$^{201}Tl^+$ is believed to be taken up by the (Na^+,K^+)ATPase as a K^+ analog. Studies by Skulskii *et al.* [2] and Britten and Blank [8] have demonstrated that Tl^+ acts as a K^+ analog and, in fact, has greater affinity for the (Na^+,K^+)ATPase. Furthermore, using cultured myocardial cells, McCall *et al.* [9] have reported that $^{201}Tl^+$ uptake is inhibited by either excess levels of non-radioactive Tl^+ or by the addition of either KCN or 2,4-DNP [9]. In related work, Zimmer *et al.* [10] reported that uptake of Tl^+ could be inhibited by 1 mM ouabain. The characteristics of the influx of $^{42}K^+$ and $^{201}Tl^+$ in the cultured neonatal rat myocytes demonstrated that the $^{201}Tl^+$ kinetics were similar to those of $^{42}K^+$ and that most of the influx was ouabain sensitive. The transport of $^{42}K^+$ and $^{201}Tl^+$ was reduced by the addition of unlabeled cation suggesting saturable transport systems. The transport of $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$, whose radius is similar to the hydrated potassium ion, differed greatly from $^{42}K^+$ and $^{201}Tl^+$. The diameter of the potassium ion is approximately 5 Å [5], and the diameter of the $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ is approximately 8 Å [4]. Although efficacy as an imaging agent has been demonstrated in laboratory animals [4, 11], the data presented here suggest that $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ is taken up by a mechanism different from that of K^+ and Tl^+. The rate and magnitude of $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ uptake were greater than those of $^{42}K^+$ and were not inhibited by ouabain. Moreover, there was no competitive effect from either unlabeled K^+ or Tl^+.

A similar study using the (Na^+,K^+)ATPase of human erythrocytes confirmed the results obtained using the cultured neonatal rat myocardial cells [6]. The results reported here do not support the hypothesis that $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ represents a K^+ analogue which is taken up by the myocardium by a mechanism similar to that of either $^{201}Tl^+$ or $^{42}K^+$, and myocardial imaging efficiency shown in animals is apparently not reflective of (Na^+,K^+)ATPase activity. Thus, an alternative mechanism independent of the (Na^+,K^+)ATPase is apparently involved in the $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ transport into both human erythrocytes and neonatal rat myocytes.

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REFERENCES

1. J. L. Johnson and D. L. Abernathy, *Radiology* **146**, 851 (1983).
2. I. A. Skulskii, V. Marrinen and J. Jarnefelt, *Biochim. biophys. Acta* **506**, 233 (1978).
3. B. M. Gallagher and E. Lebowitz, in *Radiopharmaceuticals: Structure and Activity Relationship* (Ed. R. P. Spencer), p. 619. Grune & Stratton, New York (1981).
4. E. Deutsch, W. Bushong, K. A. Glavan, R. C. Elder, V. J. Sodd, D. L. Fortman and S. J. Lukes, *Science* **214**, 85 (1981).
5. H. Nishiyama, E. A. Deutsch, R. J. Adolph, V. J. Sodd, K. Libson, E. L. Saenger, M. C. Gerson, M. Gabel, S. J. Lukes, J. L. Vanderheyden, D. L. Fortman, K. L. Scholz, L. W. Grossman and C. C. Williams, *J. nucl. Med.* **23**, 1093 (1982).
6. H. Sands, M. L. Delano, L. L. Camin and B. M. Gallagher, *Biochim. biophys. Acta* **812**, 665 (1985).
7. T. J. Lampidis, L. Y. Johnson and M. Israel, *Molec. cell. Cardiol.* **13**, 913 (1981).
8. J. S. Britten and M. Blank, *Biochim. biophys. Acta* **159**, 160 (1968).
9. D. McCall, L. E. Zimmer, L. D'Addabbo and K. Whitney, *Circulation* **60**, (Part II), 138 (1979).
10. L. Zimmer, D. McCall, L. D'Addabbo and K. Whitney, *Circulation* **60**, (Part II), 138 (1979).
11. E. Deutsch, K. A. Glavan, V. J. Sodd, H. Nishiyama, D. L. Ferguson and S. J. Lukes, *J. nucl. Med.* **22**, 897 (1981).

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Effect of sulfotransferase inhibitors on the 2-acetylaminofluorene-mediated lowering of rat liver *N*-hydroxy-2-acetylaminofluorene sulfotransferase activity

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2-Acetylaminofluorene (AAF) induces liver cancer in rats when administered in various feeding protocols [1–3]. *In vivo* covalent binding of AAF to cellular nucleic acids and proteins also has been found in rat liver [4]. Metabolic conversion of AAF by liver enzymes to a more reactive form, an ultimate carcinogen, is required for AAF covalent binding to cellular molecules [5]. A principal metabolic pathway implicated in the formation of protein–AAF and DNA–AAF adducts involves conversion of AAF to *N*-hydroxy-AAF (*N*-OH-AAF) by the hepatic microsomal cytochrome P-450-dependent monooxygenases [6, 7], followed by conversion of *N*-OH-AAF to the *N*-*O*-sulfate ester of *N*-OH-AAF by an *N*-OH-AAF sulfotransferase [8, 9] believed to be aryl sulfotransferase IV [10–12]. Higher levels of hepatic *N*-OH-AAF sulfotransferase activity in male rats have been correlated with increased hepatotoxicity [13] and higher hepatic cancer incidence [14] during *N*-OH-AAF administration. When *N*-OH-AAF sulfotransferase inhibitors, viz. acetanilide or pentachlorophenol (PCP), are administered with AAF or *N*-OH-AAF, hepatic cancer incidence decreases [15–18] as does carcinogen binding to macromolecules and evidence for hepatotoxicities [19–22].

Experiments to assess the role of *N*-OH-AAF sulfotransferase activity in the carcinogenic and cytotoxic actions of AAF showed that rats undergo a rapid loss of *N*-OH-AAF sulfotransferase activity when fed a diet containing AAF [23]. AAF-mediated losses in sulfotransferase activity were dose dependent, reversible, and occurred without evidence of accompanying AAF

hepatotoxicity. To further assess the significance of the AAF-mediated loss in sulfotransferase activity with respect to carcinogenic and cytotoxic roles, we have administered sulfotransferase inhibitors during AAF feeding to rats. Our findings indicate that the activity loss could be blocked by sulfotransferase inhibitors and suggest that sulfotransferase activity was required for AAF-mediated lowering of *N*-OH-AAF sulfotransferase activity.

Materials and methods

Male Holtzman rats (200–250 g) were fed a semi-synthetic diet [24] for 1 week prior to use in experiments. Rats then were fed this diet alone or supplemented with 0.05% AAF (Aldrich Chemical Co., Milwaukee, WI), 0.05% PCP (Fluka Chemical Corp., Hauppauge, NY), 0.88% acetanilide (Aldrich Chemical Co., Milwaukee, WI), 0.88% *p*-hydroxyacetanilide (Sigma Chemical Co., St. Louis, MO) or the combination of AAF and any one of the latter three xenobiotics for 1 additional week. Post-microsomal fractions were prepared from livers of these rats and their *N*-OH-AAF sulfotransferase activities were determined as previously described [23]. A typical reaction mixture contained in 0.5 ml: 100 mM Tris (pH 8.0), 10 mM *p*-nitrophenylsulfate, 20 μM PAP, 0.5 mM *N*-OH-AAF, 5% (v/v) ethanol, and 300 μg protein from post-microsomal supernatant fraction. Absorbancy at 405 nm was monitored continuously with a Gilford 250 spectrophotometer during a 10-min incubation at 31°. The rate of *p*-nitrophenol formation in control incubation mixtures, lacking *N*-OH-AAF, was subtracted from values for experimental mixtures to give an *N*-OH-AAF-dependent measure of sulfotransferase activity. Sulfotransferase activity is expressed as units per mg of post-microsomal supernatant protein; 1 unit of sulfotransferase activity was that causing 1 nmole of *p*-nitrophenol to be produced per min.

Results and discussion

Effects of supplementing control and AAF-containing diets with sulfotransferase inhibitors on rat liver *N*-OH-AAF sulfotransferase activity are shown in Table 1. As previously reported [23], rats fed an AAF-containing diet for 1 week showed a marked loss (80%) in sulfotransferase activity. The addition of acetanilide or *p*-hydroxyacetanilide to diets of control-fed rats caused little or no change in enzyme activity, while PCP caused a decrease of about 30%. Addition of the three inhibitors to AAF-containing diets resulted in hepatic sulfotransferase activities that were 2- to 3-fold greater than those among rats fed only AAF. Notwithstanding its inhibitory effect on sulfotransferase activity among rats fed control diet, inclusion of PCP in the AAF-containing diet appeared to give the greatest reversal of the AAF-mediated lowering of sulfotransferase activity. In light of its effectiveness, PCP was employed in further studies. A dose–response study of PCP reversal of the AAF-mediated loss in sulfotransferase activity (Fig. 1) indicated a dose-dependent response at PCP concentrations lower than 0.05% (w/w), but complete recovery of sulfotransferase activity was not obtained at higher PCP concentrations. Since PCP alone caused a dose-related reduction in sulfotransferase values among rats fed control diets (Fig. 1), perhaps this effect of PCP caused the decline in sulfotransferase activities at PCP concentrations >0.05% (w/w). If data from Fig. 1 are expressed as the ratio of AAF-fed : control-fed, then recovery of sulfotransferase activity as a function of increasing PCP concentration appears more complete, e.g. for PCP concentrations shown in Fig. 1, the AAF-fed : control-fed ratios were 0.18, 0.47, 0.70, 0.75 and 0.75 respectively. To determine whether PCP reversal of the AAF-mediated loss in sulfotransferase activity required simultaneous presence of PCP, experiments were conducted to examine the effect of conditioning rats with PCP prior to their exposure to AAF. When rats were pre-conditioned for 1 week on either control diet or control diet supplemented with 0.05% (w/w) PCP and then fed the AAF-containing diet for another week, sulfotransferase activities among rats in either group were similar, viz. 27 ± 5 and 28 ± 4% of activities among rats fed only control diet. Thus, no PCP-mediated recovery of activity was detected. Furthermore, no enhancement of

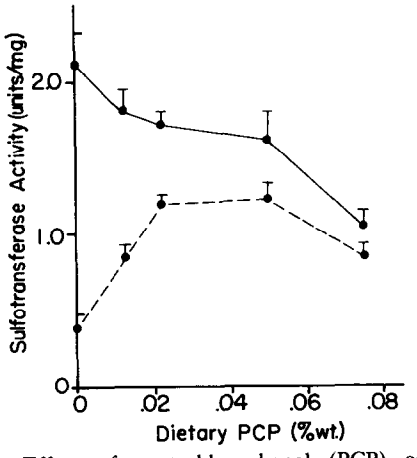

Fig. 1. Effect of pentachlorophenol (PCP) on sulfotransferase activity of livers from rats fed control diet or control diet supplemented with AAF (0.05%). Rats were fed control or AAF-supplemented diets containing 0, 0.0125, 0.025, 0.05 or 0.075% (w/w) PCP for 1 week. Post-microsomal supernatant fractions were prepared, and sulfotransferase activities were determined as described in Materials and Methods. Each point represents mean ± S.E. of values for nine individual rats. Key: control-fed (●—●) and AAF-fed (●- -●).

Table 1. Influence of sulfotransferase inhibitors on AAF-mediated loss of sulfotransferase activity in rat liver

Dietary supplement	*N*-OH-AAF sulfotransferase activity* (units/mg)	
	Control-diet	AAF-diet
None	1.81 ± 0.10	0.29 ± 0.11
Acetanilide (0.88%)	1.65 ± 0.20	0.69 ± 0.06†
p-Hydroxyacetanilide (0.88%)	1.81 ± 0.14	0.50 ± 0.03†
Pentachlorophenol (0.05%)	1.28 ± 0.04‡	0.76 ± 0.09†

* Values are mean ± S.E. of nine individual rats.
† Significantly ($P < 0.05$) different from AAF alone.
‡ Significantly ($P < 0.05$) different from control alone.

Table 2. Paired feeding study of pentachlorophenol (PCP) reversal of AAF-mediated lowering of rat liver *N*-OH-AAF sulfotransferase activity*

Diet	Amount available (g/rat)	Amount eaten (g/rat)	Sulfotransferase activity (units/mg)
AAF (0.05%) + PCP (0.05%)	19.0	12.0 ± 0.5	0.98 ± 0.13
AAF (0.05%)	12.0	9.6 ± 0.6	0.38 ± 0.07
Control	10.0	8.8 ± 0.4	1.85 ± 0.11

* Values are mean ± S.E. of four individual rats.

the PCP reversal of AAF-mediated loss in *N*-OH-AAF sulfotransferase activity was observed when, following 1 week of PCP-diet or control-diet pre-conditioning, rats were fed for 1 week a diet containing both AAF and PCP. Under these circumstances, sulfotransferase activity values were 68 ± 7 and 66 ± 6% of control-fed values respectively. These data suggest that PCP reversal of the AAF-mediated loss of sulfotransferase activity requires the simultaneous administration of PCP and AAF.

Since the AAF-mediated loss of *N*-OH-AAF sulfotransferase activity was AAF-dose-dependent [23], a paired feeding study was conducted to ensure that the PCP reversal of the AAF-mediated decrease in sulfotransferase activity was not the result of decreases in the amount of AAF ingested when sulfotransferase inhibitor also was present. As shown in Table 2, when AAF-fed rats were restricted to amounts of diet consumed by rats fed both AAF plus PCP (12 g/rat), they consumed less diet (9.6 g/rat) but still displayed a 2- to 3-fold lower sulfotransferase activity than did rats fed AAF plus PCP. Furthermore, when control-fed rats were restricted to amounts consumed by AAF-fed rats, they still displayed the high, normal values of activity displayed by rats fed control diet *ad lib.* These data indicated that neither the AAF-mediated lowering of rat liver *N*-OH-AAF sulfotransferase activity nor its reversal by simultaneous PCP administration resulted from differences in diet consumption.

Two mechanisms advanced to explain the reduction of AAF carcinogenesis and AAF cytotoxicity by *N*-OH-AAF sulfotransferase inhibitors are: (1) the acetanilide sulfate-trap mechanism [16], whereby acetanilide metabolites combine with sulfate to deplete sulfate and thereby reduce enzymatic production of the ultimate carcinogenic form of AAF, the *N*-*O*-sulfate ester of *N*-OH-AAF, and (2) the PCP dead-end inhibitor mechanism [25], wherein PCP binds to *N*-OH-AAF sulfotransferase and inhibits enzymatic production of the *N*-*O*-sulfate ester of *N*-OH-AAF. Both proposed mechanisms involve a proximal influence of inhibitor at the enzyme level which leads to reduced *N*-*O*-sulfation of *N*-OH-AAF. Our data also suggest a proximal influence of PCP in its reversal of the AAF-mediated decrease in sulfotransferase activity, i.e. a lack of influence of PCP pre-conditioning on the AAF lowering of enzyme activity and a PCP dose-responsiveness for the reversal of the AAF-mediated loss. In light of the above, it is possible to offer an explanation for the loss in *N*-OH-AAF sulfotransferase activity observed upon AAF administration and the reversal of this loss observed during the simultaneous administration of *N*-OH-AAF sulfotransferase inhibitors. The AAF-mediated decrease in *N*-OH-AAF sulfotransferase activity could be due to a loss in enzyme activity resulting from an inactivation of the enzyme by reaction with its own highly reactive reaction product, the *N*-*O*-sulfate ester of *N*-OH-AAF. The presence of inhibitors would prevent inactivations of the enzyme by preventing formation of the highly reactive *N*-*O*-sulfate conjugate. The possibility also exists that PCP could have caused the lowering of the sulfate ester of *N*-OH-AAF by inhibiting cytochrome P-450 catalyzed *N*-hydroxylation of AAF, thereby diminishing the amount of *N*-OH-AAF available for sulfation. However, cytochrome P-450 activity has been reported previously not to be lowered but increased slightly during PCP dietary administration [26]. Thus, the primary inhibitory action of PCP would be restricted to the level of the sulfotransferase reaction. Future studies using antibody to *N*-OH-AAF sulfotransferase to determine whether decreases in enzyme activity resulted from decreased enzyme specific activity or enzyme amount should aid in establishing whether the above explanation is valid.

In summary, this study demonstrated that the simultaneous administration of the sulfotransferase inhibitors, acetanilide, its metabolite, *p*-hydroxyacetanilide, or PCP with AAF, resulted in a reversal of the AAF-mediated loss in *N*-OH-AAF sulfotransferase activity. Furthermore, in studies using PCP, this reversal was shown to be inhibitor dose-dependent and to occur during coadministration of PCP with AAF, but not following pre-conditioning with PCP. We suggest these results indicate that *N*-OH-AAF sulfotransferase activity is required for the AAF-mediated lowering of sulfotransferase activity observed in the liver of rats fed AAF.

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REFERENCES

1. G. W. Teebor and F. F. Becker, *Cancer Res.* **31**, 1 (1971).
2. C. Peraino, R. J. M. Fry and E. Staffeldt, *Cancer Res.* **31**, 1506 (1971).
3. B. Lombardi and H. Shinozuka, *Int. J. Cancer* **23**, 565 (1979).
4. E. C. Miller and J. A. Miller, *Pharmac. Rev.* **18**, 805 (1966).
5. E. C. Miller and J. A. Miller, *Cancer, N.Y.* **47**, 2327 (1981).
6. J. A. Miller, J. W. Cramer and E. C. Miller, *Cancer Res.* **20**, 950 (1960).
7. S. S. Thorgeirsson, J. S. Felton and D. W. Nebert, *Molec. Pharmac.* **11**, 159 (1975).
8. C. M. King and B. Phillips, *Science* **159**, 1351 (1968).
9. J. R. DeBaun, J. Y. Rowley, E. C. Miller and J. A. Miller, *Proc. Soc. exp. Biol. Med.* **129**, 268 (1968).
10. W. B. Jakoby, *Meth. Enzym.* 77, 197 (1981).
11. W. B. Jakoby, R. D. Sekura, E. S. Lyon, C. L. Marcus and J-L. Wang, in *Enzymatic Basis of Detoxication* (Ed. W. B. Jakoby), Vol. II, pp. 199–228. Academic Press, New York (1980).
12. R. D. Sekura and W. B. Jakoby, *Archs Biochem. Biophys.* **211**, 352 (1981).

13. C. C. Irving, *Cancer Res.* **35**, 2959 (1975).
14. J. R. DeBaun, E. C. Miller and J. A. Miller, *Cancer Res.* **30**, 577 (1970).
15. R. S. Yamamoto, R. M. Glass, H. H. Frankel, E. K. Weisburger and J. H. Weisburger, *Toxic. appl. Pharmac.* **13**, 108 (1968).
16. J. H. Weisburger, R. S. Yamamoto, G. M. Williams, P. H. Grantham, T. Matsushima and E. K. Weisburger, *Cancer Res.* **32**, 491 (1972).
17. R. S. Yamamoto, G. M. Williams, H. L. Richardson, E. K. Weisburger and J. H. Weisburger, *Cancer Res.* **33**, 454 (1973).
18. J. H. Weisburger, E. K. Weisburger, R. M. Madison, M. L. Wenk and D. S. Klein, *J. natn. Cancer Inst.* **51**, 235 (1973).
19. L. C. Mohan, P. H. Grantham, E. K. Weisburger, J. H. Weisburger and J. B. Idoine, *J. natn. Cancer Inst.* **56**, 763 (1976).
20. J. H. N. Meerman, A. B. D. van Doorn and G. J. Mulder, *Cancer Res.* **40**, 3772 (1980).
21. J. H. N. Meerman, F. A. Beland and G. J. Mulder, *Carcinogenesis* **2**, 413 (1981).
22. J. H. N. Meerman and G. J. Mulder, *Life Sci.* **28**, 2361 (1981).
23. D. P. Ringer, K. Kampschmidt, R. L. King, Jr., S. Jackson and D. E. Kizer, *Biochem. Pharmac.* **32**, 315 (1983).
24. G. Medes, B. Friedmann and S. Weinhouse, *Cancer Res.* **16**, 57 (1956).
25. M. W. Duffel and W. B. Jakoby, *J. biol. Chem.* **256**, 11123 (1981).
26. F. M. H. Debets, J. J. T. W. A. Strik and K. Olie, *Toxicology* **15**, 181 (1980).

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Hymenoxon: biologic and toxic effects

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Hymenoxys odorata (bitterweed) is an economically important poisonous range plant indigenous to the southwestern United States [1]. Hymenoxon, a sesquiterpene lactone, has been isolated from bitterweed, and laboratory studies have demonstrated that this compound elicits toxic symptoms in goats, rabbits and sheep which resemble those reported for bitterweed poisoning in the field [2, 3]. Previous studies on the mechanism of action of hymenoxon have been equivocal [4–12]. For example, pretreatment of mice with carbon tetrachloride, a compound which partially inactivates hepatic microsomal monooxygenases, affords some protection from the acute toxicity of this compound [9]. However, pretreatment of mice with either phenobarbital or Aroclor 1254, two inducers of hepatic monooxygenases and drug-metabolizing enzymes, does not increase the toxicity of hymenoxon [9]. Kim and coworkers have also reported that the antioxidant ethoxyquin (EQ) [10] and cysteine [11] offer some protection from the toxicity of hymenoxon in sheep and mice. In contrast, butylated hydroxyanisole (BHA) increases the LD_{50} of hymenoxon in mice but not sheep [10]. Since BHA and EQ induce hepatic glutathione transferases, it is possible that alterations in hepatic thiol levels and their conjugating enzymes may be important determinants in the mechanism of action of hymenoxon [13, 14]. However, BHA and EQ also act as cellular radical scavengers and induce microsomal cytochrome P-450-dependent monooxygenases, and these processes may also be involved in hymenoxon toxicity [15].

This study probes the mechanism of action of hymenoxon by determining the effects of a toxic dose of this compound (20 mg/kg) on mouse hepatic drug-metabolizing enzymes and related activities, including the microsomal monooxygenases, cytochrome P-450 levels, relative hepatic levels of reduced and oxidized glutathione (GSH and GSSG, respectively), GSH *S*-transferase (GSH Tase), GSSG reductase (GSSG Red), GSH peroxidase (GSH Px) and lipid peroxidation (LP) (determined as malondialdehyde levels in liver tissue).

Materials and methods

Hymenoxon (m.p. 135.5–136.5°, molecular weight 282.3) was extracted from dried ground bitterweed by the procedure of Kim *et al.* [3]. Corn oil/dimethyl sulfoxide (DMSO) (10 ml/kg) and hymenoxon (20 mg/kg) in corn oil/DMSO) were administered i.p. to immature male ICR mice (25–30 g, four mice for each time point) as a single dose 4, 8, 12, 72, 192 and 240 hr before the animals were killed. The mice were maintained on food and water *ad lib.* and were fasted 12 hr before sacrifice. Mice were killed by cervical dislocation, and the livers were immediately perfused with cold isotonic saline, pH 7.3.The livers were excised, weighed and minced in 0.25 M sucrose, pH 7.4. The microsomal (100,000 *g*) and soluble (supernatant) fractions were isolated by differential centrifugation as described by Mazel [16] and stored at 10°. Biochemical determinations were performed on the fresh preparations.

The cytochrome P-450 content was determined spectrophotometrically by difference spectrum using the molar extinction coefficient of 91 mM^{-1} cm^{-1} [17]. Dimethylaminoantipyrine (DMAP) *N*-demethylase and arylhydrocarbon hydroxylase (AHH) were measured as previously described [18]. GSH Tase activity towards 1-chloro-2,4-dinitrobenzene (CDNB) was measured spectrophotometrically at 340 nm [19]. GSH Px activity towards H_2O_2 and GSSG Red activity were assayed spectrophotometrically by determining the rate of NADPH oxidation at 340 nm, using the molar extinction coefficient for NADPH of 6.22×10^6 M^{-1} cm^{-1} [20]. The extent of microsomal lipoperoxidation was determined by measuring malondialdehyde production as thiobarbituric acid (TBA)-reactive substances at 535 nm [21]. The protein content of microsomal and soluble fractions was determined by the method of Lowry *et al.* [22]. Level of GSH and GSSG were measured in a tissue homogenate using an enzymatic recycling procedure [23]. All biochemical determinations were measured in fasted mice. Hymenoxon was added to the *in vitro* incubation of different compounds varying in sulfhydryl content. Hymenoxon (0.75 mg) in 0.1 ml of DMSO was added to 0.9 ml of 0.25 M phosphate buffer, pH 6.5, containing the polypeptide (3.0 mg). The solution was incubated at 20° for 30 min, and the reaction was terminated by the addition of ethyl acetate (400 μl) which was used to extract the unreacted hymenoxon. The amount of hymenoxon reacting with the polypeptides was derived from the quantitation of the unreacted compound using the appropriate standard curves determined for recoveries of hymenoxon employing a Tracor 565 gas chromatograph equipped with a flame ionization detector containing a 3 ft column packed with 3% OV-1 on Chromasorb W. The