mobilizable renal mercury over a wide range of kidney mercury concentrations. In previous studies (Woods *et al.*, 1991), significant increases in urinary porphyrin excretion rates during prolonged MMH exposure were observed at renal mercury concentrations as low as 3–6  $\mu\text{g/g}$ , an order of magnitude less than that at which proteinuria and other more conventional indices of mercury-induced nephrotoxicity are observed (Buchet *et al.*, 1980; Rosenman *et al.*, 1986). Additionally, urinary porphyrin levels increased in a dose-related manner with renal mercury content through the end of renal viability at cortical mercury concentrations in excess of 100  $\mu\text{g/g}$  (Woods *et al.*, 1991). The present findings that urinary porphyrin levels fluctuate as a reversible function of kidney mercury concentration support their sensitivity as a biomarker both of renal mercury content as well as a biochemical measure of reversible effects of mercury in kidney cells.

This study is the first, to our knowledge, to employ the metal chelator, DMPS, as a pharmacologic tool to modulate tissue metal levels for the purpose of evaluating tissue (kidney)-derived urinary metabolites (porphyrins) as a surrogate measure (biomarker) of renal mercury content and body burden. These findings are consistent with the efficacy of DMPS in mobilizing both  $\text{CH}_3\text{Hg}^+$  and  $\text{Hg}^+$  from kidney cells, as previously described (Pingree *et al.*, 2001). Notably, DMPS treatment did not significantly reduce kidney porphyrin concentrations when administered to MMH-unexposed ( $\text{dH}_2\text{O}$ -exposed) rats, supporting the view that changes in kidney and urinary porphyrin levels during MMH exposure are attributable principally to renal mercury accumulation (Woods *et al.*, 1984, 1991; Woods and Southern, 1989). Although the possible depletion of metals other than mercury from the kidney by DMPS was not assessed in the present study, no endogenous metals that are susceptible to chelation by DMPS from tissue sources (e.g., Cu, Zn; Aposhian, 1983) are known to affect porphyrin metabolism.

In conclusion, the present studies support a quantitative relationship between renal mercury content and urinary porphyrin concentrations that further characterize urinary porphyrin profiles as a biochemical measure of renal mercury content and potential toxicity over a wide range of mercury exposure. These findings also demonstrate the efficacy of DMPS as a pharmacologic tool for modulating tissue metal content as an investigative approach toward defining the etiology of biochemical measures of metal exposure.

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## REFERENCES

- Aposhian, H. V. (1983). DMSA and DMPS—Water soluble antidotes for heavy metal poisoning. *Annu. Rev. Pharmacol. Toxicol.* **23**, 193–215.
- Atallah, R. H., and Kalman, D. A. (1993). Selective determination of inorganic mercury and methylmercury in tissues by continuous flow and cold vapor atomic absorption spectrometry. *J. Anal. Toxicol.* **17**, 87–92.
- Buchet, J., Roels, H. M., Bernard, A., and Lauwerys, R. (1980). Assessment of renal function of workers exposed to inorganic lead, cadmium, or mercury vapor. *J. Occup. Med.* **22**, 741–750.
- Bowers, M. A., Aicher, L. D., Davis, H. A., and Woods, J. S. (1992). Quantitative determination of porphyrins in rat and human urine and evaluation of urinary porphyrin profiles during mercury and lead exposures. *J. Lab. Clin. Med.* **120**, 272–281.
- Clarkson, T. W., Hursh, J. B., Sager, P. R., and Syversen, T. L. M. (1986). Mercury. In *Biological Monitoring of Toxic Metals* (T. W. Clarkson, L. Friberg, G. F. Nordberg, and P. R. Sager, Eds.), pp. 199–246. Plenum Press, New York.
- Corns, W., Stockwell, P., and Jameel, M. (1994). Rapid method for the determination of total mercury in urine samples using cold vapour atomic fluorescence spectrometry. *Analyst* **119**, 2481–2484.
- Fowler, B. A., Oskarsson, A., and Woods, J. S. (1987). Metal- and metalloid-induced porphyriurias. Relationships to cell injury. *Ann. N.Y. Acad. Sci.* **514**, 172–182.
- Fowler, B. A., and Woods, J. S. (1977). Ultrastructural and biochemical changes in renal mitochondria during chronic oral methyl mercury exposure: The relationship to renal function. *Exp. Mol. Pathol.* **27**, 403–412.
- Gonzalez-Ramirez, D., Maiorino, R. M., Zuniga-Charles, M. Z., Hurlbut, K. M., Junco-Munoz, P., Aposhian, M. M., Dart, R. C., Gama, J. H. D., Echeverria, D., Woods, J. S., and Aposhian, H. V. (1995). Sodium 2,3-dimercaptopropane-1-sulfonate challenge test for mercury in humans: II. Urinary mercury, porphyrins and neurobehavioral changes of dental workers in Monterrey, Mexico. *J. Pharmacol. Exp. Ther.* **272**, 264–274.
- Lund, B.-O., Miller, D. M., and Woods, J. S. (1993). Studies on Hg(II)-induced H<sub>2</sub>O<sub>2</sub> formation and oxidative stress *in vivo* and *in vitro* in rat kidney mitochondria. *Biochem. Pharmacol.* **45**, 2017–2024.
- Marks, G. S. (1985). Exposure to toxic agents: The heme biosynthetic pathway and hemoproteins as indicator. *Crit. Rev. Toxicol.* **15**, 151–179.
- Miller, D. M., and Woods, J. S. (1993). Redox activities of mercury-thiol complexes: Implications for mercury-induced porphyria and toxicity. *Chem Biol. Interact.* **88**, 23–35.
- Pingree, S. D., Simmonds, P. L., and Woods, J. S. (2001). Effects of 2,3-dimercapto-1-propanesulfonic acid (DMPS) on tissue and urine mercury levels following prolonged methylmercury exposure in rats. *Toxicol. Sci.* **61**, 224–233.
- Rosenman, A., Valciukas, J. A., Glickman, L. Meyers, B. R., and Cinotti, A. (1986). Sensitive indicators of inorganic mercury toxicity. *Arch. Environ. Health* **41**, 208–215.
- Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Goeke, N. M., Olson, B. J., and Klenk, D. C. (1985). Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **150**, 76–85.
- Woods, J. S. (1995). Porphyrin metabolism as indicator of metal exposure and toxicity. In *Handbook of Experimental Pharmacology* (R. A. Goyer and M. G. Cherian, Eds.) Vol. 115, pp. 19–52. Springer-Verlag, Berlin.
- Woods, J. S., Bowers, M. A., and Davis, H. A. (1991). Urinary porphyrin profiles as biomarkers of trace metal exposure and toxicity: Studies on urinary porphyrin excretion patterns in rats during prolonged exposure to methyl mercury. *Toxicol. Appl. Pharmacol.* **110**, 464–476.
- Woods, J. S., Calas, C. A., Aicher, L. D., Robinson, B. H., and Mailer, C. (1990a). Stimulation of porphyrinogen oxidation by mercuric ion. I. Evidence of free radical formation in the presence of thiols and hydrogen peroxide. *Mol. Pharmacol.* **38**, 253–260.
- Woods, J. S., Calas, C. A., and Aicher, L. D. (1990b). Stimulation of porphyrinogen oxidation by mercuric ion. II. Promotion of oxidation from the interaction of mercuric ion, glutathione, and mitochondria-generated hydrogen peroxide. *Mol. Pharmacol.* **38**, 261–266.
- Woods, J. S., Eaton, D. L., and Lukens, C. B. (1984). Studies on porphyrin metabolism in the kidney. Effects of trace metals and glutathione of renal uroporphyrinogen decarboxylase. *Mol. Pharmacol.* **26**, 336–341.
- Woods, J. S., and Fowler, B. A. (1977). Renal porphyriuria during chronic methyl mercury exposure. *J. Lab. Clin. Med.* **90**, 266–272.
- Woods, J. S., Martin, M. D., Naleway, C. A., and Echeverria, D. (1993). Urinary porphyrin profiles as a biomarker of mercury exposure: Studies on dentists with occupational exposure to mercury vapor. *J. Toxicol. Environ. Health* **40**, 235–246.
- Woods, J. S., and Miller, H. D. (1993). Quantitative measurement of porphyrins in biological tissues and evaluation of tissue porphyrins during toxicant exposures. *Fundam. Appl. Toxicol.* **21**, 291–297.
- Woods, J. S., and Southern, M. R. (1989). Studies on the etiology of trace metal-induced porphyria: Effect of porphyrinogenic metals on coproporphyrinogen oxidase in rat liver and kidney. *Toxicol. Appl. Pharmacol.* **97**, 183–190.