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Studies on Porphyrin Metabolism in the Kidney

Effects of Trace Metals and Glutathione on Renal Uroporphyrinogen Decarboxylase

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SUMMARY

Uroporphyrinogen (urogen) decarboxylase catalyzes the decarboxylation of 8- to 4-carboxyl porphyrinogen during heme biosynthesis in mammalian tissues. The specific activity of renal urogen decarboxylase was shown to be approximately one-third that of the hepatic enzyme and to be readily inactivated by $HgCl_2$ following acute treatment or at concentrations as low as 50 μM *in vitro*. $HgCl_2$ differentially inhibited the decarboxylation of 8- to 7- and 7- to lesser-carboxylated porphyrinogens in the kidney, suggesting that at least a two-stage process is involved in the catalytic action of the renal enzyme. In contrast, neither lead nor iron compounds inhibited renal urogen decarboxylase in concentrations as high as 1 mM in the reaction mixture. GSH increased renal but not hepatic urogen decarboxylase activity by over 4-fold *in vitro* when measured as total porphyrinogen products produced, and preferentially accelerated the decarboxylation of 7- to 4-carboxyl porphyrinogen. GSH also protected the renal enzyme from $HgCl_2$ inhibition. These findings suggest that renal urogen decarboxylase catalyzes porphyrin decarboxylation significantly less rapidly than the hepatic enzyme, is readily inactivated by mercuric chloride, and may be GSH-dependent with respect to achieving optimal catalytic activity. These observations may be useful in characterizing the contribution of the kidney to the clinical manifestations of the inherited porphyrias and environmentally induced disorders of porphyrin metabolism.

INTRODUCTION

Previous studies from these laboratories (1) have demonstrated that prolonged exposure of rats to mercury as MMH[2] elicits a significant increase in the urinary concentrations of both uroporphyrin and coproporphyrin, the former rising, for example, from 52 to 649 pmol/ml (12.5-fold increase) over a 6-week course of exposure to 10 ppm MMH in the drinking water. This porphyrinogenic change occurs progressively, with the initial manifestation of porphyrinuria observed between 1 and 2 weeks following commencement of exposure. Biochemically, the development of MMH-induced porphyrinuria was found to be characterized by changes in specific enzymes of the renal heme biosynthetic pathway. These changes include an initial decrease, followed by a subsequent increase (to over 2.5 times control levels), in the activity of renal δ-aminolaevulinate synthase (EC 2.3.1.37), the rate-limiting enzyme in this process, and a gradual decrease in the activities of uroporphyrinogen (urogen) I synthetase and ferrochelatase to 60–70% of control values during the 6-week course of exposure. More recently, we have observed that the activity of renal urogen decarboxylase (porphyrin carboxyl-lyase, EC 4.1.1.37), the enzyme which catalyzes the sequential decarboxylation of 8-carboxyl to 4-carboxyl porphyrinogen (Fig. 1), also undergoes a progressive decline to

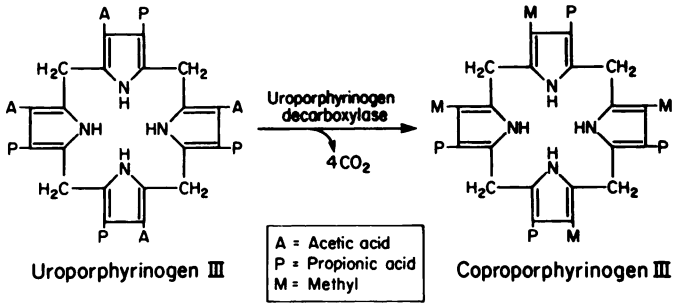

FIG. 1. *Decarboxylation of uroporphyrinogen (8-carboxyl porphyrinogen) to coproporphyrinogen (4-carboxyl porphyrinogen) by urogen decarboxylase*

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[2] The abbreviations used are: MMH, methyl mercury hydroxide; HPLC, high-performance liquid chromatography; PCT, porphyria cutanea tarda.

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approximately 50% of control activity during methyl mercury exposure, concomitant with the appearance of increased urinary uroporphyrin levels. In contrast, no significant changes are observed in any heme biosynthetic pathway enzymes measured in liver during the course of mercury treatment.

These observations support the concept that the kidney, a principal target organ for mercury and various other metals of toxicologic importance, may be the likely source of the increased urinary porphyrin concentrations observed during mercury exposure. Moreover, they suggest that mercury inhibition of renal urogen decarboxylase may contribute to this effect.

The present studies were undertaken to evaluate more fully this prospect through an investigation of the effects of mercury and other metals on renal urogen decarboxylase. Mercury, as mercuric chloride, was employed to study the mechanisms of porphyrin decarboxylation by renal urogen decarboxylase *in vitro* and to evaluate the effects of acute treatment with mercury on the renal enzyme *in vivo*. The effects of iron and lead compounds (which do not elicit uroporphyrinuria) on renal urogen decarboxylase were also evaluated. Finally, the effects of glutathione on renal urogen decarboxylase *in vitro* were investigated.

EXPERIMENTAL PROCEDURES

Materials. Porphyrin standards were acquired from Porphyrin Products (Logan, Utah). Male Sprague-Dawley rats (150–200 g) were supplied by Tyler Laboratories, Inc. (Bellevue, Wash.) Metal salts, glutathione, and reagents utilized in these studies were obtained from standard commercial sources.

Treatment of animals. In studies to assess the effects of mercury on urogen decarboxylase *in vivo*, rats were placed in individual metabolism cages and treated 18-hr prior to sacrifice by i.p. injection of mercuric chloride dissolved in distilled water. Controls received water injection only. At least three animals were utilized in each dose group. Urine samples were collected over the 18-hr treatment period for porphyrin analysis.

Preparation of tissues. All animals were killed by decapitation. Liver and kidneys were rapidly excised, washed, perfused with buffered saline, minced, and homogenized in 9 volumes of 0.25 M sucrose containing 0.05 M Tris buffer (pH 7.5), using a Potter-Elvehjem homogenizer fitted with a Teflon pestle. Tissues from animals in each dose group were pooled for each experimental point. All assays were performed in duplicate on fresh tissues immediately following sacrifice. Subcellular fractions were prepared by differential centrifugation, as previously described (2, 3).

Uroporphyrinogen decarboxylase assay. Uroporphyrinogen decarboxylase activity in mitochondria-free extracts of rat liver or kidney was assayed using the following modification of our previously described method (4). Assays were performed in 25-ml Erlenmeyer flasks wrapped tightly in foil to exclude light with slow shaking in a metabolic incubator set at 37°. Flasks were kept under a constant stream of nitrogen to prevent oxidation of porphyrinogens during the assay. The assay was conducted in two steps. In the first step, uroporphyrinogen I was enzymatically synthesized in reaction mixtures containing 2 ml of 25,000 × *g* rat spleen supernatant solution, previously heated at 65° for 15 min to destroy splenic decarboxylase, and 1 ml of 50 mM potassium phosphate buffer (pH 7.65) containing 160 nmol of porphobilinogen. (Inasmuch as preheating of the splenic supernatant containing urogen I synthetase destroys urogen III cosynthetase as well as urogen decarboxylase, only urogen I is formed in this step. In preliminary studies, urogen decarboxylase from liver or kidney was equally active when either the I or III isomer of urogen was used as substrate under the present assay conditions.) Immediately following the 60-min substrate generation step, during which urogen I is generated with a final concentration of 3–5 μM, the pH of the assay medium was lowered to 6.8 by addition of 0.125 ml of 0.025 M phosphoric acid into the reaction mixture. One milliliter of tissue fraction containing urogen decarboxylase (unheated 25,000 × *g* supernatant from liver or kidney) was then added to each flask, and the reaction was allowed to proceed under nitrogen at 37° with shaking for 1 hr more. Assays were terminated by addition of 2 volumes of ice-cold 95% methanol to the reaction flask, and porphyrinogens were oxidized to the corresponding porphyrins by exposure to room air and fluorescent light for 2–3 hr. Mixtures were then centrifuged at 10,000 × *g* for 10 min to remove protein. Porphyrins, which remain in the supernatant solution, were separated and quantitated using reversed-phase HPLC. The chromatographic system used was a Hewlett-Packard HPLC model 1084B equipped with two programmable solvent-metering pumps, a gradient programmer/recorder, automatic variable volume sample injector, and an Alltech Econosphere C18 bonded column (spherical 5-μm, 4.6 mm inner diameter × 25 cm) from Alltech Associates Inc. (Deerfield, Ill.). A C18 bonded-guard column (spherical 5-μm, 4.6 mm inner diameter × 3 cm) from Brownless Labs (Santa Clara, Calif.) was located between the pump and the analytical column. This system was interfaced with a Schoeffel fluorometer Model FS970. The continuously variable excitation wavelength was set at 400 nm, and a secondary filter with a cutoff of 470 nm was used.

The analytical procedure for porphyrin quantitation in both reaction mixture and urine samples utilized a modification of the method of Ford *et al.* (5) for liquid chromatographic analysis of urinary porphyrins. Reservoir A contained 0.05 M phosphate buffer (pH 3.5), and Reservoir B contained methanol. A flow rate of 1.0 ml/min was used for most assays. A programmed gradient was used, with an initial 10% methanol (B) concentration at time 0, 50% B at 3 min, 70% B at 10 min, and 99% B from 15 to 23 min. The gradient was returned to 10% B at 25 min, and the column was allowed to re-equilibrate at 10% B for 5 min before the program was terminated at 30 min. The columns and reservoirs were maintained at 40°. The peak integration system was calibrated by injecting 50 μl of the combined porphyrin standard onto the column, with each of the six porphyrin peaks representing 50 pmol. This system provides for the separation and quanitation of 8-, 7-, 6-, 5-, 4-, and 2-carboxyl porphyrins with a sensitivity to detect 0.5 pmol. Complete resolution of all porphyrin isomers by HPLC is achieved within 20 min following sample injection.

Other assays. Glutathione concentrations in reaction mixtures were measured by the method of Jollow *et al.* (6). Tissue mercury concentrations were assayed by the cold vapor method (7), using a Perkin-Elmer Model 103 atomic absorption spectrophotometer equipped with a 10-cm quartz cell. Protein concentrations were measured by the method of Lowry *et al.* (8), using bovine serum albumin (Fraction V) as a standard.

Studies in vitro with metal salts and glutathione. Studies to ascertain the effect of trace metals and glutathione on urogen decarboxylase activity *in vitro* were performed by adding appropriate concentrations of aqueous solution (0.01 ml) of the test substance directly to the incubation mixture at the same time the enzyme source was added. Control reaction mixtures received distilled water only. Final concentrations of test substances are given in the table and figure legends.

Statistical analyses. Analysis of significance of differences between groups or experimental points was performed by means of Student's *t*-test. The level of significance was chosen as $p < 0.05$.

RESULTS

Studies were first conducted to measure the specific activities of urogen decarboxylase in both kidney and liver and to compare the production of carboxylated porphyrinogens by each tissue. These measurements, presented in Table 1, show that the specific activity of urogen decarboxylase in the kidney is approximately one-

TABLE 1

Specific activities and patterns of porphyrin production by renal and hepatic urogen decarboxylase

Assays were conducted as described under Experimental Procedures. Values in this and other tables and figures represent the means ± standard error of the mean of at least four separate determinations.

Porphyrinogen	Urogen decarboxylase activity	
	Kidney	Liver
	(pmol porphyrinogen/mg protein) hr^{-1}	
7-COOH	59.6 ± 17.2	26.2 ± 5.3
6-COOH	9.9 ± 4.3	3.6 ± 0.7
5-COOH	25.9 ± 3.1	39.1 ± 8.8
4-COOH	97.1 ± 9.0	296.5 ± 17.8

TABLE 2

Effects of acute treatment with $HgCl_2$ on renal and hepatic urogen decarboxylase activity

Male rats (three per dose group) were treated 18 hr prior to sacrifice by i.p. injection of $HgCl_2$ in aqueous solution at a dose of 0.75 or 1.5 mg/kg. Tissues were prepared and analyses performed as described under Experimental Procedures.

$HgCl_2$	Urogen decarboxylase	Tissue Hg
mg/kg	*(pmol coprogen/mg) hr^{-1}*	*μg/g*
Kidney		
0	96.3 ± 7	ND[a]
0.75	52.0 ± 2[b]	10.9 ± 0.2
1.5	37.5 ± 6[b]	15.3 ± 0.1
Liver		
0	287.5 ± 17	ND
0.75	244.4 ± 35	0.41 ± 0.03
1.5	299.0 ± 11	0.98 ± 0.02

[a] ND, Not detectable.
[b] $p < 0.05$.

third that of the liver when expressed in terms of 4-carboxyl porphyrinogen produced under identical assay conditions. Interestingly, substantially greater proportions of porphyrinogen intermediates with 7-, 6-, and 5-carboxyl groups accumulate in the renal enzyme assay mixture than is observed in the case of the liver enzyme. Decarboxylase activity with enzyme preparations from both tissues was approximately linear over at least a 2-hr time course with respect to production of all carboxylated porphyrinogens.

The acute effects of mercury treatment on the activity of urogen decarboxylase in kidney and liver *in vivo* were assessed by measuring enzyme activity in both tissues from animals treated with $HgCl_2$ (0.75 or 1.5 mg/kg) 18 hr prior to sacrifice. Results of these experiments, presented in Table 2, indicate that renal urogen decarboxylase was decreased to 54% and 39% of control values in animals treated with $HgCl_2$, 0.75 and 1.5 mg/kg, respectively. In contrast, hepatic urogen decarboxylase was not significantly inhibited at either dose level. Tissue mercury concentrations from control and treated animals are also presented in Table 2. As anticipated, renal mercury concentrations substantially exceeded those of the liver. Urinary uroporphyrin concentrations increased from 99 pmol/ml in controls to 138 pmol/ml at the highest dose level. However, this change was not statistically significant ($p < 0.05$). Notable, but statistically insignificant, increases in urinary levels of coproporphyrin were also seen.

Studies were next conducted to evaluate the effects of $HgCl_2$ on renal urogen decarboxylase *in vitro*. As shown in Fig. 2, the production of 6-, 5-, and 4-carboxyl porphyrinogen was inhibited at mercury concentrations greater than 10^{-5} M (10 μM), and the production of 4-carboxyl porphyrinogen was effectively eliminated at 10^{-4} M (100 μM). In contrast, the production of 4-carboxyl porphyrinogen by hepatic urogen decarboxylase was inhibited to the same extent only at mercury concentrations greater than 10^{-3} M under similar assay conditions (data not shown). Interestingly, the decarboxylation of 8- to 7-carboxyl porphyrinogen by renal urogen decarboxylase was not substantially inhibited until the mercury concentration equaled or exceeded 10^{-4} M (100 μM) in the reaction mixture (Fig. 2). This observation suggests that a two-stage enzymatic process is responsible for porphyrin decarboxylation in which mercury differentially inhibits the conversion of uroporphyrinogen to coproporphyrinogen at 8- to 7-carboxyl and 7- to lesser-carboxyl porphyrinogens, respectively.

The kidney is also a principal target organ for lead (9). In contrast to mercury, however, lead neither produces uroporphyrinuria during prolonged exposure nor inhibits renal urogen decarboxylase *in vitro*. When added to enzyme assay medium in final concentrations of 0.01, 0.1, or 1 mM, lead acetate was ineffective in altering the decarboxylation of 8- to 4-carboxyl porphyrinogen or the production of porphyrinogen intermediates in renal decarboxylase reaction mixtures. Similar results have been observed with respect to effects of lead on hepatic urogen decarboxylase (10).

The GSH concentration of the kidney is approximately one-half that of the liver (3–5 mM versus 8–10 mM, respectively), and is susceptible to alteration by various metals (11, 12). It was therefore of interest to determine whether the activity of renal urogen decarboxylase *in vitro* varies with the concentration of GSH in the reaction mixture. As indicated in Table 3, when GSH was added in various concentrations to reaction mixtures of the renal enzyme to produce the concentrations indi-

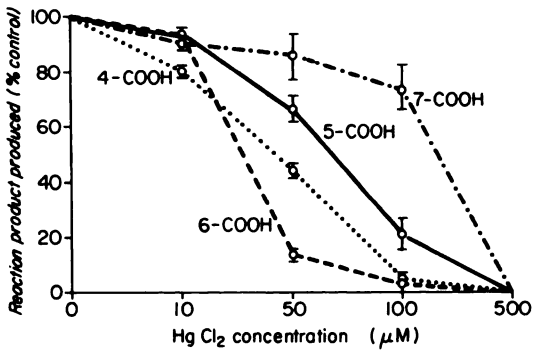

FIG. 2. *Effects of $HgCl_2$ on renal urogen decarboxylase in vitro*

$HgCl_2$ in aqueous solution was injected into enzyme reaction mixtures to achieve the final concentration indicated at the same time urogen decarboxylase fractions were added. Assays were conducted as described under Experimental Procedures.

TABLE 3

Effects of GSH on renal urogen decarboxylase activity in vitro

GSH in aqueous solution was added to each enzyme assay flask to achieve the concentration indicated at the same time urogen decarboxylase fractions were added.

GSH	Enzyme activity as isomer formed[a]			
	7-COOH	6-COOH	5-COOH	4-COOH
mM	*% control*			
0	100	100	100	100
1	168	136	132	135
2.5	186	227	203	199
5	176	279	259	246
10	186	469	381	320
20	102	221	244	308

[a] Enzyme activity in control flasks, expressed in terms of porphyrinogen isomer formed (picomoles per milligram of protein per hour), was as follows: 4-COOH, 83.1; 5-COOH, 27.0; 6-COOH, 7.4; 7-COOH, 52.5.

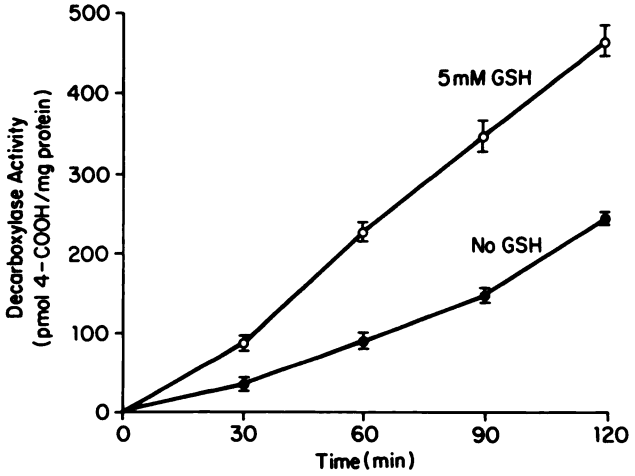

FIG. 3. *Effects of GSH on renal urogen decarboxylase in vitro*

GSH in aqueous solution was added to enzyme reaction mixtures to achieve the concentrations indicated at the same time urogen decarboxylase fractions were added. Assays were conducted as described under Experimental Procedures.

cated, urogen decarboxylase was markedly increased. A final GSH concentration of 10 mM was most effective in increasing renal urogen decarboxylase activity when measured in terms of total porphyrinogen isomers produced. In contrast, hepatic urogen decarboxylase was unaltered at any GSH concentration tested.

GSH appeared to act principally by direct stimulation of renal enzyme activity, rather than by stabilizing or protecting porphyrin products from degradation in the reaction mixture. As shown in Fig. 3, renal decarboxylase activity, measured as the production of 4-carboxyl porphyrinogen, increased linearly over a 2-hr period, in both the presence and absence of GSH. If GSH were acting merely by protecting products of the enzyme reaction from degradation, it might be anticipated that the lower curve, representing enzyme activity in the absence of added GSH, would level out or even decrease over time as reaction products disappeared. In contrast, a steady increase in enzyme activity was observed. Since this increase occurred more rapidly in the presence of added GSH, we consider the effect of GSH to reflect direct stimulation of enzyme activity, rather than an effect on the reaction products.

GSH also altered the proportion of porphyrinogen intermediates produced during the enzyme reaction. As seen in Fig. 4, 7-carboxyl porphyrinogen constitutes about 30% of total reaction products formed in the absence of added GSH, whereas 4-carboxyl porphyrinogen constitutes approximately 50% of products formed. As the concentration of GSH in the reaction mixture increased, the proportion of 7-carboxyl porphyrinogen remaining in the reaction mixture decreased substantially, whereas the proportion of 4-carboxyl porphyrinogen increased. This pattern approaches that produced by the liver enzyme, where 4-carboxyl porphyrinogen constitutes approximately 80% of total reaction products. This observation suggests that decarboxylation of 7- to 4-carboxyl porphyrinogen by the renal enzyme is stimulated by GSH and proceeds at a relatively slow pace at lower concentrations or in its absence.

GSH was also highly effective in preventing inhibition of renal urogen decarboxylase by $HgCl_2$ *in vitro*. As shown in Table 4, the production of 6- through 4-carboxyl porphyrinogens was essentially eliminated at mercury concentrations exceeding 5×10^{-5} M in the reaction mixture. This effect was largely prevented in the presence of 5 mM GSH. Interestingly, this effect appears to involve more than mere complex formation between mercury and GSH *in vitro*, since stimulation of over-all enzyme activity by GSH, as well as a shifting of the reaction toward more complete production of 4-carboxyl porphyrinogen, occurs even in the presence of $HgCl_2$.

Finally, iron has been reported to inhibit urogen decarboxylase in pig liver extracts *in vitro* (13), and iron repletion has been implicated in the exacerbation of PCT, a condition characterized by a deficiency of urogen decarboxylase in various tissues. However, previous studies from these laboratories (10) have failed to demonstrate an effect of iron *in vitro* on rat liver urogen decarboxylase. Inasmuch as the present findings implicate the kidney in the clinical presentation of metal-induced porphyrinopathies, it was of interest to assess the effects of iron compounds on urogen decarboxylase activity in that organ. In these experiments, ferrous

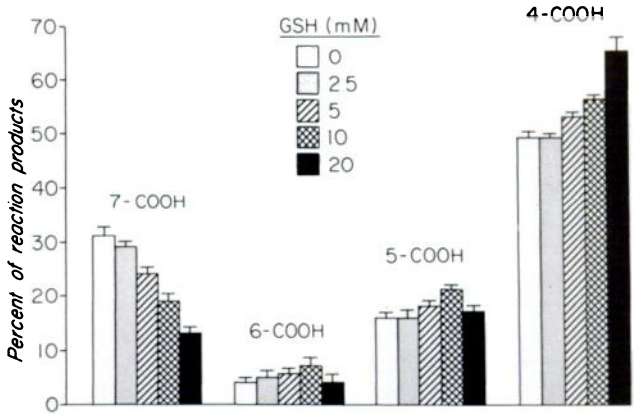

FIG. 4. *Percentage of porphyrinogen isomers formed by renal urogen decarboxylase at various concentrations of GSH in the reaction mixture*

Assays were conducted as described under Experimental Procedures.

TABLE 4

Effects of glutathione (5 mM) on mercury inhibition of renal urogen decarboxylase

GSH and $HgCl_2$ were added to enzyme reaction mixtures to achieve the concentrations indicated.

Condition	Enzyme activity as isomer formed[a]			
	7-COOH	6-COOH	5-COOH	4-COOH
	% control			
No GSH				
Control	100	100	100	100
$HgCl_2$				
10^{-5} M	95	78	82	76
5×10^{-5} M	92	0	38	31
10^{-4} M	81	0	7	6
GSH (5 mM)				
Control	100	100	100	100
$HgCl_2$				
10^{-5} M	100	100	99	100
5×10^{-5} M	91	111	90	85
10^{-4} M	78	99	56	69

[a] Control enzyme activity, expressed in terms of porphyrinogen isomer formed (picomoles per milligram of protein per hour) with no GSH added, was as follows: 4-COOH, 97.1; 5-COOH, 28.4; 6-COOH, 7.4; 7-COOH, 49.9. Control activity in the presence of 5 mM GSH was as follows: 4-COOH, 238.2; 5-COOH, 81.0; 6-COOH, 24.8; 7-COOH, 95.0.

ammonium sulfate or ferric sulfate were dissolved in water and added to enzyme reaction mixtures at the same time urogen decarboxylase was added to achieve concentrations of 0.1, 0.5, or 1 mM, respectively. Neither ferrous nor ferric iron significantly altered renal urogen decarboxylase activity *in vitro* at any concentration tested.

DISCUSSION

Numerous studies have appeared in recent years describing the properties of urogen decarboxylase in various tissues (14–18). This report is the first to our knowledge in which urogen decarboxylase in mammalian kidney has been investigated. The findings presented in this paper suggest that renal urogen decarboxylase catalyzes the decarboxylation of 8- to 4-carboxyl porphyrinogen with approximately one-third the specific activity of the liver enzyme when measured under identical assay conditions, and is significantly more readily inactivated by mercuric chloride *in vitro*.

Although the observations made in this study do not directly explain the etiology of uroporphyrinuria which occurs during prolonged mercury exposure, the data presented in Table 2 demonstrate that renal decarboxylase is substantially more readily inhibited than the hepatic enzyme following acute mercury treatment. The preferential accumulation of mercury by the kidney as well as the endogenously lower GSH concentration in that organ may contribute to this result. Although urinary uroporphyrin levels were not significantly elevated in mercury-treated rats in the acute studies conducted here, it has been previously observed (1) that uroporphyrinuria does not develop until at least 1 week of continuous exposure to mercury has ensued. Similar findings have been reported with respect to increased urinary uroporphyrin levels associated with both hexachlorobenzene- and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced porphyria (19, 20). This observation suggests that a sustained reduction in urogen decarboxylase activity may be required before tissue porphyrin levels become sufficiently increased for overt uroporphyrinuria to be elicited. Moreover, a sustained increase in renal ALA synthetase activity, as is observed during prolonged mercury exposure, may be required for porphyrinuria to develop. The notable but not statistically significant increases in urinary porphyrin levels seen in rats acutely treated with $HgCl_2$ might therefore reflect general damage to the renal proximal tubule cell membrane induced by acute inorganic mercury treatment. These effects are considered to occur independently of the porphyrinogenic response elicited during more prolonged exposure to lower mercury levels. Further studies are required to determine the temporal sequence of the events which occur in relation to prolonged metal exposure leading to overt porphyrinuria.

The accumulation of substantial amounts of 7- and lesser-carboxyl porphyrinogen in the reaction mixture during the renal urogen decarboxylase assay suggests that the decarboxylation of the first acetic acid side chain occurs at a considerably faster rate than that of the subsequent three porphyrinogens. This observation suggests that at least two steps are involved in the catalytic action of the renal enzyme, a concept supported by the differential inhibitory effects of mercury on this process (Fig. 2). This finding is different from that reported for the mouse spleen decarboxylase (15), for which stoichiometric conversion of 8- to 4-carboxyl porphyrinogen occurs without accumulation of intermediates with 7-, 6-, or 5-carboxyl groups. In contrast, accumulation of partially decarboxylated porphyrins, similar to that found in the present study with the kidney enzyme, has been observed in reaction mixtures containing enzymes from rabbit (21), chicken (22), and human (23) erythrocytes. Intermediate porphyrinogens have also been reported to accumulate in *in vitro* assays of rat liver decarboxylase (24), although to a considerably lesser extent than with kidney, similar to results observed in these studies (Table 1). While the reasons for these differences remain unknown, it seems unlikely that enzymes from different tissues differ with respect to the number of catalytic sites per mole of enzyme or in other physiochemical properties. A more likely possibility, suggested by the observation of accelerated renal decarboxylase activity in the presence of increased GSH concentrations (Fig. 3), is that tissue-specific factors, including endogenous GSH levels, underlie these differences, possibly by affecting the mode of binding of partially decarboxylated porphyrinogens to the enzyme in different tissues. Alternatively, GSH may act to stabilize thiol groups of the renal enzyme, which, because of slight differences in primary or secondary enzyme structure, could be more susceptible to oxidation or inactivation than occurs with the liver enzyme. A similar mechanism has been suggested to explain differences observed between the properties of urogen decarboxylase from liver and erythrocytes (17). Further evaluation of this issue is required to determine the apparent requirement of the renal enzyme for GSH

in vitro and the relationship of lower endogenous renal GSH levels to porphyrin decarboxylation *in vivo*.

It is of interest to note that neither lead nor iron significantly altered renal urogen decarboxylase activity *in vitro*. Lead, although preferentially accumulated by the kidney, neither inhibited over-all renal decarboxylase activity nor altered the patterns of porphyrin accumulation in enzyme reaction mixtures from that tissue. Similar observations have been made with respect to the enzyme from liver (10) and erythrocytes (14), and are consistent with the failure of lead to induce uroporphyrinuria during prolonged exposure.

The failure of either ferrous or ferric iron to alter renal urogen decarboxylase *in vitro* is consistent with previous observations made with respect to both hepatic and erythrocyte enzyme preparations (2, 14, 17), although more recent studies on purified decarboxylase from bovine liver (18) demonstrate inhibition by Fe^{2+} (0.5–10 mM) at high substrate concentrations (urogen I = 30 μM). These observations suggest that the enzyme-to-substrate ratio may in some way affect the susceptibility of the enzyme to direct inhibition by iron *in vivo*, a prospect of considerable relevance to the clinical presentation of PCT, which is characterized by a deficiency of decarboxylase in various tissues (25, 26). Thus, although iron compounds did not alter renal decarboxylase activity *in vitro* in the present studies, the question of a potential effect of iron in the kidney *in vivo* remains highly interesting in light of the relatively low decarboxylase activity and low GSH levels measured in that organ. Evidence that the kidney serves as the principal source of urinary uroporphyrin during PCT attacks has been provided by several investigators (27, 28).

In conclusion, the present studies demonstrate that renal urogen decarboxylase catalyzes the metabolism of 8- to 4-carboxyl porphyrinogen with one-third the specific activity of the liver enzyme and is significantly more readily inhibited by mercuric chloride both *in vivo* and *in vitro*. These effects may reflect the preferential accumulation of mercury by the kidney, the potency of mercury with respect to sulfhydryl binding capacity, and the lower endogenous concentration of GSH in that organ. These findings suggest, also, that renal urogen decarboxylase may differ from the hepatic enzyme with respect to both catalytic properties and requirements for endogenous factors such as glutathione. Further characterization of these differences may be useful in understanding the renal contribution to the clinical manifestations of the inherited porphyrias and to environmentally induced disorders of porphyrin metabolism.

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