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Stimulation of Porphyrinogen Oxidation by Mercuric Ion. II. Promotion of Oxidation from the Interaction of Mercuric Ion, Glutathione, and Mitochondria-Generated Hydrogen Peroxide

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SUMMARY

Previous studies have shown that mercuric ion (Hg^{2+}) reacts with GSH and H_2O_2 *in vitro* to form reactive species capable of oxidizing reduced porphyrins (porphyrinogens). This effect is independent of the presence of iron in the reaction mixture. The present studies demonstrate that Hg^{2+} and GSH can interact in biologically relevant concentrations with H_2O_2 generated by the mitochondrial electron transport chain to promote oxidation of porphyrinogens via comparable mechanisms. Mitochondria from rat liver or kidney readily oxidize uroporphyrinogen when H_2O_2 production is stimulated by the presence of a respiratory chain substrate (NADH, succinate) and an electron transport inhibitor (e.g., NaN_3). Porphyrinogen oxidation by mitochondria is significantly increased by the addition of Hg^{2+} and GSH, in a molar ratio of approximately 3:5, to the reaction mixture. Stimulation of porphyrinogen oxidation in the presence of Hg^{2+} plus GSH increases proportionately with the concentration of mitochondrial protein in the reaction cuvettes but decreases with diminished H_2O_2 production by the electron transport chain. Studies with reactive oxidant scavengers suggest the participation of reactive oxygen species in Hg plus GSH stimulation of mitochondrial porphyrinogen oxidation. These findings support the hypothesis that Hg^{2+} and GSH interact with mitochondria-generated H_2O_2 to promote propagation of reactive oxidants or other free radical species, which, in turn, oxidize reduced porphyrins proximal to mitochondrial membranes. These results suggest a mechanistic explanation for the porphyrinogenic action of mercury compounds, as well as for the oxidative damage to target cell constituents associated with mercury exposure.

In the accompanying paper (1), we described the stimulation of oxidation of reduced porphyrins (porphyrinogens) *in vitro* by the interaction of mercuric ions (Hg^{2+}) and thiols, particularly GSH, in the presence of H_2O_2. Evidence is presented to support the view that this action may be mediated by free radical species derived from the interaction of Hg^{2+} and thiols with H_2O_2. Evidence is also presented that the stimulation of H_2O_2-dependent porphyrinogen oxidation by Hg plus GSH is independent of that mediated by iron chelates.

In the present studies, we investigated the possibility that Hg^{2+} and GSH can interact with H_2O_2 generated endogenously by the mitochondrial electron transport chain to promote oxidation of reduced porphyrins. Mitochondria are a principal source of H_2O_2 *in vivo* (2–5), as well as the primary subcellular target of mercuric ions (6). The mitochondria are also a principal locus of porphyrinogen decarboxylation during heme biosynthesis. Hence, events facilitating the production of free radicals or other oxidizing species proximal to mitochondrial membranes could be reasonably expected to result in oxidation of porphyrinogens, leading to excess porphyrin excretion, as is observed during prolonged exposure to mercury and other metals (7–9). The oxidation of higher carboxylated porphyrinogens by reactive oxidizing species generated by tissue microsomes has been postulated to contribute to the etiology of hepatic uroporphyria (10, 11).

The present studies demonstrate that Hg^{2+} and GSH readily react under physiological conditions with H_2O_2 generated by the mitochondrial electron transport chain from rat liver and kidney to promote porphyrinogen oxidation, similar to results observed in studies using exogenous H_2O_2 (1). These findings suggest a plausible mechanism by which Hg^{2+} facilitates the oxidation of reduced porphyrins in target tissue cells, leading to excess porphyrin excretion observed during prolonged mercury exposure. These findings also suggest a possible mechanistic explanation for the oxidative damage to tissue mitochondria and other subcellular constituents that characterizes mercury toxicity (6, 12–14).

Experimental Procedures

Materials. Male Sprague-Dawley rats (175–200 g) were obtained from Tyler Laboratories, Inc. (Bellevue, WA) and were maintained in

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ABBREVIATIONS: SOD, superoxide dismutase; CAT, catalase; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

individual wire-bottom cages, with free access to food (Lab Chow) and water, until used. NADH, NaN_3, sodium succinate, EDTA, GSH, SOD, and CAT were purchased from Sigma Chemical Co. (St. Louis, MO). Porphyrins (free carboxylic acids of the I isomeric configuration) were purchased from Porphyrin Products (Logan, UT). All other chemicals and reagents were obtained from standard commercial sources and were of the highest available purity. All solutions were prepared with metal-free deionized water.

Preparation of iron chelates and porphyrinogens. Iron-EDTA chelates were prepared by mixing a 40 mM solution of $FeCl_3$ with an equal volume of 41 mM EDTA, and appropriate dilutions of this solution were made with deionized water. Uroporphyrinogen or coproporphyrinogen was prepared by reduction of the corresponding porphyrin with freshly ground 3% sodium amalgam under N_2 and was neutralized to pH 7.5 before use.

Preparation of mitochondria. Mitochondria were prepared from rat liver or kidney cortex essentially as described by Johnson and Lardy (15), using 0.25 M sucrose, 0.05 M Tris buffer, pH 7.5. Pellets were washed twice in the same solution and were suspended 1:1 with respect to original tissue weight in 140 mM KCl. Suspensions were frozen at −80° and thawed before use. In preliminary studies, it was determined that freeze-thawing enhanced the permeability of mitochondria to respiratory chain substrates but did not impair oxidative capability. Protein concentrations were determined by the method of Smith *et al.* (16).

Oxidation of porphyrinogens. Oxidation of porphyrinogens was monitored spectrofluorometrically using a Shimadzu model RF-5000U recording spectrofluorometer, as previously described (1, 17). Reaction mixtures contained 0.1 M HEPES or 0.1 M potassium phosphate (K_2HPO_4/KH_2PO_4) buffer, pH 7.45, 600–800 μg of mitochondrial protein, 0.4 mM NADH or 5 mM succinate, and 1 mM NaN_3, in a total volume of 3 ml. Hg^{2+}, GSH, Fe^{3+}-EDTA, and other components were added as indicated in the tables and figures. Porphyrinogen was added to the sample cuvette after a 5-min incubation in a final concentration of 1 μM. Rates presented represent the maximum rates observed under the conditions described.

Statistical analyses. Statistical differences between groups were determined by means of Student's *t* test.

Results

Reduced porphyrins are oxidized by the mitochondrial electron transport chain. Previous studies from these laboratories and others (2, 17–19) have demonstrated the capacity of the mitochondrial electron transport chain from liver and kidney to generate reduced oxygen species, e.g., $O_2\cdot^-$ and H_2O_2, in the presence of specific respiratory chain substrates (NADH, succinate) and an electron transport inhibitor. In preliminary experiments for the present studies, H_2O_2 production by succinate- and NaN_3 (50 μM)-supplemented liver mitochondria was 0.2 to 0.4 nmol of H_2O_2/min/mg of protein, as determined by the method of Loschen *et al.* (5). Addition of trace amounts of iron as Fe^{2+}-EDTA or Fe^{3+}-EDTA to the mitochondrial reaction mixture greatly facilitates the oxidation of reduced porphyrins by reactive oxidants generated under these conditions, and this effect is significantly attenuated by GSH and OH· radical scavengers (17). [Stimulation of mitochondrial OH· production by Fe^{3+}-EDTA most likely proceeds from mitochondrial $O_2\cdot^-$-mediated reduction of Fe^{3+} to Fe^{2+}, which, in turn, reduces H_2O_2 to OH· (20).] In the present studies, the possibility that Hg^{2+} and GSH could react with mitochondria-generated H_2O_2 to stimulate free radical-mediated porphyrinogen oxidation, as suggested from studies *in vitro* using exogenously added H_2O_2, was investigated.

Initial studies were conducted to demonstrate the capacity of mitochondria to oxidize reduced porphyrinogens in the presence of constituents known to promote reactive oxidant generation by the mitochondrial electron transport chain. As in the previous study (1), uroporphyrinogen was routinely employed as the model porphyrinogen. In preliminary studies, it was determined that coproporphyrinogen is actually oxidized by mitochondrial preparations at a rate slightly greater than that of uroporphyrinogen, e.g., 90–150 versus 75–90 pmol/min/mg of protein, respectively. The reasons for this difference are unknown. However, increases in the rate of oxidation of either porphyrinogen when Fe^{3+}-EDTA or Hg^{2+} plus GSH were added to reaction mixtures were comparable.

As shown in Table 1, uroporphyrinogen oxidation proceeded at a slow but measurable rate in the absence of a respiratory chain substrate and electron transport inhibitor in the reaction mixture. Rates were not measurably affected by the addition of a respiratory chain substrate (NADH or succinate) to the reaction mixture. However, addition of either NADH or succinate and NaN_3 as electron transport inhibitor resulted in approximately 2-fold increased rates of oxidation of porphyrinogens by mitochondria from either tissue. Further addition of iron as Fe^{3+} (100 μM)-EDTA to reaction mixtures significantly increased the rates of porphyrinogen oxidation by another 4- to 5-fold. No porphyrinogen oxidation was observed if mitochondria were omitted from the reaction mixture.

GSH attenuates mitochondrial porphyrinogen oxidation. Addition of GSH significantly reduced the rate of Fe^{3+}-EDTA-stimulated uroporphyrinogen oxidation by tissue mitochondria. As seen in Fig. 1, GSH reduced the rate of porphyrinogen oxidation by mitochondria from either liver or kidney in a dose-related fashion. Of interest is the observation that GSH at concentrations as low as 0.5 mM significantly reduced the rate of uroporphyrinogen oxidation by the mitochondrial electron transport chain. Previous studies (17) have demonstrated the attenuation of Fe^{3+}-EDTA-stimulated H_2O_2-dependent porphyrinogen oxidation by tissue mitochondria by various reactive oxidant scavengers.

Hg^{2+} plus GSH stimulate mitochondrial porphyrinogen oxidation. Although mitochondria-mediated porphyrinogen oxidation is substantially attenuated by GSH alone, GSH

TABLE 1

Effects of electron transport chain constituents and iron on mitochondria-mediated porphyrinogen oxidation

Reaction mixtures contained 0.4 mM NADH or 5 mM succinate, 1 mM NaN_3, 600–800 μg of mitochondrial protein, and 0.1 M phosphate buffer, pH 7.5, in a total volume of 3 ml. Fe as Fe^{3+} (100 μM)-EDTA was added where indicated. Uroporphyrinogen was added after a 5-min incubation of the reaction mixture at 37°, at a final concentration of 1 μM. In this and subsequent tables, porphyrinogen oxidation was followed spectrofluorometrically, as described in Experimental Procedures. Values are expressed as means ± standard errors of at least three replicate experiments.

Addition	Porphyrinogen oxidation rate	
	Liver	Kidney
	pmol/min/mg of protein	
None	30 ± 8	42 ± 10
NADH	34 ± 7	39 ± 11
NADH, NaN_3	61 ± 17[a]	88 ± 13[a]
NADH, NaN_3, Fe	256 ± 23[a]	344 ± 25[a]
Succinate	46 ± 9	41 ± 18
Succinate, NaN_3	67 ± 12[b]	75 ± 11[b]
Succinate, NaN_3, Fe	301 ± 33[b]	340 ± 52[b]

[a] Values differ significantly ($p < 0.05$) from NADH alone.
[b] Values differ significantly ($p < 0.05$) from succinate alone.

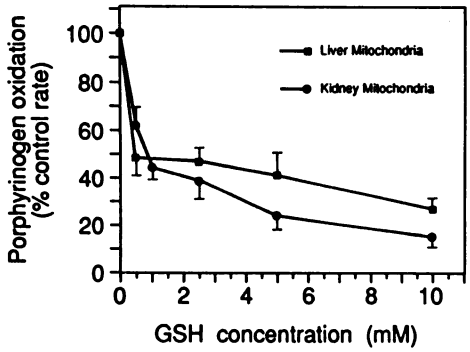

Fig. 1. Effects of GSH concentration on uroporphyrinogen oxidation by hepatic and renal mitochondria. Reaction mixtures contained 600–800 μg of mitochondrial protein, 0.4 mM NADH, 1 mM NaN_3, 100 μM Fe^{3+}-EDTA, and 0.1 M HEPES buffer, pH 7.45, in a total volume of 3 ml. GSH was added at the concentrations indicated. Uroporphyrinogen was added after a 5-min incubation of the reaction mixture at 37°, at a final concentration of 1 μM. Porphyrinogen oxidation in this and subsequent figures was followed spectrofluorometrically, as described in Experimental Procedures. Values in all figures are expressed as means ± standard errors of at least three replicate experiments.

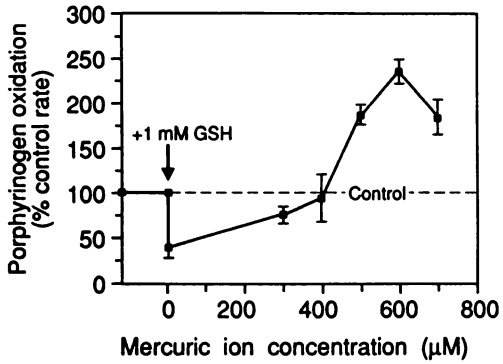

Fig. 2. Effect of Hg:GSH complex on porphyrinogen oxidation by rat liver mitochondria. Reaction mixtures contained 600–800 μg of hepatic mitochondria, 5 mM succinate, 1 mM NaN_3, 1 mM GSH, $HgCl_2$ at the concentrations indicated, and 0.1 M HEPES buffer, pH 7.45, in a total volume of 3 ml. Uroporphyrinogen was added after a 5-min incubation of the reaction mixture at 37°, at a final concentration of 1 μM. The initial rate of oxidation (100% rate) was 175 ± 12 pmol/min/mg of protein.

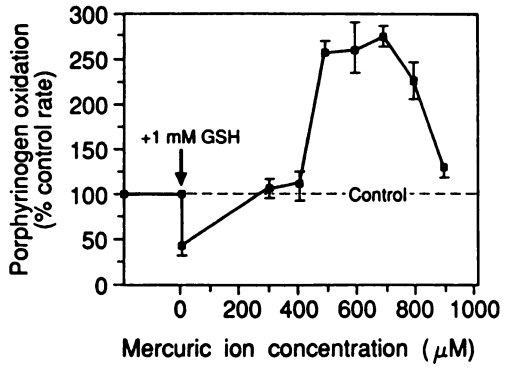

Fig. 3. Effect of Hg:GSH complex on porphyrinogen oxidation by rat kidney mitochondria. Reaction mixtures contained 600–800 μg of renal cortical mitochondria and other components, as indicated in the legend to Fig. 2.

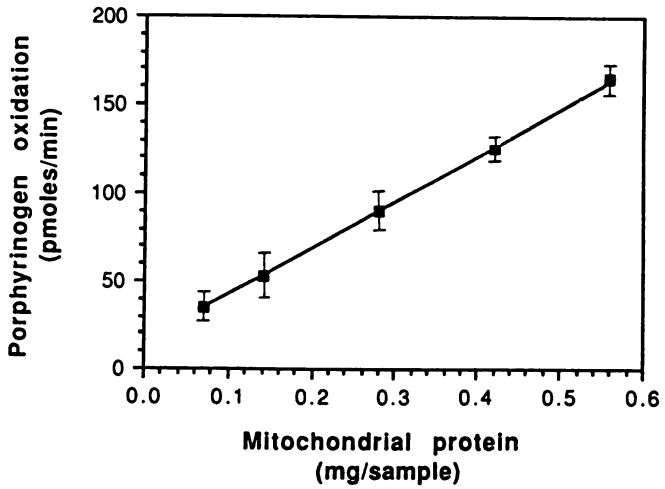

Fig. 4. Correlation of Hg:GSH-promoted porphyrinogen oxidation with mitochondrial protein concentration. Reaction mixtures contained 5 mM succinate, 1 mM NaN_3, 3 mM $HgCl_2$, 5 mM GSH, and hepatic mitochondria at the protein concentrations indicated, in 0.1 M phosphate buffer, pH 7.5. Uroporphyrinogen was added after a 5-min incubation of the reaction mixture at 37°, at a final concentration of 1 μM.

in the presence of Hg^{2+} promotes porphyrinogen oxidation. As seen in Fig. 2, uroporphyrinogen oxidation by liver mitochondria proceeded at a slow but measurable rate when succinate was used as the respiratory chain substrate. This process was significantly attenuated, by approximately 60%, by the addition of GSH in a final concentration of 1 mM to the reaction mixture. Addition of Hg^{2+} in low concentrations relative to that of GSH in the reaction cuvette reversed the antioxidant action of GSH. Moreover, as the ratio of Hg to GSH approached 1:2, the stoichiometric binding ratio of Hg^{2+} to GSH, the rate of porphyrinogen oxidation increased and exceeded that mediated by the mitochondrial system alone, reaching 2.5 times the control rate at a final molar ratio of Hg to GSH of approximately 3:5. Similar observations were made when NADH (0.4 mM) was employed as the respiratory chain substrate for liver mitochondria. As in the case with succinate as substrate, the maximal rate of porphyrinogen oxidation occurred at a molar ratio of Hg to GSH of approximately 3:5.

Kidney mitochondria responded to Hg^{2+} and GSH as did mitochondria from liver. As demonstrated in Fig. 3, when Hg and GSH were added to renal mitochondrial reaction mixtures, porphyrinogen oxidation increased another 2.7 times, with a maximal rate again achieved at a 3:5 molar ratio of Hg to GSH. Similar results were observed when NADH was employed as the respiratory chain substrate for kidney mitochondria.

Stimulation of porphyrinogen oxidation by Hg and GSH was directly proportional to the concentration of mitochondrial protein in the reaction mixture. As shown in Fig. 4, the rate of uroporphyrinogen oxidation by succinate- and NaN_3-supplemented liver mitochondria increased linearly in the presence of 3 mM Hg^{2+} plus 5 mM GSH as the concentration of mitochondrial protein increased from 20 to 200 μg/ml in the reaction cuvette. Hg^{2+} and GSH did not cause a measurable increase in the rate of porphyrinogen oxidation by mitochondria in the absence of a stimulus for H_2O_2 production, i.e., if a respiratory chain substrate or electron transport inhibitor was omitted from the reaction mixture.

As described in the accompanying paper (1), Fe^{3+}-EDTA and Hg plus GSH appear to act synergistically with exogenously added H_2O_2 to promote porphyrinogen oxidation *in vitro*. Therefore, studies were conducted to determine whether a comparable interaction between Fe^{3+} and Hg plus GSH could be observed with respect to mitochondria-mediated porphy-

rinogen oxidation. As shown in Table 2, when Fe^{3+} (100 μM)-EDTA was added to reaction mixtures containing renal mitochondria (250 μg/ml), the rate of porphyrinogen oxidation increased 6-fold above that mediated by mitochondria alone. However, concomitant addition of Hg and GSH (3 and 5 mM, respectively), to reaction mixtures caused the rate of porphyrinogen oxidation to proceed only slightly more rapidly than that observed in the presence of Fe^{3+}-EDTA alone. Thus, unlike results observed in studies using exogenously added H_2O_2, the combined effects of Fe and Hg plus GSH systems were not more than additive with respect to stimulation of porphyrinogen oxidation by mitochondria.

Hg:GSH-stimulated mitochondrial porphyrinogen oxidation is moderately attenuated by free radical scavengers. Further studies were conducted to confirm the participation of free radical oxidant species in Hg:GSH-stimulated mitochondrial porphyrinogen oxidation by measurement of the extent to which oxidation could be attenuated by various reactive oxidant scavengers. The results of these studies, presented in Table 3, demonstrate that Hg:GSH-stimulated mitochondrial porphyrinogen oxidation was moderately attenuated by the OH· radical scavengers deoxyribose and dimethyl sulfoxide, although only the effect of deoxyribose in the kidney was statistically significant. SOD (750 units) was moderately effective in attenuating Hg:GSH-stimulated porphyrinogen oxidation by renal mitochondria but was not effective in this regard with liver. CAT also moderately reduced the rate of Hg:GSH-stimulated porphyrinogen oxidation by both liver and kidney mitochondria, although only at a very high enzyme concentration. In experiments with CAT, NaN_3 was omitted from the reaction mixture to avoid direct inhibition of the enzyme by the azide.

TABLE 2

Combined effects of Fe^{3+}-EDTA and Hg plus GSH on mitochondria-mediated porphyrinogen oxidation

Reaction mixtures contained 5 mM succinate, 1 mM NaN_3, 600–800 μg of kidney mitochondrial protein, and 0.1 M phosphate buffer, pH 7.5, in a total volume of 3 ml. Other components were added at the concentrations indicated. Uroporphyrinogen was added after a 5-min incubation of the reaction mixture at 37°, at a final concentration of 1 μM. Values are expressed as means ± standard errors of at least three replicate experiments.

Addition	Porphyrinogen oxidation rate
	pmol/min/mg of protein
Succinate, NaN_3	70 ± 17
Succinate, NaN_3, Hg^{2+} (0.6 mM), GSH (1 mM)	110 ± 12
Succinate, NaN_3, Fe^{3+} (100 μM)-EDTA	427 ± 38
Succinate, NaN_3, Fe-EDTA, Hg^{2+} (0.6 mM), GSH (1 mM)	512 ± 40

TABLE 3

Effects of reactive oxidant scavengers on Hg:GSH-stimulated porphyrinogen oxidation by tissue mitochondria

Reaction mixtures contained 5 mM succinate, 1 mM NaN_3 (except where noted), 600 μM $HgCl_2$, 1 mM GSH, 600–800 μg of mitochondrial protein, and reactive oxidant scavengers at the concentrations indicated, in a total of 3 ml of 0.1 M HEPES buffer, pH 7.45. Uroporphyrinogen was added at a final concentration of 1 μM after a 5-min incubation of the reaction mixture at 37°. Values are expressed as means ± standard errors of three replicate experiments.

Addition	Porphyrinogen oxidation rate	
	Liver	Kidney
	pmol/min/mg of protein	
Succinate, NaN_3	74 ± 14	73 ± 17
+ Hg, GSH (control)	168 ± 23	108 ± 21
+ Hg, GSH, deoxyribose (20 mM)	151 ± 83	93 ± 4[a]
+ Hg, GSH, dimethyl sulfoxide (5 mM)	158 ± 22	98 ± 13
+ Hg, GSH, SOD (750 units)	157 ± 17	86 ± 24[a]
Succinate, Hg, GSH (control 2) (no NaN_3)	111 ± 21	115 ± 32
+ CAT (14,000 units)	81 ± 10[b]	86 ± 8[b]

[a] Value differs significantly from control 1 ($p < 0.05$).
[b] Value differs significantly from control 2 ($p < 0.05$).

Discussion

The capacity of mitochondria from various tissues to generate $O_2\cdot^-$ and H_2O_2 has been described by a number of investigators and is known to occur at several sites along the electron transport chain (2, 18, 19, 21). As schematically depicted in Fig. 5, principal sites of generation of reduced oxygen species are the flavoprotein-NADH dehydrogenase (rotenone-inhibited) and the ubisemiquinone-cytochrome *b* (antimycin-inhibited) regions. At pH 7.4, NADH dehydrogenase contributes about one third and ubisemiquinone about two thirds of total $O_2\cdot^-$ and H_2O_2 production (21). In previous studies (17), we have demonstrated that iron as either Fe^{2+}-EDTA or Fe^{3+}-EDTA significantly stimulates H_2O_2-dependent oxidation of reduced porphyrins by hepatic and renal mitochondria. The results of the present investigation confirm these findings and demonstrate, further, that Hg^{2+} and GSH also interact with mitochondria-generated reduced oxygen species to promote porphyrinogen oxidation. This action, coupled with compromised mitochondrial porphyrinogen metabolism by mercury compounds (7, 8), provides a plausible explanation for the substantial increase in the excretion of oxidized porphyrins, particularly coproporphyrin, observed during prolonged mercury exposure.

The precise mechanism(s) by which Hg^{2+} acts to facilitate porphyrinogen oxidation in the presence of GSH and mitochondria-generated H_2O_2 remains to be fully delineated. However, a plausible mechanism could involve the interaction of Hg^{2+} and GSH with mitochondria-generated H_2O_2 to produce both thiol- and oxygen-derived free radical species capable of porphyrinogen oxidation, as proposed in the preceding paper (1). In this respect, it is hypothesized that Hg^{2+} reacts with the SH^- groups of GSH via mechanisms involving ligand exchange to form GS· radicals. These, in turn, could react with GSH or with reduced oxygen species (e.g., H_2O_2) to promote formation of reactive oxidants, possibly including the highly reactive OH· radical (22–25). The formation of multiple radical species during the interaction of Hg^{2+}, GSH, and H_2O_2 under the experimental conditions employed is supported by both free radical scavenger data and ESR studies (1). The direct oxidation of

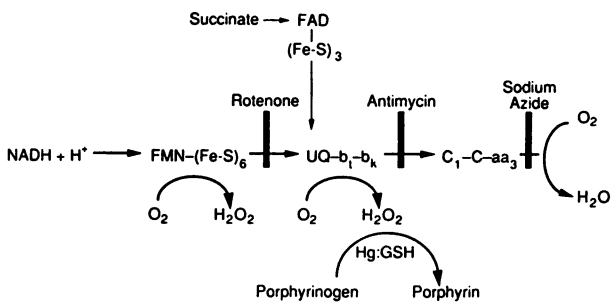

Fig. 5. Schematic representation of the mitochondrial electron transport chain, showing principal sites of H_2O_2 formation. The promotion of reactive oxidants from the interaction of Hg^{2+}, GSH, and mitochondria-generated H_2O_2, which in turn oxidize reduced porphyrins, is proposed.

reduced porphyrins by $O_2 \cdot^-$ and OH· radicals has been previously demonstrated (10, 11, 17, 26, 27).

The not-more-than additive interaction of Hg:GSH- and Fe-mediated porphyrinogen oxidation in the mitochondrial system differs from the apparent synergistic interaction observed between Fe and Hg plus GSH in the presence of exogenous H_2O_2 *in vitro*, suggesting that mitochondria-generated H_2O_2 is limiting in this process. This explanation would be consistent with studies from these laboratories and others (28) demonstrating the maximal capacity of H_2O_2 generation by the mitochondria from rat liver as being on the order of 0.5 nmol/min/mg of protein. This rate would be expected to produce considerably less H_2O_2 than the concentrations employed in exogenous H_2O_2 studies (1). The oxidative potential of both Fe^{3+}-EDTA/H_2O_2 (15) and Hg:GSH/H_2O_2 (1) systems has been previously shown to increase proportionately with the concentration of H_2O_2 in the reaction medium.

Alternatively, the diminished interaction between Fe^{3+}-EDTA and Hg plus GSH systems in mitochondria, as compared with the tissue-free system, could reflect the possible attenuation of reactive species by endogenous antioxidants or by the mitochondrial membrane *per se*. Although CAT is not found in mitochondria *in vivo*, the contribution of $O_2 \cdot^-$ to porphyrinogen oxidation reactions could have been attenuated by the presence of active SOD in mitochondrial preparations (29). The relative ineffectiveness of exogenously added SOD or CAT in attenuating mitochondrial porphyrinogen oxidation even at high concentrations (Table 3) argues against this possibility, however. It is also possible that mitochondrial membranes *per se* could have reduced the interaction of reactive species with reduced porphyrins in the reaction medium. The probability that free radical-mediated oxidative events occur within the immediate vicinity of reactive oxidant production and the direct attenuation of reactive oxidants by mitochondrial membranes has been suggested by various investigators (20, 30–32).

The finding that renal, as well as hepatic, mitochondria have a substantial capacity to oxidize reduced porphyrins and that this effect is significantly increased in the presence of Hg and GSH is of considerable interest with respect to the etiology of Hg-induced porphyria, inasmuch as the kidney is the principal target organ of mercury compounds. Studies of prolonged exposure of rats to mercury as methyl mercury hydroxide at 5 or 10 ppm in drinking water (7, 8) have demonstrated that mercury concentrations can reach 100 μM or greater in renal proximal tubule cells during such exposure. Because renal mitochondrial GSH levels have been found in this laboratory to be at least 10-fold less than those of the soluble cellular fraction, it is feasible that intramitochondrial mercury and glutathione could readily reach concentrations optimally conducive to promotion of porphyrinogen oxidation via the mechanisms proposed (1). The capacity of mercuric ions to disrupt mitochondrial biological oxidation reactions (10, 33), as well as to deplete endogenous GSH levels (34, 35), increases the likelihood of these events. Evidence that the kidney is the principal source of urinary porphyrins excreted during exposure to mercury, as well as to various other porphyrogenic chemicals, has been provided by numerous investigators (36).

In summary, the present studies demonstrate that mercuric ion in the presence of GSH interacts with H_2O_2 generated by the mitochondrial electron transport chain to stimulate the oxidation of reduced porphyrins. Findings from this and the preceding paper (1) suggest that this reaction is mediated by free radical species resulting from this interaction. These results could account for the pronounced porphyrinuria observed during prolonged exposure to mercury compounds. These findings also suggest a plausible mechanistic explanation of the oxidative tissue damage associated with prolonged mercury exposure.

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