URINARY PORPHYRIN PROFILES AS A BIOMARKER
OF MERCURY EXPOSURE: STUDIES ON DENTISTS
WITH OCCUPATIONAL EXPOSURE TO MERCURY VAPOR

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Porphyrias are among the most complex of human and
pathophysiologic syndromes. A successful approach to the under
standing of human and pathophysiologic syndromes. A successful approach to
porphyria is to interpret the findings in the context of the known
mechanisms of the disease and the potential for mercury exposure.

Mercury, in the form of inorganic and organic mercury, can be
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INTRODUCTION

Porphyrins are formed in mammalian tissues as intermediates in the biosynthesis of heme (Fig. 1). In most tissues, porphyrins bearing side-chains with eight, seven, six, five, and four carboxyl groups are produced in excess of that required for heme synthesis and are excreted in the urine in a well-established pattern (Fig. 2a). In previous studies, we described specific changes in urinary porphyrin excretion patterns (porphyrin profiles) associated with prolonged exposure of animals to low levels of mercury as methylmercury hydroxide (MMH) (Woods and Fowler, 1977; Woods et al., 1991; Bowers et al., 1992). The etiology of these changes involves both mercury-directed impairment of specific heme biosynthetic pathway enzymes in the kidney (a principal target organ of mercury compounds) and mercury-promoted oxidation of reduced porphyrins that accumulate in kidney cells because of impaired porphyrin metabolism (Woods et al., 1990a, 1990b; Woods, 1989; Muller and Woods, 1993). These observa-

![Diagram of heme biosynthesis pathways](image)

FIGURE 1: Heme biosynthetic pathways. Steps are catalyzed by: 1) glycine amidinotransferase; 2) 5-aminolevulinic acid synthase; 3) 6-aminolevulinic acid dehydratase; 4) porphobilinogen deaminase; 5) uroporphyrinogen I synthase; 6) uroporphyrinogen III cosynthase; 7) coproporphyrinogen oxidase; 8) protoporphyrinogen oxidase; and 9) heme synthase.
FIGURE 2. HPLC retention profiles of urinary porphyrins (a) from unexposed Fischer rats or (b) from rats exposed to methylmercury chloride (MMD) at 10 ppm for 5 wk. Porphyrin concentrations in urine and serum were determined as described in Materials and Methods. Porphyrin concentrations (pmol/24 h, mean ± SD) of unexposed control rats were (a) 555 ± 81; b)γ; 554 ± 71; and (c) 28 ± 8; pentac. 28 ± 8; and (d) 28 ± 8. Porphyrin concentrations of methylmercury rats were: (a) 162 ± 65; b)γ; 1.7 ± 2.7; and (c) 28 ± 8. Pentac. 330 ± 85; and (d) 15.19 ± 14.29. Values for pentac. and triprotoporphyrin were significantly different from control value (p < .05).

(Images and text not fully visible in the provided frame.)
tions support the suggestion that porphyrin profile measurements may serve as a biomarker of metal exposure and their effects in human subjects (Marks, 1985; Fowler et al., 1987; Woods, 1989).

Dentists and other dental professionals are subjected to chronic low-level exposure to mercury arising from the use of elemental mercury in the preparation of dental amalgam restorative material (Langan et al., 1987; Fang and Molvar, 1992). Practicing dentists have been found to have from 1.5 (Chang et al., 1987) to 2 (Mackert, 1991) times higher blood mercury levels than nondentists. The mean concentrations of urinary mercury among dentists practicing in the Health Screening Program (HSP) conducted during the American Dental Association (ADA) annual meetings from 1975 to 1983 was 14.2 μg/L (range 0–556 μg/L) (Naleway et al., 1985), whereas mean urinary mercury levels among HSP participants from the 1985 and 1986 meetings were 5.8 and 7.6 μg/L, respectively (range 0–115 μg/L) (Naleway et al., 1991). Approximately 10% of HSP participants exhibited urinary mercury concentrations above 20 μg/L. The World Health Organization (1993) has set 25 μg Hg/L of urine as the upper limit of acceptable exposure.

In the present studies we measured urinary porphyrin excretion patterns among dentists participating in the Health Screening Programs conducted during the 1991 and 1992 annual meetings of the ADA, and compared these with mercury levels in urine of the same subjects. The findings are similar to those observed in experimental animal studies and support the potential utility of urinary porphyrin profile measurements in the assessment of occupational mercury exposure among human subjects.

MATERIALS AND METHODS

Study Subjects

Subjects were drawn from male dentists attending the 1991 American Dental Association (ADA) annual meeting in Seattle, Wash., and the 1992 ADA Meeting in Orlando, Fla., and who participated in a voluntary Health Screening Program (HSP) conducted during the meetings. Approximately 1500 subjects participated in the HSP at each meeting. Participants provided spot urine samples for mercury, creatinine, and porphyrin analyses and participated in a number of physiological tests, including a blood chemistry panel, electrocardiogram (EKG) and neurobehavioral assessments. Subjects also completed a questionnaire regarding dietary and health habits, as well as dental practice characteristics.

Collection of Urine Samples

Urine collections were made in sterile 100 ml plastic cups with screw-on lids. Immediately following collection, 0.5 ml aliquots were taken for on-site mercury analysis, while 20-ml aliquots were transferred to fal-
RESULTS

We have previously reported (Buckley et al., 1971) that mercury metabolism in the rat is influenced by dietary factors. In this study, rats were divided into two groups: one receiving a high-mercury diet and the other a low-mercury diet. The levels of mercury in the urine of both groups were measured.

FIG. 1 shows the results of these measurements. The group receiving the high-mercury diet had significantly higher levels of mercury in their urine compared to the group receiving the low-mercury diet.

Urinary creatinine excretion rates were also measured. There was no significant difference between the two groups in terms of creatinine excretion.

Statistical Analysis

The data were analyzed using the t-test for independent samples. The level of significance was set at 0.05.

Conclusions

The results of this study suggest that dietary factors can significantly influence mercury metabolism in rats. Further studies are needed to elucidate the mechanisms behind this effect.

References


after 10 wk of MMH exposure at 10 ppm, demonstrating the time-dependent relationship of urinary porphyrin changes with duration of mercury exposure (Woods et al., 1991). Additionally, a porphyrin with as yet unknown chemical characteristics that elutes approximately midway between free- and five-carboxyl porphyrins on HPLC ("precoproporphyrin") is observed in urine of MMH-treated rats. The concentration of this porphyrin also varies in direct proportion to duration of mercury treatment.

Changes in urinary porphyrin concentrations also vary directly in proportion to the dose of mercury administered. Animals exposed to 5 ppm MMH displayed a urinary porphyrin profile with comparable characteristics to those exposed at 10 ppm, although the magnitude of the changes in four- and five-carboxyl porphyrins, as well as in precoproporphyrin, were approximately half those observed at the higher dose level (Woods et al., 1991).

Urinary porphyrin profiles are strongly correlated with renal mercury content. Regression analyses demonstrated a highly significant correlation between urinary porphyrin concentrations and renal mercury content at each dose level over the course of prolonged MMH treatment in rats (Woods et al., 1991). Correlation coefficients for each regression ranged from .72 to .94, suggesting a close association of each of the three elevated porphyrins with renal mercury content. These observations from animal studies demonstrated that urinary porphyrin profiles are an accurate measure both of the renal mercury content and of the biological effects of mercury in the kidney over a wide range of mercury exposure.

The porphyrin profile from a healthy adult male human subject is shown in Figure 3, and mean urinary porphyrin concentrations from 24-h

![FIGURE 3. HPLC elution profile of urinary porphyrins from a healthy adult male human subject.](image-url)
Table 1: Normal Human Urine Porphyrin Concentrations from 24-Hour and Randomly Collected (spot) Urine Samples

<table>
<thead>
<tr>
<th>Porphyrin</th>
<th>24-h samples (μmol/mol Cr)</th>
<th>Random spot samples (μmol/mol Cr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UROH</td>
<td>3.9 ± 1.5 (2.2–8.4)</td>
<td>2.5 ± 1.0 (1.4–7.6)</td>
</tr>
<tr>
<td>HEU</td>
<td>2.8 ± 1.2 (0.6–5.4)</td>
<td>3.2 ± 1.2 (0.6–8.4)</td>
</tr>
<tr>
<td>PRO</td>
<td>1.0 ± 0.1 (0.3–3.5)</td>
<td>1.5 ± 0.2 (1.0–3.7)</td>
</tr>
<tr>
<td>COPRO</td>
<td>3.1 ± 1.3 (0.4–15.0)</td>
<td>2.1 ± 1.5 (0.4–17.5)</td>
</tr>
<tr>
<td>COPRO A</td>
<td>52.6 ± 11.4 (29.9–97.4)</td>
<td>27.5 ± 16.9 (11.1–51.1)</td>
</tr>
</tbody>
</table>

Note: Porphyrin levels were measured in urine samples collected from healthy adult male subjects presenting to this laboratory during the period 1990–1992 for research purposes. Values represent the mean and standard deviations of 34 individual determinations for 24-h samples and 21 individual determinations for spot samples.

and randomly collected (spot) samples from a group of adult male human subjects are presented in Table 1. Porphyrins with eight, seven, six, five, and four carbonyl groups are readily measured in human urine, although are present at concentrations approximately 25 times less than those measured in rat urine. The ranges in porphyrin concentrations found in 24-h and random urine samples are also presented.

Table 2 presents the mean porphyrin concentrations in spot urine samples of dentists participating in the HSP as a comparison of those having no detectable urinary mercury with those having mercury concentrations ≥20 μg/L.

Table 2: Urinary Porphyrin Concentrations in Dentists

<table>
<thead>
<tr>
<th>Urinary porphyrin concentration (μmol/mol Cr)</th>
<th>Urinary mercury (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No urinary mercury</td>
</tr>
<tr>
<td>PRO</td>
<td>4.76 ± 0.88</td>
</tr>
<tr>
<td>PRO-axanthoporphyrin</td>
<td>7.36 ± 1.37</td>
</tr>
<tr>
<td>PRO-coproporphyrin</td>
<td>22.9 ± 7.50</td>
</tr>
</tbody>
</table>

Note: Porphyrin concentrations were measured in spot urine samples, obtained from dentists whose urine contained no detectable mercury in a 57- and whose urine had total mercury concentrations equal to or greater than 20 μg/L (n = 56). Urinary porphyrin and mercury concentrations were measured as described in Materials and Methods. Values represent mean ± standard deviations of individual determinations.

*Significantly different from urinary porphyrin concentrations in samples, with no detectable mercury (p < .05).
The concentrations of only the three porphyrins that have been observed to be altered during mercury exposure in animal studies (i.e., pentacarboxyl-, prepro- and coproporphyrin) are presented. No changes were observed in the concentrations of eight, seven-, or six-carboxyl porphyrins. Urinary mercury values among exposed ≥20 μg Hg/L subjects ranged from 20.34 to 135.65 μg/L with a mean of 36.42 μg/L. Pentacarboxyl porphyrin was highly variable among unexposed subjects in the present study population, and the mean concentration was somewhat lower than that observed among the reference group (Table 1), although within the normally expected range (0-47). However, the mean concentrations of all 3 porphyrins were significantly elevated among dentists with urinary mercury content ≥20 μg/L, exceeding the unexposed group values by three- to four-fold.

Differences in urinary porphyrin levels among mercury-exposed and unexposed dentists were less marked when adjusted for urinary creatinine concentrations (Table 3). However, the mean concentrations of prepro- and coproporphyrin remained significantly elevated over those found in the unexposed group by 1.5- to 2-fold. When adjusted for creatinine, urinary mercury values ranged from 5.29 to 45.95 ng/mg creatinine, with a mean concentration of 22.90 ng/mg.

**DISCUSSION**

Previous studies from this laboratory have demonstrated that urinary porphyrin changes associated with prolonged mercury exposure meet established criteria by which an effective biomarker of toxicant exposure may be judged, as listed in Table 4. In terms of specificity, the unique change in

**TABLE 3. Urinary Porphyrin Concentrations in Dentists with Adjustment for Urinary Creatinine Levels**

<table>
<thead>
<tr>
<th>Porphyrin</th>
<th>Urinary mercury concentration (ng/mg creatinine)</th>
</tr>
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<tbody>
<tr>
<td>Pentacarboxyl-porphyrin</td>
<td>0.47 ± 0.04</td>
</tr>
<tr>
<td>Prepro-porphyrin</td>
<td>2.14 ± 1.28</td>
</tr>
<tr>
<td>Coproporphyrin</td>
<td>28.36 ± 11.52</td>
</tr>
</tbody>
</table>

Note: Porphyrin concentrations were measured in urine samples obtained from dentists whose urine contained no detectable mercury in < 7% and whose urine had total mercury concentrations equal to or greater than 20 μg/L to < 60 μg/L. Urinary porphyrin, mercury, and creatinine concentrations were measured as described in Materials and Methods. Values represent means ± standard deviations of individual determinations.

*Significantly different from urinary porphyrin concentrations in samples with no
the porphyrin excretion pattern, characterized by elevated levels of five- and four-carboxyl porphyrins, as well as by the expression of the atypical porphyrin, preprocoproporphyrin, is, to our knowledge, unique to mercury exposure. This pattern is different from that which characterizes any of the known forms of inherited porphyrias, and also differs distinctly from those elicited by exposure to other metals (Woods, 1989) or porphyrogenic aromatic hydrocarbons (Mann, 1989). The biochemical etiology of changes in five- and four-carboxyl porphyrins observed during mercury exposure has previously been shown to occur as a result of the formation of Hg(II) or CHHg(I) directed impairment of the specific enzyme pathways enzymes, coproporphyrinogen decarboxylase (Woods et al., 1984) and coproporphyrinogen oxidase (Woods and Southern, 1989), in kidney cells. The first-order binding constant (K > 10^5) for mercaptoacetate formation by Hg is highest for any metal, predisposing to the likely impairment of SH-dependent enzyme functions at concentrations of mercury likely to accumulate in kidney cells during low-level occupational or environmental exposures. The porphyrogenic action of mercury is also attributable to the prooxidant property of Hg^2+ to promote formation of H_2O and other reactive oxidants (Woods et al., 1990a, 1990b; Lund et al., 1991, 1993; Miller and Woods, 1993), which facilitates the conversion of reduced porphyrins (porphyrinogenes) that accumulate in kidney cells to the oxidized, readily excreted porphyrins. Since these properties of mercury may ultimately underlie cell injury and toxicity during prolonged mercury exposure, a mechanistic association between mercury-induced porphyrin and toxicity involving oxidative stress reactions is suggested. The demonstration that porphyrin profiles mechanically and quantitatively reflect a cellular response to mercury-induced oxidative stress in target tissue cells could have substantial significance in the detection, monitoring, and prevention of mercury-related toxicity in human subjects. The mechanisms underlying the expression of porphyrinogen expression as a unique response to mercury exposure remain to be determined.

The criterion of an effective biomarker that it permit an assessment of past as well as current toxicant exposure is also met by the porphyrogenic response to mercury treatment. As demonstrated in previous studies (Woods et al., 1991), urinary concentrations of five- and four-carboxyl porphyrins, as well as of preprocoproporphyrin, remain substantially elevated for up to 25 wk following cessation of mercury treatment, and decline at a rate consis-
tent with the clearance of organic mercury from the kidney (t 1/2 70 d). These observations are in contrast to those pertaining to Hg levels in the urine, which decline rapidly following cessation of mercury exposure, despite potentially high residual tissue mercury levels.

Urinary porphyrin profile changes were less well correlated with urinary mercury levels when adjusted for urinary creatinine concentrations (Table 3). In the present study creatinine concentrations varied widely among individual spot urine samples, ranging from 25.0 to 410.4 mg/dL. Vestergaard and Leverett (1958) have previously reported substantial variability in creatinine excretion among individual men and women over both 24-hr and shorter urine collection periods, and have concluded that creatinine should not be used to normalize or compare rates of metabolic excretion unless a preliminary assessment of individual variability in creatinine excretion is performed. The present studies, in which a substantially stronger correlation between creatinine-unadjusted porphyrin and mercury concentrations in spot urine samples is obtained, support this conclusion.

Some potential applications of the urinary porphyrin profile method to the assessment of mercury exposure in human subjects are listed in Table 5. Since the change in the urinary porphyrin excretion pattern is unique to mercury exposure and occurs in subtoxic as well as potentially toxic mercury levels, the urinary porphyrin profile method could be employed in the clinical setting to indicate whether a biological response to mercury has occurred, or to monitor the effectiveness of treatment regimens for facilitating mercury clearance. The porphyrin profile method could be further used for population monitoring of current or past mercury exposure at hazardous waste sites or in the workplace. Finally, the changes in urinary porphyrin excretion patterns might be used to identify individuals or subgroups who might be uniquely susceptible to mercury toxicity or to verify biological responses to mercury predicted by risk assessment models. A positive correlation between changes in urinary porphyrin profiles and neurobehavioral test parameters among dentists with urinary mercury ≥20 μg/L observed in preliminary assessment of the present study population (Fehervari et al., 1991) supports this view.

In conclusion, the present studies describe characteristic changes in the

| TABLE 5: Potential Applications of the Porphyrin Profile Method as a Biomarker of Human Mercury Exposure |
| 1. Establish a biological response to occupational or environmental mercury exposure. |
| 2. Monitor the effectiveness of treatment regimens for mercury poisoning and toxicity. |
| 3. Population monitoring of mercury exposure at hazardous waste sites or from occupational sources. |
| 4. Correlate past mercury exposure in clinical and epidemiologic studies and identify susceptible subgroups. |
| 5. Verify biological responses predicted by risk assessment models. |
The urinary porphyrin excretion pattern among dentists with occupational mercury exposure and demonstrate that these changes are comparable to those associated with prolonged mercury exposure in animal studies. These findings suggest the predictive and diagnostic potential of urinary porphyrin profile measurements as a specific biomarker of mercury exposure and potential effects in human populations.

REFERENCES


