0026-895X/88/010015-08$02.00/0

MOLECULAR PHARMACOLOGY, 34:15-22



# Synthesis and Nephrotoxicity of 6-Bromo-2,5-Dihydroxy-Thiophenol

TERRENCE J. MONKS, ROBERT J. HIGHET, PING S. CHU, and SERRINE S. LAU

*The University of Texas System Cancer Center, Science Park-Research Division, Smithville, Texas (T.J.M.), National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland (R.J.H.), M. D. Anderson Hospital, Houston, Texas (P.S.C.), and the College of Pharmacy, University of Texas at Austin, Texas (S.S.L.)*

Received January 28, 1988; Accepted April 19, 1988

**SUMMARY**

The formation of potentially reactive thiols has been postulated to play a role in the nephrotoxicity caused by a number of glutathione and/or cysteine conjugates. However, the inherent reactivity of such compounds has precluded both their identification in biological systems and a determination of their actual toxicity. To this end we have synthesized 6-bromo-2,5-dihydroxy-thiophenol as a putative metabolite of nephrotoxic 2-bromohydroquinone-glutathione conjugates.The compound was prepared by the addition of sodium thiosulfate to 2-bromo-1,4-benzoquinone followed by reduction of the *S*-arylthiosulfate to the thiophenol. 2,5-Dihydroxy-thiophenol was similarly prepared. Structural identification was confirmed by mass spectroscopy and nuclear magnetic resonance spectroscopy. Administration of 6-bromo-2,5-dihydroxy-thiophenol to rats (0.35 mmol/kg; intraperitoneally) caused an increase in blood urea nitrogen and histological alterations similar to those observed after 2-bromo-(diglutathion-*S*-yl)hydroquinone administration. 2,5-Dihydroxy-thiophenol was also nephrotoxic but at a dose of 0.6 mmol/kg. In contrast, no effects on liver pathology were observed after administration of either 6-bromo-2,5-dihydroxy-thiophenol or 2,5-dihydroxy-thiophenol and serum glutamate pyruvate transaminase levels were normal. Neither 2-, 3-, nor 4-bromothiophenol had any effect on blood urea nitrogen at doses between 0.2 and 0.8 mmol/kg (intraperitoneally) and no apparent alterations were seen in kidney slices prepared from bromothiophenol-treated rats. These findings suggest that the quinone function of 6-bromo-2,5-dihydroxy-thiophenol is necessary for the expression of toxicity. In this respect, the lower activity of NAD(P)H quinone oxidoreductase (EC 1.6.99.2) in renal cortex may be of toxicological significance.

The kidney is a frequent target of toxic chemicals although, as with most other extrahepatic toxicities, the biochemical mechanisms of such tissue injury remain to be elucidated (1). Chloroform nephrotoxicity is probably mediated by its metabolism to phosgene, catalyzed by renal and/or hepatic cytochrome P-450 (1–6). In contrast, cephaloridine nephrotoxicity may be a consequence of lipid peroxidation initiated by the redox cycling of cephaloridine, catalyzed by renal mitochondrial P-450 reductase (7–10). However, mechanisms other than those involving P-450 are capable of mediating chemically induced nephrotoxicity. In this respect, only detoxifying functions had until recently been attributed to conjugations with GSH because these conjugates are usually less toxic than their parent compounds and are readily excreted in bile or urine as their corresponding mercapturic acids. However, conjugation with GSH has now been implicated in the activation of a number of chemicals to mutagenic and carcinogenic electrophiles (11–14) and evidence is accumulating that GSH conjugates of a variety of compounds and/or their corresponding cysteine conjugates are nephrotoxic (15–17).

We have recently provided evidence that the renal necrosis observed after bromobenzene administration (18) is probably mediated by the formation of 2-bromo-(diglutathion-*S*-yl) hydroquinone (19) via the pathway illustrated in Fig. 1. Three monosubstituted GSH conjugates of 2-bromohydroquinone are also formed, which exhibit differentially less toxicity than the disubstituted conjugate. A possible mechanism by which GSH conjugates elicit toxicity involves metabolism to the cysteine conjugate and subsequent cleavage by cysteine conjugate $\beta$-lyase to give a potentially reactive thiol. For example *S*-1,2-dichlorovinyl-L-cysteine, which is a potent nephrotoxicant (20) is also an excellent substrate for the enzyme $\beta$-lyase (21) and is metabolized to a sulfur-containing alkylating metabolite that reacts covalently with tissue components (22–25). Although the vinyl thiol generated by $\beta$-lyase would be electrophilic and react covalently with cellular macromolecules, the exact mechanism of toxicity is still a matter for conjecture because such compounds have yet to be synthesized. $\beta$-Lyase has also been

This work was supported in part by United States Public Health Service Grant ES 04662 from the National Institute of Environmental Health Sciences.

**ABBREVIATIONS:** GSH, glutathione; HPLC, high performance liquid chromatography; BUN, blood urea nitrogen; SGPT, serum glutamate pyruvate transaminase; IP, intraperitoneal.

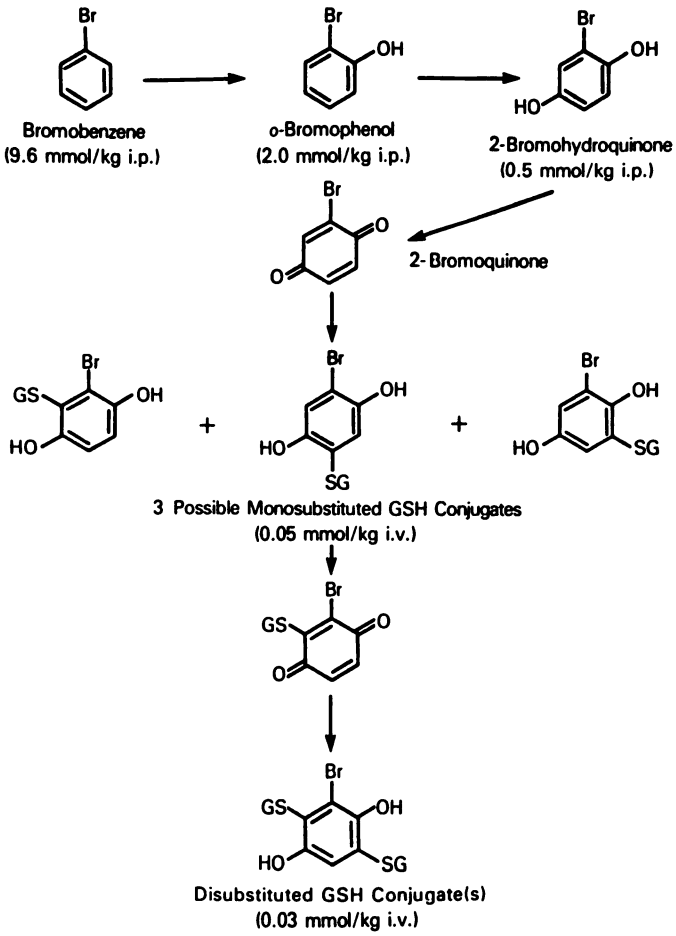


**Fig. 1.** The metabolism of bromobenzene to nephrotoxic metabolites. This pathway involves two successive cytochrome P-450-catalyzed oxidations to give *o*-bromophenol and then 2-bromohydroquinone. Oxidation of 2-bromohydroquinone occurs by an as yet unknown mechanism, followed by conjugation with GSH to give three possible monosubstituted isomers. These GSH conjugates may undergo further oxidation and conjugation with a second molecule of GSH to give rise to 2-bromo-(diglutathion-*S*-yl)-hydroquinone, a potent nephrotoxicant in rats. *Figures in parentheses* represent the dose of each compound required to elicit a similar degree of nephrotoxicity. *i.v.*, intravenous.

postulated to be involved in the nephrotoxicity of the cysteine conjugates of chlorodifluoroethylene, chlorotrifluoroethylene (26, 27), and hexachlorobutadiene (16).

Whether metabolism of 2-bromohydroquinone GSH conjugates to reactive thiols contributes to their nephrotoxicity is not known. Indeed, in most instances in which formation of a reactive thiol has been postulated to play an important role in toxicity, the positive identification and a determination of the toxicity of these putative reactive metabolites are missing. We therefore now report on the synthesis and toxicity of 6-bromo-2,5-dihydroxy-thiophenol, a putative metabolite of 2-bromohydroquinone-GSH conjugates. Structure activity studies suggest that the quinone function of this compound is a more important determinant of toxicity than the thiol group.

## Materials and Methods

**Chemicals.** 2-,3-, and 4-bromothiophenol were obtained from the Aldrich Chemical Company (Milwaukee, WI). 2-Bromo-1,4-benzoquinone was prepared as previously described (19). *S*-Adenosyl-L-[*methyl*-$^{14}$C]methionine (>45mCi/mmol) was purchased from Amersham (Arlington Heights, IL). All other reagents were of the highest grade commercially available.

**Synthesis of 6-bromo-2,5-dihydroxy-thiophenol.** 6-Bromo-2,5-dihydroxy-thiophenol was synthesized by modification of the method of Alcalay (28) as follows. To a stirred and cooled (ice bath) solution of sodium thiosulfate pentahydrate (1.8 g; 7.3 mmol) in water (4 ml) was added dropwise a solution of 2-bromo-1,4-benzoquinone (0.73 g; 4.3 mmol) in acetic acid (8 ml). The color of the quinone was discharged almost immediately upon addition. After the addition was completed, the ice bath was removed and stirring was continued for an additional 5 min. Concentrated hydrochloric acid (10 M, 9.0 ml) and water (5 ml) was then added to dilute the solution. A thermometer was inserted and zinc dust (2.3 g) was added in small portions such that the temperature was maintained between 40–50°. After addition was completed, the solution was allowed to cool, with stirring, to room temperature (approximately 15 min). The solution was then partitioned between ice-cold water (100 ml) and ether (100 ml). The aqueous layer was separated and extracted twice with ether (50 ml). The combined ether extracts were washed twice with ice-cold water (100 ml), dried over anhydrous magnesium sulfate, filtered, and concentrated under vacuum with a rotory-evaporator to yield a white solid product. The product was readily soluble in ether and was crystalized in a minimum amount of acetone or methylene chloride. The weight of the recrystallized product was approximately 300 mg, which was stored under argon. This same procedure was used to synthesize 2,5-dihydroxy-thiophenol using 1,4-benzoquinone as the starting material. The yields for both 6-bromo-2,5-dihydroxy-thiophenol and 2,5-dihydroxy-thiophenol were approximately 10%. Melting points were determined using a Thomas Hoover Uni-melt Capillary Melting Point Apparatus (Arthur H. Thomas Co., Philadelphia, PA) and the values for 6-bromo-2,5-dihydroxy-thiophenol and 2,5-dihydroxy-thiophenol were 143–145° and 110–112°, respectively. A similar melting point for 2,5-dihydroxy-thiophenol was reported (118°) (28). The purity of the compounds (>98%) was further determined by HPLC analysis.

**Structural identification of 6-bromo-2,5-dihydroxy-thiophenol and 2,5-dihydroxy-thiophenol.** Proton NMR spectra were obtained on a Varian XL-200 at 200 MHz, using solutions of approximately 10 mM in $CDCl_3$. Typically, 128 free induction decays of 20,000 points were acquired with a sweep width of 2500 Hz and transformed without weighting. Mass spectra were obtained on a LKB 9000 mass spectrometer by electron impact ionization of a sample introduced directly into the probe.

**Toxicity studies.** Male Sprague-Dawley rats (100–150 g; Taconic Farms, Inc., Germantown, NY) were used for all experiments and were allowed food and water *ad libitum* before the experiments. 6-Bromo-2,5-dihydroxy-thiophenol, 2,5-dihydroxy-thiophenol, and the various bromothiophenols were dissolved in ethanol/phosphate buffered saline (40:60; v/v). These compounds were injected IP (0.5 ml) into rats and 24 or 48 hr later a sample of blood (400 μl) was taken from the retro-orbital sinus. Control animals received 0.5 ml of the vehicle. Plasma was separated by centrifugation and the degree of renal damage was assessed by measuring BUN levels using Sigma kit 535A according to Sigma technical bulletin 535 (Sigma Chemical Co., St. Louis, MO). Hepatic damage was assessed by measuring the level of SGPT activity using Sigma kit 505P according to Sigma technical bulletin 505. Livers and kidneys were excised and histology slides prepared and stained with hematoxylin and eosin by American Histolabs (Rockville, MD).

**Determination of renal S-methyl transferase activity.** Rats were killed by cervical dislocation, the kidneys were perfused *in situ*, excised, and immediately homogenized with Tris-KCl buffer, pH 7.4 (0.15 M KCl; 20 mM Tris) and microsomes prepared as described by Hinson *et al.* (29). Renal microsomal *S*-methyltransferase activity was determined with freshly prepared microsomes only. Incubation mixtures consisted of 2 mg/ml microsomal protein, 0.5 mM 6-bromo-2,5-dihydroxy-thiophenol or 0.5 mM 2,5-dihydroxy-thiophenol, 1 mM *S*-adenosyl-L-[*methyl*-$^{14}$C]methionine (2200 dpm/nmol) in 0.1 M potassium phosphate buffer, pH 7.9, containing 1 mM EDTA and 0.5%

Triton X-100 in a total volume of 2 ml. Control incubations were prepared without substrate but with radiolabeled cofactor. The mixtures were incubated at 37° for 30 min and terminated by placing on ice. A 0.5-ml aliquot from each incubation was immediately extracted with 3 ml of ether. A 1-ml aliquot of ether was then used for the determination of radioactivity by liquid scintillation counting. Enzyme activity is expressed as nmol of [$^{14}$C]$SCH_3$ formed/mg of microsomal protein/30 min and results are corrected with the appropriate control and for incomplete recovery. A second aliquot of ether was evaporated to dryness under nitrogen and reconstituted in 200 μl of methanol and analyzed by HPLC. A 50-μl aliquot of methanol was injected onto a Whatman ODS-3 Partisil reverse phase analytical column and eluted with a linear gradient of water/methanol/acetic acid (89:10:1, v/v/v) to water/methanol/acetic acid (0:99:1, v/v/v) over 60 min at a flow rate of 1 ml/min. The eluate was collected at 30-sec intervals and radioactivity was determined by liquid scintillation counting. Under these conditions each of the substrates gave rise to a single major radioactive peak, which presumably corresponded to the *S-methyl*-$^{14}$C-derivative.

**Determination of renal quinone reductase activity.** Cytosol fractions from renal cortex, medullae, and papillae were prepared at 0–4°. Assays were performed by modification of the procedures of Ernster (30). The reaction mixture contained, in a final volume of 1 ml, 25 mM Tris·HCl (pH 7.4), 0.7 mg of bovine serum albumin (Sigma); 5 μM FAD (Sigma), 0.2 mM β-NAD(P)H (Sigma), 40 μM menadione as the electron acceptor in 10 μl of ethanol, and an appropriate amount of cytosol such that enzymatic activity remained linear for 1 min. Assays were carried out at 25° in the presence or absence of 10 μM dicoumarol. The dicoumarol-sensitive portion of the activity was taken as a measure of the quinone reductase (DT-diaphorase) activity. The initial velocity of the reduction of menadione was measured spectrophotometrically by following the oxidation of the pyridine nucleotide at 340 nm. Enzyme activity (units/mg) is expressed as nmol of NAD(P)H oxidized/min/mg of protein. All values are corrected for nonenzymatic rates. Under the above conditions, the dicoumarol-sensitive quinone reductase activity represented greater than 98% of the activity assayed.

## Results

**Structural identification of 6-bromo-2,5-dihydroxy-thiophenol and 2,5-dihydroxy-thiophenol.** Fig. 2A shows the mass spectrum of 6-bromo-2,5-dihydroxy-thiophenol. The major ions in this spectrum were *m/z* 220, 222 ($M^+$); 191, 193 (M−COH); 141 (M−Br); and 113 (M−Br− CO). Fig. 2B shows the mass spectrum of 2,5-dihydroxy-thiophenol. The major ions in this spectrum were M/Z 142 ($M^+$); 113 (M−COH); and 81 (M−CO−SH). The $^1$H NMR spectra of the two synthetic products are shown in Figs. 3 and 4. The spectrum of the brominated analog (Fig. 3) has two aromatic protons at δ 6.89 and 6.84 ppm, which exhibit an *ortho*-coupling of $J$ = 8.5 Hz. The signals at δ 3.69, 5.18, and 5.56 ppm represent the two hydroxyl substituents and the mercaptan. Because the hydroxyl substituents are *para* to each other, the thiol and bromo groups must be *ortho* to each other. The spectrum of 2,5-dihydroxy-thiophenol is shown in Fig. 4 and has three aromatic protons at δ 6.72, 6.84, and 6.95 ppm. The signals at δ 3.10, 4.50, and 5.67 ppm represent the two hydroxyl substituents and the mercaptan. Therefore, based upon both the mass spectrometric and $^1$H NMR analyses, the structures of the two chemically synthesized thiols were confirmed as 6-bromo-2,5-dihydroxy-thiophenol and 2,5-dihydroxy-thiophenol. $^{13}$C NMR spectra of the latter compound were also consistent with this structural assignment (in $CDCl_3$: C-2, and -5, 149.9, 145.4; C-1, 118.9; C-3, -4, and -6, 115.3, 115.2, and 112.4). The limited solubility of 6-bromo-2,5-dihydroxy-thiophenol in $CDCl_3$ precluded a similar analysis by $^{13}$C NMR. Moreover, in a dimethylsulfoxide solution the brominated compound apparently formed the disulfide. Evidence for disulfide formation was provided by the $^1$H NMR spectra, which exhibited a change in the chemical shifts on standing, and the mass spectra, which showed the parent ion of the disulfide.

**Toxicity of 6-bromo-2,5-dihydroxy-thiophenol and 2,5-dihydroxy-thiophenol.** Administration of 6-bromo-2,5-dihydroxy-thiophenol and 2,5-dihydroxy-thiophenol IP to rats caused a dose-dependent elevation of BUN (Fig. 5). Elevated BUN concentrations corresponded to histological alterations in the kidney as evaluated by the method of Reid (18) and as previously described by us (31). The histopathological alterations in the kidney were similar to those observed after bromobenzene, *o*-bromophenol, 2-bromohydroquinone, or 2-bromo-hydroquinone-glutathione conjugate administration. The alterations were characterized by a striking coagulative necrosis of the proximal renal tubules in the corticomedullary region. As shown in Fig. 6, severe necrosis was observed in the S3 segments of the proximal tubules, which contained eosinophilic cells with pyknotic nuclei. The collecting ducts and tubules exhibited occasional hyaline casts whereas some of the tubules in the mid and outer cortex were markedly dilated and contained proteinaceous fluid. However, the dose of 2,5-dihydroxy-thiophenol required to cause a maximum elevation of

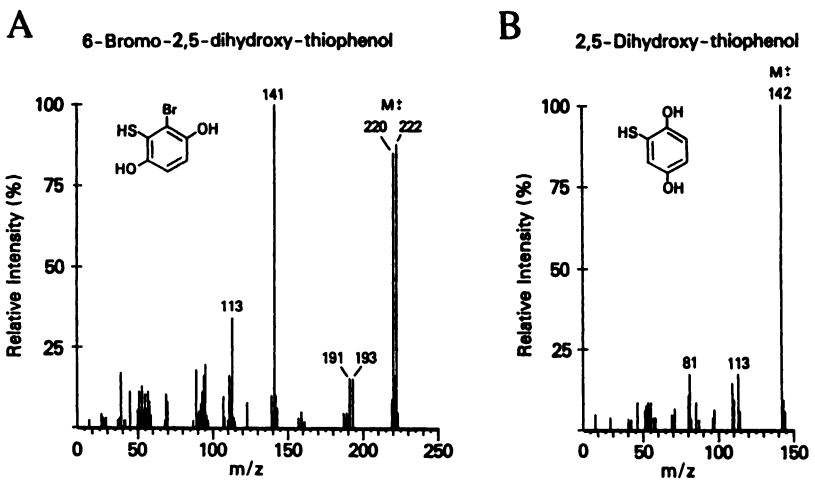


**Fig. 2.** Mass spectra of (A) 6-bromo-2,5-dihydroxy-thiophenol and (B) 2,5-dihydroxy-thiophenol. Mass spectra were obtained on an LKB 9000 mass spectrometer by electron impact ionization of a sample introduced directly onto the probe.

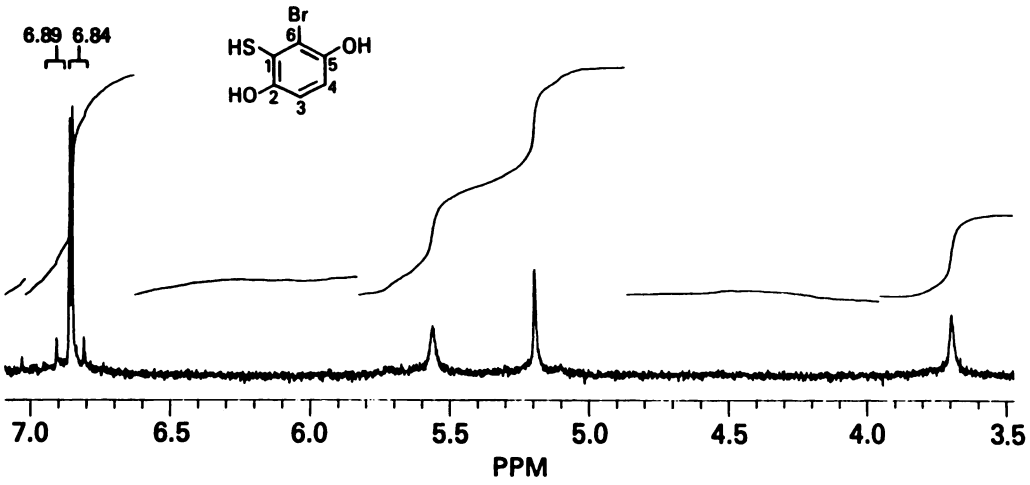


**Fig. 3.** $^1$H NMR spectrum of 6-bromo-2,5-dihydroxy-thiophenol. Spectra were obtained on a Varian XL-200 spectrometer at 200 MHz on a 10 mM solution in $CDCl_3$. Typically, 128 free induction decays of 20,000 points were acquired with a sweep width of 250 Hz and were transformed without weighting.

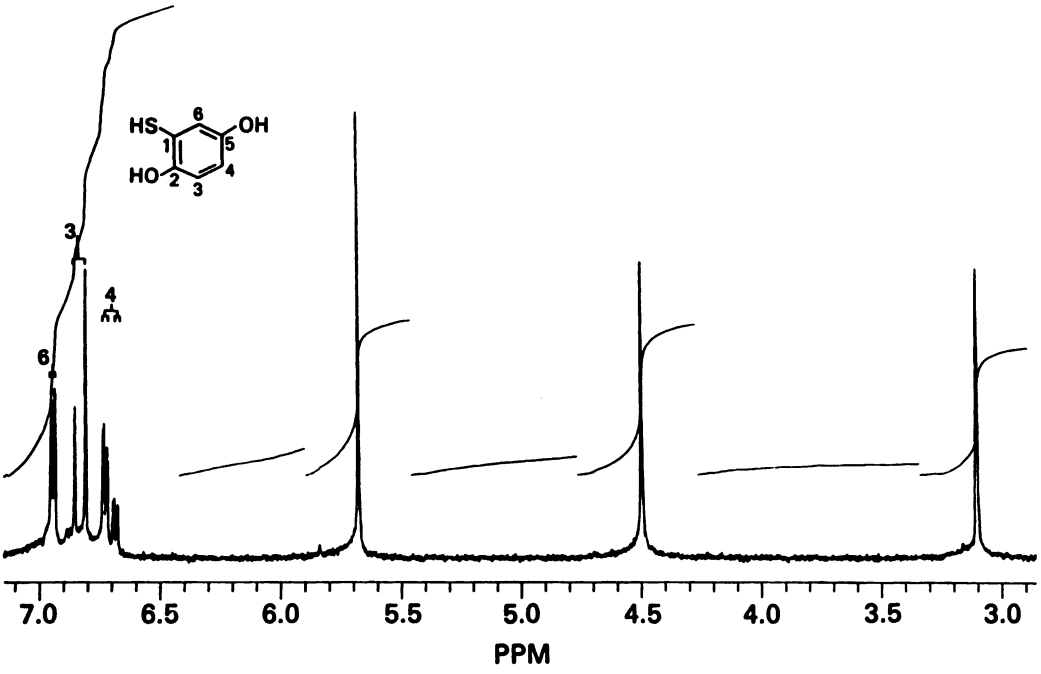


**Fig. 4.** $^1$H NMR spectrum of 2,5-dihydroxy-thiophenol. Conditions are as described in the legend to Fig. 3.

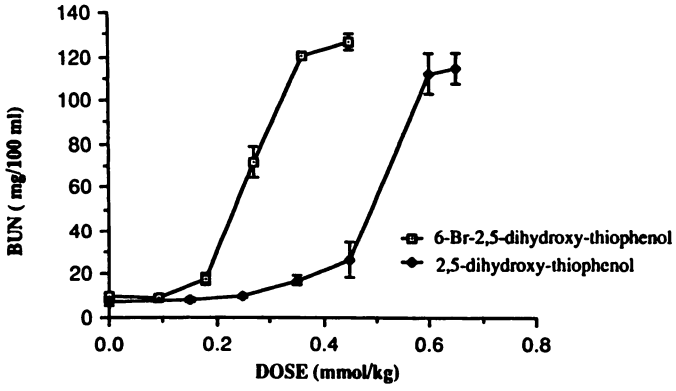


**Fig. 5.** The *in vivo* nephrotoxicity of 2,5-dihydroxy-thiophenol and 6-bromo-2,5-dihydroxy-thiophenol. The compounds were given by IP injection in 0.5 ml of ethanol/phosphate-buffered saline (40:60 v/v). After 24 hr a sample of blood was obtained via the retroorbital sinus, plasma was separated by centrifugation, and renal damage was assessed by measuring BUN concentrations.

BUN (0.6 mmol/kg) was approximately twice that of the brominated analog (0.35 mmol/kg).

In contrast to the severe renal necrosis caused by 6-bromo-2,5-dihydroxy-thiophenol, examination of liver sections from these animals showed no apparent alterations in liver pathology and SGPT levels were in the normal range (data not shown). However, high doses (nonlethal) of 2,5-dihydroxy-thiophenol did cause slight elevations in SGPT values (control, 20.8 ± 6.0 units/liter; 0.6 mmol/kg, 62.0 ± 19.8 units/liter).

**Toxicity of isomeric bromothiophenols.** The demonstration that the dihydroxy-thiophenols were capable of causing a tissue-specific toxicity led us to subsequently determine those features of the molecule that contribute the most toward its biological activity. In this respect, either the quinone moiety or the thiol moiety (or both) might be responsible for the observed nephrotoxicity of the dihydroxy-thiophenols. We therefore examined the potential toxicity of a series of bromothiophenols, which differ from the presently synthesized compounds by the absence of the quinone function. Interestingly, neither 2-, 3-, or 4-bromothiophenol had any effect on BUN at doses between

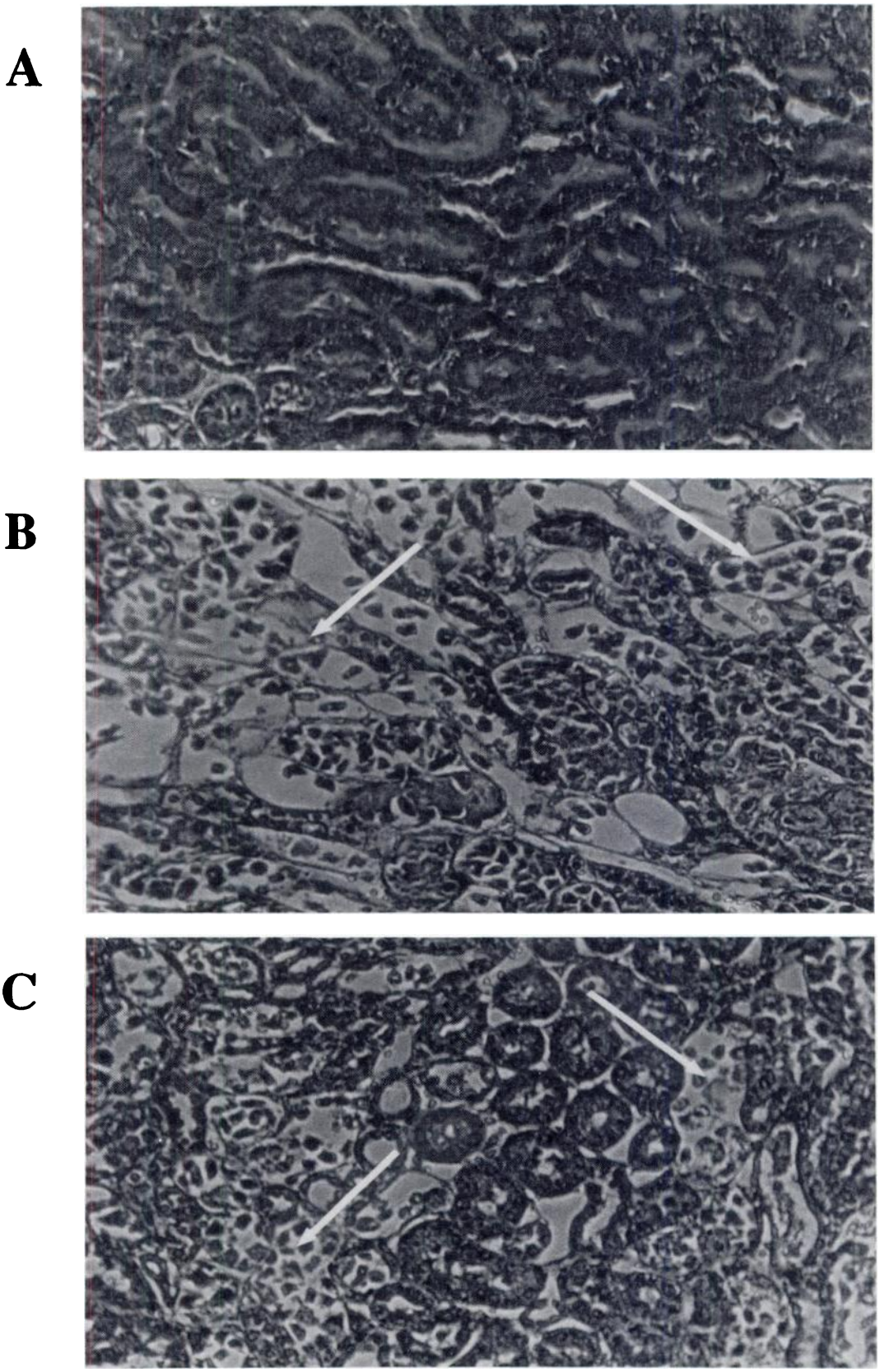


**Fig. 6.** Kidney sections obtained from rats 24 hr after treatment with (A) the vehicle, ethanol/phosphate-buffered saline (40:60 v/v), (B) 6-bromo-2,5-dihydroxy-thiophenol (0.35 mmol/kg, IP), and (C) 2,6-dihydroxy-thiophenol (0.6 mmol/kg, IP). All sections were stained with hematoxylin and eosin and the magnification was 50×. Each photograph was taken of the corticomedullary junction. Severe necrosis was observed in the $S_3$ segments of the proximal tubules, which contained eosinophilic cells with pyknotic nuclei (*arrows*).

TABLE 1

**Lack of *in vivo* toxicity of various bromothiophenols**

Numbers represent mean ± SD; $n = 6$. Various doses of bromothiophenols were administered IP to rats in 0.5 ml of ethanol/phosphate-buffered saline (40:60, v/v). Control animals received vehicle only. BUN and SGPT levels were determined 24 and 48 hr after injection.

| Dose | BUN | | SGPT | |
|---|---|---|---|---|
| | 24 hr | 48 hr | 24 hr | 48 hr |
| *mmol/kg* | *mg/100 ml* | | *units/liter* | |
| 4-Br-thiophenol[a] | | | | |
| 0.6 | 15.2 ± 1.2 | 9.8 ± 1.0 | 17.6 ± 2.0 | 14.8 ± 2.0 |
| 0.4 | 14.6 ± 2.3 | 6.9 ± 2.6 | 24.8 ± 14.4 | 16.6 ± 10.5 |
| 0.3 | 18.6 ± 3.7 | 7.0 ± 3.3 | 17.2 ± 13.9 | 15.8 ± 12.3 |
| 0.2 | 17.8 ± 3.7 | 9.7 ± 5.7 | 35.8 ± 15.5 | 10.8 ± 7.6 |
| 3-Br-thiophenol | | | | |
| 0.8 | 19.9 ± 7.1 | 21.6 ± 10.5 | 29.2 ± 19.8 | 23.1 ± 13.6 |
| 0.4 | 19.0 ± 2.6 | 16.0 ± 16.1 | 17.7 ± 14.8 | 17.7 ± 9.5 |
| 0.3 | 22.2 ± 10.2 | 27.6 ± 18.2 | 7.6 ± 5.2 | 15.9 ± 2.7 |
| 0.2 | 19.9 ± 3.2 | 37.2 ± 8.6 | 10.7 ± 5.6 | 25.1 ± 5.8 |
| 2-Br-thiophenol | | | | |
| 0.8 | 15.3 ± 5.1 | 18.4 ± 15.6 | 15.1 ± 13.3 | 9.2 ± 3.4 |
| 0.4 | 16.5 ± 5.4 | 16.6 ± 4.3 | 6.1 ± 5.0 | 25.1 ± 6.5 |
| 0.3 | 17.4 ± 6.8 | 11.1 ± 4.8 | 18.2 ± 1.5 | 27.8 ± 4.3 |
| 0.2 | 14.9 ± 1.3 | 18.1 ± 8.9 | 11.0 ± 5.3 | 8.5 ± 2.0 |
| Control | 19.1 ± 5.7 | 15.6 ± 4.3 | 20.0 ± 4.2 | 17.1 ± 5.3 |

[a] Dose at 0.8 mmol/kg caused death in five out of six rats within 24 hr.

0.2 and 0.8 mmol/kg IP (Table 1) and no pathological alterations were seen in kidney slices from bromothiophenol-treated rats. SGPT values were also within the normal range (see Table 3).

**Enzymatic determinants of 6-bromo-2,5-dihydroxy-thiophenol and 2,5-dihydroxy-thiophenol nephrotoxicity.** The amount of a potentially reactive thiol generated from a cysteine conjugate will be dependent upon a variety of factors, including the relative activities of *N*-acetyltransferase(s), deacetylase(s), and cysteine conjugate $\beta$-lyase. The thiol may or may not alkylate tissue components, which may or may not lead to subsequent necrosis. Alternatively, the thiol can be a substrate for thiol *S*-methyl transferase (32–34) to give rise to the thiomethyl derivative. Such a reaction might be expected to alter the biological activity of the parent compound. We therefore measured the activity of freshly prepared renal microsomal *S*-methyl transferase toward 0.5 mM 6-bromo-2,5-dihydroxy-thiophenol and 0.5 mM 2,5-dihydroxy-thiophenol (Table 2). Enzyme activity toward 2,5-dihydroxy-thiophenol was 7 times that toward the brominated analog. It should be noted that the enzyme has a far higher affinity for aromatic thiols than for aliphatic thiols (32) and the substrate concentrations used in the present experiments (0.5 mM) are above those required for saturation. The formation of the *S*-methyl derivatives were further confirmed by HPLC. Thus, analysis of the ether extract from renal microsomal incubations containing either of the dihydroxy-thiophenols and *S*-adenosyl-L-[*methyl*-$^{14}$C]methionine, gave rise to a single major radioactive peak, which presumably corresponded to the *S*-[*methyl*-$^{14}$C]dihydroxy-thiophenol. This radioactive peak was absent in incubations containing boiled renal microsomes (Fig. 7).

The lack of renal toxicity of the isomeric bromothiophenols suggests that the quinone function of the dihydroxy-thiophenols is an important determinant of their toxicity. We therefore measured the tissue distribution of renal quinone reductase activity in order to determine whether possible differences might contribute to the observed localization of dihydroxy-thiophenol renal toxicity. Renal quinone reductase activity was found to be highest in the papilla (269.3 ± 5.4 units/mg) and lowest in the cortex (38.2 ± 1.1 units/mg) with intermediate activity in the medulla (142.4 ± 5.7 units/mg) (Table 3).

TABLE 2

**Formation of *S*-methylthioether from 6-bromo-2,5-dihydroxy-thiophenol and 2,5-dihydroxy-thiophenol in rat renal microsomes**

Mixtures contained 2 mg/ml freshly isolated kidney microsomes, 1.0 mM [$^{14}$C]*S*-adenosyl-L-methionine (2200 dpm/nmole), 1 mM EDTA, and 0.5% Triton X-100 in the presence and absence of 0.5 mM substrate in 0.1 M potassium phosphate buffer, pH 7.9, and were incubated at 37° for 30 min. The figures represent the mean ± SD of triplicate incubations.

| Substrate | *S*-Methylthioether Formed |
|---|---|
| | *nmol/mg/30 min* |
| 6-Bromo-2,5-dihydroxy-thiophenol | 1.7 ± 0.1 |
| 2,5-Dihydroxy-thiophenol | 11.9 ± 0.7 |

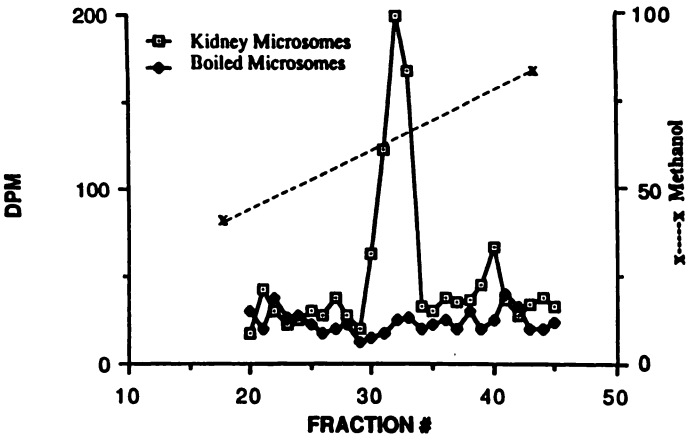


**Fig. 7.** *S*-Methylation of 6-bromo-2,5-dihydroxy-thiophenol. Rat renal microsomes (2 mg/ml) were incubated with 0.5 mM 6-bromo-2,5-dihydroxy-thiophenol and 1 mM *S*-adenosyl-L-[*methyl*-$^{14}$C]methionine in 0.1 M potassium phosphate buffer (pH 7.9) containing 1 mM EDTA and 0.5% Triton X-100 at 37° for 30 min. An aliquot of an ether extract from this incubation was analyzed by HPLC using a Whatman, reverse phase ODS-3 Partisil column and eluted with a linear gradient of water/methanol/acetic acid (89:10:1, v/v/v) to water/methanol/acetic acid (0:99:1, v/v/v) over 60 min at a flow rate of 1 ml/min.

TABLE 3

**Rat renal quinone reductase activity**

Mixtures contained, in a final volume of 1 ml, 25 mM Tris·HCl (pH 7.4), 0.7 mg of bovine serum albumin 5 μM FAD, 0.2 mM NAD(P)H, 40 μM menadione, and an appropriate amount of 10,000 × *g* supernatant of cortex, medulla, and papillae in the presence or absence of 10 μM dicumarol and were incubated at 25° for 1 min. The figures represent the mean ± SD of triplicate incubations. Enzyme activity is expressed as nmol of NAD(P)H oxidized/min.

| | Activity |
|---|---|
| | *units/mg* |
| Cortex | 38.2 ± 1.1 |
| Medulla | 142.4 ± 5.7 |
| Papillae | 269.3 ± 5.4 |

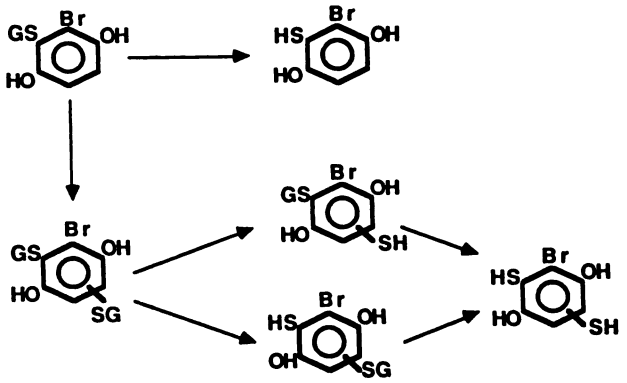

**Fig. 8.** The metabolism of 2-bromo-(glutathion-*S*-yl)hydroquinone(s) to dihydroxy-thiophenols.

## Discussion

In the present study we have synthesized 6-bromo-2,5-dihydroxy-thiophenol as a putative metabolite of nephrotoxic 2-bromohydroquinone-GSH conjugates (Fig. 8). Administration of purified 6-bromo-2,5-dihydroxy-thiophenol to rats resulted in a dose-dependent increase in BUN (Fig. 4) and histological changes in the kidney consistent with those observed after 2-bromohydroquinone-GSH intoxication (19). Interestingly, SGPT levels remained within the normal range (data not shown) and no histopathological alterations in the liver were observed. The data suggest that bromo-dihydroxy-thiophenols may contribute to the nephrotoxicity of 2-bromohydroquinone-GSH conjugates. Indeed, preliminary experiments on the *in vivo* disposition of [$^{14}$C]2-bromohydroquinone indicate the presence of thiomethyl metabolites in urine (35). This suggests that dihydroxy-thiophenols are formed *in vivo*, probably via the processing of 2-bromohydroquinone-GSH conjugates through the mercapturic acid pathway.

In contrast to the nephrotoxicity of 6-bromo-2,5-dihydroxy-thiophenol, neither 2-, 3-, or 4-bromothiophenol had any effect on the livers or kidneys of rats at doses between 0.2 and 0.8 mmol/kg (IP) (Table 1). 4-Bromothiophenol at a dose of 0.8 mmol/kg caused five of six rats to die within 24 hr. None of these deaths, however, were related to either liver or kidney damage. It appears likely, therefore, that the presence of the hydroquinone moiety of 6-bromo-2,5-dihydroxy-thiophenol is essential for the expression of nephrotoxicity inasmuch as analogs lacking this function are not toxic. By inference, the thiol group is clearly not a prerequisite for toxicity. Therefore, it is possible that the entire cascade of the mercapturic acid pathway metabolites contribute to the toxicity observed after administration of the corresponding glutathione conjugates, including the *S*-methyl derivative.

The mechanism of toxicity of the dihydroxy-thiophenols is not known but is probably related to their oxidation to the corresponding quinones. However, the mechanism of this oxidation is unclear. Oxidation could conceivably give rise to either the 1,4-quinone or a 4-hydroxy-1,2(thio) quinone (Fig. 9). In either case, if the nonenzymatically mediated oxidation of the dihydroxy-thiophenols were responsible for their nephrotoxicity, their effects might be expected to be more general in nature. The kidney-specific toxicity of the corresponding GSH conjugates may be a consequence of their selective accumulation by renal proximal tubular cells, mediated by γ-glutamyl transpeptidase (19). In support of this idea, the rate and extent of uptake of 2-bromo-(diglutathion-*S*-yl)hydroquinone into renal slices is greater than either of the three monosubstituted isomers (36). Whether a specific renal transport system exists for the uptake of dihydroxy-thiophenols is not known. Thus, the oxidation of the dihydroxy-thiophenols is probably enzymatically mediated rather than a nonspecific auto-oxidative process. In this respect, the electron transfer reactivity of these polyphenols may be controlled largely by enzyme-dependent activation requirements rather than by the oxidizability of the substrate. The kidney-specific toxicity of the dihydroxy-thiophenols could then be explained on the basis of the tissue distribution of the enzyme(s) responsible for catalyzing the oxidation of the hydroquinone to the quinone and/or the lack of quinone detoxification enzymes. With respect to the former possibility we have initially characterized an enzyme that catalyzed the activation of 2-bromohydroquinone to covalently bound material (37). This enzyme exhibits a higher activity in the kidney than in the liver. We are currently attempting to purify this enzyme. With respect to the latter possibility, it may be relevant that the toxicity of the GSH conjugates of 2-bromohydroquinone and of the dihydroxy-thiophenols is localized to the corticomedullary junction. We have shown the differential distribution of quinone reductase activity through the kidney (Table 3). Activity was found to be highest in the papilla and lowest in the cortex. Because the quinone moiety of the dihydroxy-thiophenol appears essential for the expression of toxicity, the differential distribution of quinone reductase activity could also be of toxicological significance.

Although the presence of the halogen atom in 6-bromo-2,5-dihydroxy-thiophenol is not a prerequisite for toxicity, the dose of 2,5-dihydroxy-thiophenol required to produce a similar toxicity is approximately twice that of the brominated compound. The reason for this difference is unclear, although, paradoxi-

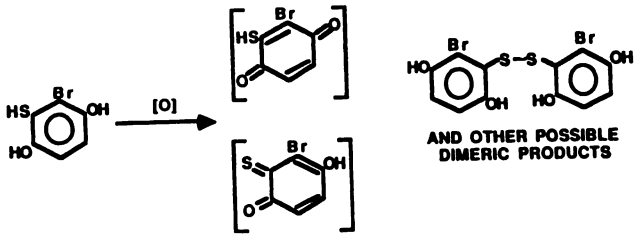


**Fig. 9.** Possible products of 6-bromo-2,5-dihydroxy-thiophenol oxidation. The dihydroxy-thiophenols could 1) undergo oxidation to the corresponding quinone(s) 2) undergo disulfide formation with either (a) a second molecule of dihydroxythiophenol, (b) glutathione, or (c) protein sulfhydryl groups, or 3) form the *S*-methyl derivative via the activity of thiol *S*-methyltransferase.

cally, the electron-withdrawing property of the halogen might be expected to make the hydroquinone less susceptible to oxidation. Another factor contributing to the more potent nephrotoxicity of 6-bromo-2,5-dihydroxy-thiophenol relative to 2,5-dihydroxy-thiophenol may be related to their different rates of *S*-methylation. Thus, although the thiol group may not be essential for toxicity it may contribute to the ease of oxidation of the hydroquinone, because the dose of 6-bromo-2,5-dihydroxy-thiophenol required to produce toxicity (0.35 mmol/kg) is less than that of 2-bromohydroquinone (0.8 mmol/kg) (38). Methylation of the thiol group might be expected to decrease its redox-enhancing effect. In this respect, the electrophilic nature of the halogen in conjunction with its bulk may explain why 2,5-dihydroxy-thiophenol methylates much faster than does 6-bromo-2,5-dihydroxy-thiophenol (Table 2). That is, the effect of the halogen is both electronic and steric.

6-Bromo-2,5-dihydroxy-thiophenol is one of several thiols that could form from the metabolism of the various isomeric 2-bromohydroquinone-GSH conjugates (Fig. 8) and in the present study it has served as a model to demonstrate that these putative metabolites do indeed exhibit toxicological activity. Attempts to synthesize the dihydroxy-dithiophenols and dihydroxy-glutathionyl-thiophenol are warranted in order to determine their relative toxicity. In conclusion, we have shown that 6-bromo-2,5-dihydroxy-thiophenol causes kidney-specific toxicity in rats. The reason for this tissue-specific toxicity is not known but may be related to its enzymatic oxidation. Dihydroxythiophenols may contribute to the nephrotoxicity caused by GSH conjugates of 2-bromohydroquinone.

**References**


1. Rush, G. F., J. H. Smith, J. F. Newton, and J. B. Hook. Chemically induced nephrotoxicity: role of metabolic activation. *Crit. Rev. Toxicol.* **13:** 99–160 (1984).
2. Smith, J. H., K. Maita, S. D. Sleight, and J. B. Hook. Mechanism of cholorform nephrotoxicity. I. Time course of chloroform toxicity in male and female mice. *Toxicol. Appl. Pharmacol.* **70:** 467–479 (1983).
3. Smith, J. H., and J. B. Hook. Mechanism of chloroform nephrotoxicity. II. *In vitro* evidence for renal metabolism of chloroform in mice. *Toxicol. Appl. Pharmacol.* **70:** 480–485 (1983).
4. Smith, J. H., and J. B. Hook. Mechanism of chloroform nephrotoxicity. III. Renal and hepatic microsomal metabolism of chloroform in mice. *Toxicol. Appl. Pharmacol.* **73:**511–524 (1984).
5. Smith, J. H., and J. B. Hook. Role of renal cytochrome P-450 in the nephrotoxicity of chloroform, in *Microsomes and Drug Oxidations* (A. R., Boobis, J. Caldwell, F. DeMatteis, and C. R. Elcombe. eds.). Taylor and Francis Press, New York, 256–264 (1985).
6. Branchflower, R. V., D. S. Nunn, R. J. Highet, J. H. Smith, J. B. Hook, and L. R. Pohl. Nephrotoxicity of chloroform: metabolism to phosgene by the mouse kidney. *Toxicol. Appl. Pharmacol.* **72:**159–168 (1984).
7. Kuo, C.-H., K. Maita, S. D. Sleight, and J. B. Hook. Lipid peroxidation: a possible mechanism of cephaloridine-induced nephrotoxicity. *Toxicol. Appl. Pharmacol.* **67:**78–88 (1983).
8. Cojocel, C., J. Hannemann, and K. Baumann. Cephalordine-induced lipid peroxidation initiated by reactive oxygen species as a possible mechanism of cephaloridine nephrotoxicity. *Biochim. Biophys. Acta.* **834:**402–410 (1985).
9. Cojocel, C., J. Hannemann, E. Inselmann, K. H. Larschke, and K. Baumann. Lipid peroxidation, a possible mechanism of cephalosporin induced nephrotoxicity. *Pflügers Arch. Eur. J. Pharmacol.* **400**(suppl.):R19, abstr. (1984).
10. Goldstein, R. S., D. A. Pasino, W. R. Hewitt, and J. B. Hook. Biochemical mechanisms of cephaloridine nephrotoxicity: time and concentration dependence of peroxidative injury. *Toxicol. Appl. Pharmacol.* **83:**261–270 (1986).
11. Hill, D. L., T. W. Shih, T. P. Johnson, and R. F. Struck. Macromolecular binding and metabolism of the carcinogen 1,2-dibromoethane. *Cancer Res.* **38:**2438–2442 (1978).
12. Rannug, U., A. Sundvall, and C. Ramel. The mutagenic effect of 1,2-dichloroethane on *Salmonella typhimurium*. I. Activation through conjugation with glutathione *in vitro*. *Chem. Biol. Interact.* **20:**1–16 (1978).
13. Ozawa, N. and F. P. Guengerich. Evidence for the formation of an *S*-[2-(*N*-guanyl) ethyl]glutathione adduct in glutathione-mediated binding of the carcinogen 1,2-dibromoethane to DNA. *Proc. Natl. Acad. Sci. USA* **80:**5266–5270 (1983).
14. van Bladeren, P. J., D. D. Breimer, G. M. T. Rotteveel-Smijs, R. A. W. de Jong, W. Buijs, A. van der Gen, and G. R. Mohn. The role of glutathione conjugation in the mutagenicity of 1,2-dibromoethane. *Biochem. Pharmacol.* **29:**2975–2982 (1980).
15. Dohn, D. R., and M. W. Anders. The enzymatic reaction of chlorotrifluoroethylene with glutathione. *Biochem. Biophys. Res. Commun.* **109:**1339–1345 (1982).
16. Wolf, R. C., P. N. Berry, J. A. Nash, T. Green, and E. A. Lock. Role of microsomal and cytosolic glutathione *S*-transferases in the conjugation of hexachloro-1,3-butadiene and its possible relevance to toxicity. *J. Pharmacol. Exp. Ther.* **228:**202–208 (1984).
17. Nash, J. A., L. J. King, E. A. Lock, and T. Green. The metabolism and disposition of hexachloro-1,3-butadiene in the rat and its relevance to nephrotoxicity. *Toxicol. Appl. Pharmacol.* **73:**124–137 (1984).
18. Reid, W. D. Mechanism of renal necrosis induced by bromobenzene or chlorobenzene. *Exp. Mol. Pathol.* **19:**197–214 (1973).
19. Monks, T. J., S. S. Lau, R. J. Highet, and J. R. Gillette. Glutathione conjugates of 2-bromohydroquinone are nephrotoxic. *Drug Metab. Dispos.* **13:**553–559 (1985).
20. Terracini, B., and V. H. Parker. A pathological study of *S*-dichlorovinyl-L-cysteine. *Food Cosmet. Toxicol.* **3:**67–74 (1965).
21. Stevens, J., and W. B. Jakoby. Cysteine conjugate β-lyase. *Mol. Pharmacol.* **23:**761–765 (1983).
22. McKinney, L. L., F. B. Weakley, A. C. Eldridge, R. E. Campbell, J. C. Cowan, J. C. Picken, Jr., and H. E. Biester. *S*-(Dichlorovinyl)-L-cysteine: an agent causing fatal aplastic anemia in calves. *J. Am. Chem. Soc.* **79:**3932–3933 (1957).
23. Schultz, M. O., P. Klubes, V. Perman, N. S. Mizuno, F. W. Bates, and J. H. Sautter. Blood dyscrasia in calves induced by *S*-(dichlorovinyl)-L-cysteine. *Blood* **14:**1015–1025 (1959).
24. Anderson, P. M., and M. O. Schultz. Cleavage of *S*-(1,2-dichlorovinyl)-L-cysteine by an enzyme of bovine origin. *Arch. Biochem. Biophys.* **111:**593–602 (1965).
25. Bhattacharya, R. K., and M. O. Schultze. Enzymes from bovine and turkey kidneys which cleave *S*-(1,2-dichlorovinyl)-L-cysteine. *Comp. Biochem. Physiol.* **22:**723–735 (1967).
26. Gandolfi, A. J., R. B. Nagle, J. J. Soltis, and F. H. Plescia. Nephrotoxicity of halogenated vinyl cysteine compounds. *Res. Commun. Chem. Pathol. Pharmacol.* **33:**249–261 (1981).
27. Bonhous, D. W., and A. J. Gandolfi. Conjugation and bioactivation of chlorotrifluroethylene. *Life Sci.* **29:**2399–2405 (1981).
28. Alcalay, W. Mono-alcoylmercapto-quinones: méthide de synthese simple. *Helv. Chim. Acta.* **30:**578–584 (1947).
29. Hinson, J. A., S. D. Nelson, and J. R. Mitchell. Studies on the microsomal formation of arylating metabolites of acetaminophen and phenacetin. *Mol. Pharmacol.* **13:**625–633 (1977).
30. Ernster, L. DT diaphorase. *Methods Enzymol.* **10:**309–317 (1967).
31. Lau, S. S., T. J. Monks, K. E. Greene, and J. R. Gillette. The role of orthobromophenol in the nephrotoxicity of bromobenzene in rats. *Toxicol. Appl. Pharmacol.* **72:**539–549 (1984).
32. Weisiger, R. A., and W. B. Jakoby. *S*-Methylation: thiol *S*-methyltransferase, in *Enzymatic Basis of Detoxification* (W. B. Jakoby, ed.). Academic Press, New York, 131–140 (1980).
33. Weisiger, R. A., and W. B. Jakoby. Thiol *S*-methyltransferase from rat liver. *Arch. Biochem. Biophys.* **196:**631–637 (1979).
34. Borchardt, R. T., and C. F. Cheng. Purification and characterization of rat liver thiol methyltransferase. *Biochim. Biophys. Acta* **522:**340–353 (1978).
35. Lau, S. S., and T. J. Monks. *In vivo* formation of nephrotoxic 2-bromohydroquinone-glutathione conjugates. *Pharmacologist* **29:**186, abstr. (1987).
36. Lau, S. S., M. G. McMenamin, and T. J. Monks. Differential uptake of 2-bromohydroquinone-glutathione conjugates into kidney slices. *Biochem. Biophys. Res. Commun.* **152:**223–230 (1988).
37. Monks, T. J., and S. S. Lau. Characterization of a renal quinol oxidase. *Pharmacologist* **29:**186, abstr. (1987).
38. Lau, S. S., T. J. Monks, and J. R. Gillette. Identification of 2-bromohydroquinone as a metabolite of bromobenzene and *o*-bromophenol: implication for bromobenzene-induced nephrotoxicity. *J. Pharmacol. Exp. Ther.* **230:**360–366 (1984).


**Send reprint requests to:** Serrine S. Lau, Ph.D., Division of Pharmacology and Toxicology, College of Pharmacy, University of Texas, Austin, TX 78712-1074.