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INHIBITION OF HEPATIC MIXED-FUNCTION OXIDASE ACTIVITY *IN VITRO* AND *IN VIVO* BY VARIOUS THIONO-SULFUR-CONTAINING COMPOUNDS*

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Abstract—Ten thiono-sulfur-containing compounds of varying structure were administered by intraperitoneal injection to untreated, phenobarbital-pretreated and 3-methylcholanthrene-pretreated adult male rats. Six hr later, the concentration of hepatic cytochrome P-450 and the ability of the hepatic microsomes to metabolize benzphetamine were examined. In the untreated, phenobarbital-pretreated and 3-methylcholanthrene-pretreated groups, two, four and four compounds, respectively, significantly decreased the concentration of cytochrome P-450 in the hepatic microsomes. A similar effect on benzphetamine metabolism was also seen. When examined 48 hr after the administration of the ten thiono-sulfur-containing compounds, four, five and seven of the compounds decreased both the levels of hepatic cytochrome P-450 and the rate of benzphetamine metabolism in the untreated, phenobarbital-pretreated and 3-methylcholanthrene-pretreated animals respectively. Eight of the thiono-sulfur-containing compounds were incubated in the presence of NADPH with hepatic microsomes isolated from untreated, phenobarbital-pretreated or 3-methylcholanthrene-pretreated animals. All of the compounds examined significantly decreased the concentration of cytochrome P-450 in the microsomes from each treatment group. Similar reductions in benzphetamine metabolism were also seen. When these same compounds were incubated with microsomes in the absence of NADPH, no significant reduction of cytochrome P-450 or benzphetamine metabolism was seen. When the oxygen analogs of six of the thiono-sulfur compounds were administered *in vivo* or incubated with hepatic microsomes either in the presence or absence of NADPH, no significant reduction of cytochrome P-450 or benzphetamine metabolism was seen.

Recent research [1, 2] has demonstrated that during the hepatic mixed-function oxidase-catalyzed metabolism of *O,O*-diethyl *p*-nitrophenyl phosphorothionate (parathion) *in vitro* to its oxygen analog, *O,O*-diethyl *p*-nitrophenyl phosphate (paraoxon), 75–95 per cent of the sulfur released becomes covalently bound to the microsomal macromolecules. Accompanying the release and binding of sulfur, which was shown to be NADPH dependent, was a decrease in cytochrome P-450 detectable as the carbon monoxide complex [2, 3] and a decreased ability of these microsomes to metabolize benzphetamine [2]. This decrease in cytochrome P-450 and ability to metabolize benzphetamine appeared to be roughly proportional to the amount of sulfur bound to the microsomes.

Several investigators have reported on the hepatotoxicity of carbon disulfide (CS_2) in phenobarbital-pretreated rats. It has been postulated that this toxicity is a result of the hepatic mixed-function oxidase-catalyzed metabolism of CS_2 to a more toxic metabolite [4–8]. However, it has also been suggested that the toxic effect of this compound might not be mediated through its biotransformation to a more toxic metabolite but may result from an increased susceptibility to the action of CS_2 itself [9, 10].

Recently, CS_2 has been shown to be metabolized *in vitro* by hepatic microsomes to its oxygen analog, carbonyl sulfide (COS) [6]. Analogous to parathion, the NADPH-stimulated metabolism *in vitro* of CS_2 to COS resulted in the release and covalent binding of the sulfur atom. This release and binding of sulfur from CS_2 bring about a decrease in the level of hepatic cytochrome P-450 detectable as its carbon monoxide complex [3, 8] and a decrease in the activity of the mixed-function oxidase enzyme system [8]. This decrease in cytochrome P-450 and mixed-function oxidase activity appeared to be proportional to the amount of sulfur bound [8].

The similarity of the results reported for these two thiono-sulfur-containing compounds, parathion and carbon disulfide, suggests that any sufficiently lipid soluble thiono-sulfur-containing compound could be metabolized by the hepatic mixed-function oxidase system, with the release and covalent binding of the sulfur atom resulting in a decrease in cytochrome P-450 levels and a decrease in mixed-function oxidase activity. If the substrate concentration and maximal velocity for the metabolism of these thiono-sulfur-containing compounds *in vivo* are high enough, it also appears possible that the production of hepatic necrosis, as is seen with CS_2, may result from the covalent binding of sulfur [3, 6, 8].

The purpose of the experiments reported in this study was to examine several compounds containing the thiono-sulfur group, or which have resonance

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forms containing the thiono-sulfur group, as well as the oxygen analogs of these compounds, for their effects on hepatic cytochrome P-450 and mixed-function oxidase activity, both *in vivo* and *in vitro*, and their ability to produce hepatic necrosis. Since a great deal is known about its effect on these parameters, CS_2 was included in these studies as a standard to which the biological and biochemical effects of the other thiono-sulfur-containing compounds could be compared.

METHODS

Adult male Sprague–Dawley rats (200–250 g), which were given food and water *ad lib.*, were used for all experiments. All compounds used in these studies were dissolved in distilled water or corn oil, depending on their solubility in these solvents, and were administered intraperitoneally using a solvent volume of 2·5 ml/kg. Controls received an equal volume of the appropriate solvent.

All thiono-sulfur compounds and the corresponding oxygen analogs were administered at a level of 5 m-moles/kg with the exception of methimazole, propylthiouracil and disulfiram, which were administered at a dose of 2·5 m-moles/kg. Methimazole was administered at a dose of 1·25 m-moles/kg to 3-methylcholanthrene-pretreated rats. The acute toxicity of these compounds necessitated administration of the lower doses.

Phenobarbital-treated animals received 50 mg/kg of sodium phenobarbital, i.p., in distilled water for 5 consecutive days. Twenty-four hr after the last injection, the rats were sacrificed if microsomes were to be isolated for studies *in vitro*. In the studies *in vivo* the thiono-sulfur compounds or their oxygen analogs were administered 24 hr after the last injection of phenobarbital. The compound 3-methylcholanthrene was administered, i.p., as a single dose of 20 mg/kg in corn oil (1 ml/kg) 3 days before the rats were to be sacrificed for isolation of microsomes or before thiono-sulfur administration in the studies *in vivo*.

In the studies *in vivo*, the animals were sacrificed by cervical dislocation 6 and 48 hr after administration of the thiono-sulfur-containing compounds. The livers were removed immediately, perfused with cold 1·15% KCl and microsomes were isolated as described previously [11]. Cytochrome P-450 levels were determined by the method of Omura and Sato [12] and *N*-demethylation of benzphetamine was measured according to the procedure of Cochin and Axelrod [13]. The biuret method modified to include 0·1 ml of 1% sodium deoxycholate in each sample was used to measure the amount of protein in the various microsomal preparations [14].

Incubations *in vitro* were performed in 0·05 M Hepes buffer, pH 7·8, without and with an NADPH-generating system (NADP, 5 μmoles; glucose 6-phosphate, 15 μmoles; glucose 6-phosphate dehydrogenase, 1 unit) and contained 0·5 to 2·0 mg microsomal protein/ml of incubation media. These incubations also contained 1×10^{-3} M concentrations of the thiono-sulfur compounds or their oxygen analogs added as a solution in 0·1 ml methanol or distilled water, depending on their solubility, and 1×10^{-4} M butylated hydroxytoluene (BHT) in a total volume of 5 ml. The control incubations were as described above except that the thiono-sulfur compounds and their oxygen analogs were omitted. After a 15-min incubation, the microsomes were isolated by the Ca^{2+} aggregation method as modified by Cinti *et al.* [15] and resuspended in 0·1 M phosphate buffer, pH 7·0. *N*-demethylation of benzphetamine, cytochrome P-450 levels and protein concentration were then determined.

Glucose 6-phosphate, NADP and glucose 6-phosphate dehydrogenase were purchased from Boehringer Mannheim Corp. (New York, N. Y.). Benzphetamine was a gift of the Upjohn Co., Kalamazoo, Mich. The compounds 6-*n*-propylthiouracil, 6-methylthiouracil and methyluracil were purchased from the Sigma Chemical Co., St. Louis, Mo. Acetamide, uracil, 2-imidazolidinone and 2-imidazolidinethione were obtained from the Eastman Chemical Co., Rochester, N. Y. Thiourea, urea, thioacetamide and sodium diethyldithiocarbamate were purchased from Fisher Scientific Co., Fair Lawn, N. J. Thiouracil was obtained from Nutritional Biochemical Co., Cleveland, Ohio, and 2-mercapto-1-methylimidazole (Methimazole) from the K & K Laboratories, Plainview, N. Y. Disulfiram was obtained from Aldrich Chemical Co., Metuchen, N. J.

All experiments *in vivo* were done using three animals in each treatment group. The experiments *in vitro* where the thiono-sulfur-containing substrates and their oxygen analogs were incubated with isolated microsomes were done in duplicate, using microsomes isolated from the pooled livers of eight rats. The means and standard deviation of the means were calculated and levels of significance between treatment groups or the various incubations *in vitro* and the appropriate control were determined using the Student's *t*-test.

RESULTS

An initial estimate of the rapidity with which the various thiono-sulfur compounds had an effect, if any, on the levels of hepatic cytochrome P-450 and mixed-function oxidase activity *in vivo* was obtained by administering the compounds to untreated, phenobarbital-pretreated and 3-methylcholanthrene-pretreated rats, and sacrificing the animals 6 hr later. As is illustrated in Table 1, only two of the compounds, CS_2 and diethyldithiocarbamate, significantly decreased the levels of cytochrome P-450 detectable as the carbon monoxide complex in untreated animals as compared to controls. A corresponding decrease in mixed-function oxidase activity was also seen. In addition, methylthiouracil significantly decreased mixed-function oxidase activity toward benzphetamine, while thiouracil and thioacetamide appeared to increase it.

Using phenobarbital-pretreated rats, CS_2 and diethyldithiocarbamate decreased both cytochrome P-450 levels and the ability of microsomes to demethylate benzphetamine. In addition, propylthiouracil and thiouracil brought about a decrease in cytochrome P-450 levels. Thiourea significantly decreased mixed-function oxidase activity as measured by benzphetamine metabolism.

Table 1. Effect of administration *in vivo* of thiono-sulfur compounds on the concentration of cytochrome P-450 and the metabolism of benzphetamine measured 6 hr after administration

Thiono-sulfur compound	Dose (m-moles/kg)	Untreated		Phenobarbital-pretreated		3-Methylcholanthrene-pretreated	
		Cytochrome P-450*	Benzphetamine metabolism†	Cytochrome P-450*	Benzphetamine metabolism†	Cytochrome P-450*	Benzphetamine metabolism†
Sodium diethyldithiocarbamate	5	0·34 ± 0·05‡	1·90 ± 0·21‡	0·84 ± 0·11‡	4·55 ± 0·27‡	0·38 ± 0·71‡	0·66 ± 0·30‡
Thiourea	5	0·48 ± 0·01	2·78 ± 0·29	1·09 ± 0·16	4·26 ± 0·04‡	0·73 ± 0·26	1·34 ± 0·10‡
Methimazole	2·5	0·59 ± 0·07	2·57 ± 0·25	1·47 ± 0·17	6·26 ± 0·83	0·64 ± 0·02‡	2·53 ± 0·00
Thioacetamide	5	0·59 ± 0·04	3·27 ± 0·30‡	1·45 ± 0·16	5·90 ± 1·44	0·85 ± 0·32	1·56 + 0·21‡
Control		0·52 ± 0·07§	2·46 ± 0·09§	1·35 ± 0·10§	7·17 ± 1·20§	1·06 ± 0·06§	2·49 ± 0·66§
2-Imidazolidinethione	5	0·54 ± 0·05	4·07 ± 0·29	1·32 ± 0·29	6·90 ± 2·35	0·79 ± 0·12	3·22 ± 0·56
Thiouracil	5	0·55 ± 0·03	4·18 ± 0·17‡	1·19 ± 0·00‡	7·85 ± 0·41	0·98 ± 0·21	2·49 ± 0·43
Methylthiouracil	5	0·57 ± 0·07	2·93 + 0·31‡	1·56 + 0·05	8·48 ± 0·93	1·42 ± 0·46	3·42 ± 0·56
Disulfiram	2·5	0·42 ± 0·05	3·71 ± 0·20	1·36 ± 0·07	7·99 ± 0·55	0·84 ± 0·15	2·50 ± 0·78
Propylthiouracil	2·5	0·51 ± 0·05	3·99 ± 0·76	0·97 ± 0·03‡	5·61 ± 0·73	0·45 ± 0·05‡	1·42 ± 0·17‡
Carbon disulfide	5	0·18 ± 0·05‡	1·29 ± 0·10‡	0·72 ± 0·21‡	4·60 ± 0·93‡	0·48 ± 0·15‡	1·30 ± 0·89‡
Control		0·57 ± 0·06‖	3·58 ± 0·26‖	1·47 ± 0·01‖	8·57 ± 1·83‖	0·94 ± 0·07‖	2·73 ± 0·94‖

* Cytochrome P-450 concentrations are expressed as nmoles/mg of hepatic microsomal protein. These values represent the means ± S. D. of the means of one determination with each of three animals in each treatment group.

† Benzphetamine metabolism is expressed as nmoles formaldehyde formed/mg of microsomal protein/min. These values represent the means ± S. D. of the means of one determination with each of three animals in each treatment group.

‡ Denotes significant differences at or below the 5 per cent level as compared to control animals.

§ Sodium diethyldithiocarbamate, thiourea, methimazole and thioacetamide were administered in distilled water. These control animals were injected with distilled water (2·5 ml/kg) at the time of injection of the experimental animals with various thiono-sulfur compounds.

‖ The compounds 2-imidazolidinethione, thiouracil, methylthiouracil, disulfiram, propylthiouracil and carbon disulfide were administered in corn oil. These control animals were injected with corn oil (2·5 ml/kg) at the time of injection of the experimental animals with various thiono-sulfur compounds.

Pretreatment with 3-methylcholanthrene resulted in CS_2, diethyldithiocarbamate, propylthiouracil and methimazole decreasing cytochrome P-450 levels. Likewise, CS_2, diethyldithiocarbamate, thiourea, propylthiouracil and thioacetamide significantly decreased mixed-function oxidase activity toward benzphetamine.

The results obtained when the rats were sacrificed 48 hr after the administration *in vivo* of the thiono-sulfur compounds are shown in Table 2. Increased exposure time resulted in a greater number of the compounds decreasing hepatic cytochrome P-450 levels and mixed-function oxidase activity, using benzphetamine as a substrate.

CS_2, disulfiram, thioacetamide and propylthiouracil significantly decreased cytochrome P-450 levels and produced a corresponding decrease in mixed-function oxidase activity in untreated rats. In addition, methylthiouracil significantly decreased mixed-function oxidase activity as determined by measuring benzphetamine metabolism. None of the rats in the untreated group exhibited hepatic necrosis.

Pretreatment of the rats with phenobarbital produced results similar to those observed in the untreated rats. However, in addition to those compounds which had an effect on cytochrome P-450 levels and benzphetamine metabolism in untreated rats, diethyldithiocarbamate and thiouracil decreased

Table 2. Effect of administration *in vivo* of thiono-sulfur compounds on the concentration of cytochrome P-450 and the metabolism of benzphetamine measured 48 hr after administration

Thiono-sulfur compound	Dose (m-moles/kg)	Untreated		Phenobarbital-pretreated		3-Methylcholanthrene-pretreated	
		Cytochrome P-450*	Benzphetamine metabolism†	Cytochrome P-450*	Benzphetamine metabolism†	Cytochrome P-450*	Benzphetamine metabolism†
Sodium diethyldithiocarbamate	5	0·59 ± 0·24	2·93 ± 1·00	0·34 ± 0·03‡	2·88 ± 0·31‡	0·45 ± 0·80‡	2·30 ± 0·16‡
Thiourea	5	0·52 ± 0·02	2·84 ± 0·62	0·86 ± 0·09	4·74 ± 1·22‡	0·66 ± 0·21	0·97 ± 0·12‡
Methimazole	2·5	0·48 ± 0·02	2·27 ± 0·60	0·73 ± 0·01	4·06 ± 0·50‡	0·50 ± 0·04‡	2·59 ± 0·25
Thioacetamide	5	0·37 ± 0·09‡	1·96 ± 0·36‡	0·72 ± 0·26	0·90 ± 0·41‡	0·25 ± 0·19‡	1·10 ± 0·47‡
Control		0·56 ± 0·04§	3·02 ± 0·45§	1·06 ± 0·16§	8·05 ± 0·60§	0·86 ± 0·12§	3·67 ± 1·22§
2-Imidazolidinethione	5	0·46 ± 0·05	2·12 ± 1·89	0·86 ± 0·03	4·06 ± 1·14‡	0·56 ± 0·10	1·38 ± 0·26‡
Thiouracil	5	0·44 ± 0·06	2·10 ± 0·17	0·48 ± 0·04‡	4·16 ± 1·62	0·46 ± 0·11‡	1·19 ± 0·35‡
Carbon disulfide	5	0·31 ± 0·06‡	1·50 ± 0·22‡	0·47 ± 0·14‡	2·81 ± 0·74‡	0·59 ± 0·28	2·07 ± 0·57
Methylthiouracil	5	0·44 ± 0·07	1·72 ± 0·49‡	0·65 ± 0·15	5·45 ± 0·46	0·36 ± 0·15‡	1·90 ± 0·18‡
Disulfiram	2·5	0·29 ± 0·16‡	0·91 ± 0·62‡	0·65 ± 0·04‡	1·38 ± 0·69‡	0·36 ± 0·00‡	1·00 ± 0·07‡
Propylthiouracil	2·5	0·33 ± 0·03	1·43 ± 0·49‡	0·45 ± 0·80‡	2·52 ± 1·27‡	0·27 ± 0·05‡	1·71 ± 0·11‡
Control		0·47 ± 0·05‖	2·54 ± 0·52‖	0·89 ± 0·14‖	6·94 ± 2·01‖	0·68 ± 0·07‖	3·04 ± 1·26‖

* Cytochrome P-450 concentrations are expressed as nmoles/mg of hepatic microsomal protein. These values represent the means ± S. D. of the means of one determination with each of three animals in each treatment group.

† Benzphetamine metabolism is expressed as nmoles formaldehyde formed/mg of microsomal protein/min. These values represent the means ± S. D. of the means of one determination with each of three animals in each treatment group.

‡ Denotes significant differences at or below the 5 per cent level as compared to control animals.

§ Sodium diethyldithiocarbamate, thiourea, methimazole and thioacetamide were administered in distilled water. These control animals were injected with distilled water (2·5 ml/kg) at the time of injection of the experimental animals with various thiono-sulfur compounds.

‖ The compounds 2-imidazolidinethione, thiouracil, methylthiouracil, disulfiram, propylthiouracil and carbon disulfide were administered in corn oil. These control animals were injected with corn oil (2·5 ml/kg) at the time of injection of the experimental animals with various thiono-sulfur compounds.

cytochrome P-450 levels, and diethyldithiocarbamate, thiourea, 2-imidazolidinethione and methimazole decreased the rate of benzphetamine *N*-demethylation. In addition to a greater number of the compounds studied decreasing cytochrome P-450 and benzphetamine metabolism, these two parameters were decreased to a greater degree in the phenobarbital-pretreated as compared to untreated animals. Also, varying degrees of centrilobular hepatic necrosis were produced in the phenobarbital-pretreated animals by five of the compounds tested. Listed in the order of the severity of necrosis, these are: thioacetamide > propylthiouracil ≥ diethyldithiocarbamate > methimazole ≥ CS_2.

Administration of the thiono-sulfur compounds to animals pretreated with 3-methylcholanthrene resulted in a decrease in cytochrome P-450 in the animals given diethyldithiocarbamate, disulfiram, thioacetamide, thiouracil, methylthiouracil, propylthiouracil and methimazole. With the exception of CS_2 and methimazole, all of the compounds studied decreased the ability of microsomes to metabolize benzphetamine. No hepatic necrosis was seen in the animals pretreated with 3-methylcholanthrene.

The effect of preincubation of hepatic microsomes with the various thiono-sulfur-containing compounds on cytochrome P-450 levels and the ability of these microsomes to metabolize benzphetamine were also examined (Tables 3–5).

Preincubation of the thiono-sulfur-containing compounds with microsomes isolated from untreated rats demonstrated that, in the presence of NADPH, all compounds significantly decreased cytochrome P-450 levels and all compounds decreased the ability of the microsomes to metabolize benzphetamine, in comparison with control microsomes incubated in the absence of the thiono-sulfur compounds but in the presence of NADPH (Table 3). When the thiono-sulfur compounds were preincubated with microsomes in the absence of NADPH, there was no decrease in cytochrome P-450 levels or in the ability of the microsomes to metabolize benzphetamine, as compared to control microsomes preincubated in the absence of both the thiono-sulfur compounds and NADPH.

Preincubation *in vitro* of microsomes isolated from phenobarbital-pretreated rats with the thiono-sulfur-containing compounds in the presence of NADPH (Table 4) produced results similar to those obtained with microsomes isolated from untreated rats. That is, cytochrome P-450 levels were decreased, as compared to the control incubations, by all compounds studied. Benzphetamine metabolism was also decreased by all compounds except methylthiouracil and propylthiouracil. In incubations carried out in the absence of NADPH, there was no decrease in cytochrome P-450 levels or in the ability of microsomes to metabolize benzphetamine in comparison to control incubations.

As compared to control incubations, preincubation *in vitro* of the various thiono-sulfur-containing compounds with microsomes isolated from 3-methylcholanthrene-pretreated rats in the presence of NADPH also resulted in all compounds decreasing cytochrome P-450 levels and the ability of microsomes to metabolize benzphetamine (Table 5). In the absence of an NADPH-generating system, none of the compounds decreased cytochrome P-450 or the ability of microsomes to metabolize benzphetamine. Thioacetamide significantly decreased the levels of cytochrome P-450 and the ability of microsomes from all treatment groups to metabolize benzphetamine, both in the presence and absence of NADPH (data not shown). The presence of disulfiram in the incubations *in vitro* interfered with the determination of the protein content of these incubations. Therefore, it was not possible to examine the effect of preincubation with this compound on the levels of cytochrome P-450 and the metabolism of benzphetamine *in vitro*.

In an attempt to ascertain if a thiono-sulfur moiety

Table 3. Effect of incubation *in vitro* of thiono-sulfur compounds with hepatic microsomes from untreated rats, in the presence and absence of NADPH, on cytochrome P-450 concentration and benzphetamine metabolism*

Thiono-sulfur compound	Cytochrome P-450† (with NADPH)	Cytochrome P-450† (without NADPH)	Benzphetamine metabolism‡ (with NADPH)	Benzphetamine metabolism‡ (without NADPH)
Carbon disulfide	0·35 ± 0·07§	0·74 ± 0·01	2·26 ± 0·46§	7·03 ± 0·16
Sodium diethyldithiocarbamate	0·46 ± 0·02§	0·82 ± 0·13	1·87 ± 0·01§	6·72 ± 0·35
Thiourea	0·48 ± 0·04§	0·69 ± 0·05	1·70 ± 0·09§	6·06 ± 0·41
2-Imidazolidinethione	0·56 ± 0·03§	0·80 ± 0·11	1·97 ± 0·24§	7·58 ± 1·91
2-Thiouracil	0·40 ± 0·11§	0·77 ± 0·03	1·95 ± 0·81§	6·85 ± 1·16
Methylthiouracil	0·33 ± 0·07§	0·71 ± 0·01	2·29 ± 0·04§	6·03 ± 0·01
Propylthiouracil	0·40 ± 0·01§	0·80 ± 0·03	1·61 ± 0·26§	6·73 ± 0·29
Methimazole	0·30 ± 0·01§	0·73 ± 0·05	1·29 ± 0·09§	6·89 ± 0·58
Control	0·70 ± 0·05‖	0·76 ± 0·03¶	5·66 ± 0·57‖	6·36 ± 0·35¶

* Incubation procedures are described in Methods. These values represent the means ± S. D. of the means of duplicate determinations under each experimental condition, using microsomes isolated from the same pooled livers of eight rats.

† Cytochrome P-450 concentrations are expressed as nmoles/mg of hepatic microsomal protein.

‡ Benzphetamine metabolism is expressed as nmoles formaldehyde formed/mg of microsomal protein/min.

§ Denotes significant differences at or below the 5 per cent level as compared to control incubations.

‖ Control microsomes were preincubated in the absence of the thiono-sulfur compound but in the presence of an NADPH-generating system. See Methods for details of these incubations.

¶ These control microsomes were preincubated in the absence of both the thiono-sulfur compounds and an NADPH-generating system. See Methods for the details of these incubations.

Table 4. Effect of incubation *in vitro* of thiono-sulfur compounds with hepatic microsomes from phenobarbital-pretreated rats, in the presence and absence of NADPH, on cytochrome P-450 concentration and benzphetamine metabolism*

Thiono-sulfur compound	Cytochrome P-450† (with NADPH)	Cytochrome P-450† (without NADPH)	Benzphetamine metabolism‡ (with NADPH)	Benzphetamine metabolism‡ (without NADPH)
Carbon disulfide	0·71 ± 0·02§	2·20 ± 0·02	6·45 ± 0·05§	13·09 ± 0·68
Sodium diethyldithiocarbamate	1·02 ± 0·05§	2·29 ± 0·01	7·26 ± 0·42§	12·64 ± 1·93
Thiourea	1·16 ± 0·00§	2·23 ± 0·01	7·30 ± 0·63§	13·30 ± 0·58
2-Imidazolidinethione	1·39 ± 0·17§	2·29 ± 0·05	6·47 ± 0·95§	12·42 ± 1·44
2-Thiouracil	1·36 ± 0·01§	2·46 ± 0·13	4·53 ± 1·39§	10·64 ± 0·86
Methylthiouracil	1·58 ± 0·06§	2·20 ± 0·17	11·81 ± 0·72	13·29 ± 1·15
Propylthiouracil	1·35 ± 0·04§	2·08 ± 0·04	10·42 ± 1·32	12·23 ± 0·17
Methimazole	1·15 ± 0·12§	2·08 ± 0·06	8·77 ± 0·12§	13·32 ± 1·43
Control	1·91 ± 0·33‖	2·27 ± 0·14¶	12·50 ± 1·97‖	13·16 ± 1·09¶

* Incubation procedures are described in Methods. These values represent the means ± S. D. of the means of duplicate determinations under each experimental condition, using microsomes isolated from the same pooled livers of eight rats.

† Cytochrome P-450 concentrations are expressed as nmoles/mg of hepatic microsomal protein.

‡ Benzphetamine metabolism is expressed as nmoles formaldehyde formed/mg of microsomal protein/min.

§ Denotes significant differences at or below the 5 per cent level as compared to control incubations.

‖ Control microsomes were preincubated in the absence of the thiono-sulfur compound but in the presence of an NADPH-generating system. See Methods for details of these incubations.

¶ These control microsomes were preincubated in the absence of both the thiono-sulfur compounds and an NADPH-generating system. See Methods for the details of these incubations.

is a necessary requirement for the various compounds to bring about a decrease in cytochrome P-450 and mixed-function oxidase activity, the hepatic cytochrome P-450 levels and rate of benzphetamine metabolism were measured 48 hr after the administration *in vivo* of the oxygen analogs of some of the thiono-sulfur compounds to phenobarbital-pretreated rats. In addition, the effect of preincubation of the various oxygen analogs with hepatic microsomes in the presence and absence of NADPH on the level of cytochrome P-450 and the rate of benzphetamine metabolism was also examined (Table 6). Neither the mixed-function oxidase activity nor the cytochrome P-450 levels were decreased either *in vivo* or *in vitro* by the oxygen analogs studied. In addition, no hepatic necrosis was seen with any of the oxygen analogs examined.

DISCUSSION

Except for 2-imidazolidinethione and thiourea, all of the thiono-sulfur-containing compounds administered *in vivo* were found to be capable of significantly decreasing the level of cytochrome P-450 in at least one of the treatment groups examined (untreated, phenobarbital-pretreated or 3-methylcholanthrene-pretreated). When examined 48 hr after administration,

Table 5. Effect of incubation *in vitro* of thiono-sulfur compounds with hepatic microsomes from 3-methylcholanthrene-pretreated rats, in the presence and absence of NADPH, on cytochrome P-450 concentration and benzphetamine metabolism*

Thiono-sulfur compound	Cytochrome P-450† (with NADPH)	Cytochrome P-450† (without NADPH)	Benzphetamine metabolism‡ (with NADPH)	Benzphetamine metabolism‡ (without NADPH)
Carbon disulfide	0·70 ± 0·06§	1·21 ± 0·12	2·17 ± 0·28§	3·28 ± 0·35
Sodium diethyldithiocarbamate	0·57 ± 0·07§	1·51 ± 0·23	0·99 ± 0·00§	3·95 ± 0·40
Thiourea	0·68 ± 0·08§	1·16 ± 0·00	0·79 ± 0·39§	4·10 ± 0·53
2-Imidazolidinethione	0·35 ± 0·16§	1·08 ± 0·15	1·05 ± 0·35§	3·87 ± 1·85
Thiouracil	0·81 ± 0·09§	1·13 ± 0·04	0·81 ± 0·29§	3·08 ± 0·40
Methylthiouracil	0·77 ± 0·15§	0·89 ± 0·06	2·11 ± 0·28§	2·87 ± 0·43
Propylthiouracil	0·52 ± 0·05§	1·04 ± 0·08	0·55 ± 0·01§	2·95 ± 0·40
Methimazole	0·38 ± 0·02§	1·14 ± 0·18	1·59 ± 0·61§	3·56 ± 1·27
Control	1·17 ± 0·11‖	1·15 ± 0·22¶	3·75 ± 0·11‖	3·54 ± 0·61¶

* Incubation procedures are described in Methods. These values represent the means ± S. D. of the means of duplicate determinations under each experimental condition, using microsomes isolated from the same pooled livers of eight rats.

† Cytochrome P-450 concentrations are expressed as nmoles/mg of hepatic microsomal protein.

‡ Benzphetamine metabolism is expressed as nmoles formaldehyde formed/mg of microsomal protein/min.

§ Denotes significant differences at or below the 5 per cent level as compared to control incubations.

‖ Control microsomes were preincubated in the absence of the thiono-sulfur compound but in the presence of an NADPH-generating system. See Methods for details of these incubations.

¶ These control microsomes were preincubated in the absence of both the thiono-sulfur compounds and an NADPH-generating system. See Methods for the details of these incubations.

Table 6. Effect of oxygen analogs of the thiono-sulfur compounds on P-450 concentration and benzphetamine demethylation using phenobarbital-pretreated rats

Oxygen analog	*In vitro**				*In vivo*†	
	Cytochrome P-450‡ (with NADPH)	Cytochrome P-450‡ (without NADPH)	Benzphetamine metabolism§ (with NADPH)	Benzphetamine metabolism§ (without NADPH)	Cytochrome P-450‡	Benzphetamine metabolism§
Acetamide	1·12 ± 0·10	1·06 ± 0·23	15·07 ± 2·02	14·74 ± 0·35	0·96 ± 0·30	8·86 ± 1·46
Urea	1·08 ± 0·16	1·14 ± 0·06	15·45 ± 2·13	13·91 ± 0·81	1·07 ± 0·21	8·63 ± 1·48
2-Imidazolidinone	1·01 ± 0·08	1·04 ± 0·15	14·95 ± 0·40	15·09 ± 0·06	0·96 ± 0·05	8·59 ± 1·10
Uracil	1·06 ± 0·12	1·13 ± 0·07	16·38 ± 1·91	16·62 ± 1·76	1·04 ± 0·04	7·19 ± 1·46
Methyluracil	1·02 ± 0·06	1·18 ± 0·05	16·33 ± 0·26	17·86 ± 0·30	1·23 ± 0·06	8·84 ± 1·17
Propyluracil	1·01 ± 0·21	1·17 ± 0·15	17·52 ± 3·66	15·79 ± 1·12	1·06 ± 0·16	6·43 ± 1·81
Control	1·05 ± 0·10‖	1·15 ± 0·11¶	16·36 ± 1·00‖	16·45 ± 1·55¶	1·05 ± 0·22**	7·94 ± 2·07**

* Incubation procedures are described in Methods. These values represent the means ± S. D. of the means of duplicate determinations under each experimental condition, using microsomes isolated from the same pooled livers of eight rats.

† Levels of cytochrome P-450, rate of benzphetamine metabolism and the degree of hepatic necrosis were examined 48 hr after the administration *in vivo* of 2·5 m-moles/kg of propyluracil and 5 m-moles/kg of the remaining compounds. These values represent the means ± S. D. of the means of one determination from each of three animals in each treatment group.

‡ Nmoles/mg of hepatic microsomal protein.

§ Nmoles formaldehyde formed/mg of hepatic microsomal protein/min.

‖ Control microsomes were preincubated in the absence of the various oxygen analogs but in the presence of NADPH. See Methods for the details of these incubations.

¶ Control microsomes were preincubated in the absence of both the oxygen analogs and NADPH.

** All of the oxygen analogs were administered as a solution or suspension in distilled water. The control animals received distilled water (2·5 ml/kg) at the time of the injection of the experimental animals with the various oxygen analogs.

all of the compounds caused a significant decrease in benzphetamine metabolism in at least one treatment group. Both the phenobarbital-pretreated and 3-methylcholanthrene-pretreated animals were susceptible to the inhibitory effect of a greater number of the various thiono-sulfur compounds than the untreated animals. In addition, the degree of inhibition by the various thiono-sulfur compounds in the phenobarbital-pretreated animals appeared, in general, to be greater than in the 3-methylcholanthrene-pretreated and untreated groups. Of the various compounds examined, disulfiram, thioacetamide and propylthiouracil appeared to be the most consistent in terms of their ability to decrease the levels of hepatic cytochrome P-450 and inhibit benzphetamine metabolism in the various treatment groups when measured 48 hr after administration.

Five of the ten compounds examined caused varying degrees of centrilobular hepatic necrosis. The most effective of these were diethyldithiocarbamate, propylthiouracil and thioacetamide. Only those animals pretreated with phenobarbital exhibited hepatic necrosis. On examination of the data, it does not appear that there is a direct relationship between the ability of the various compounds to decrease the levels of cytochrome P-450, inhibit benzphetamine metabolism or cause centrilobular hepatic necrosis. For example, although thioacetamide markedly inhibited the rate of benzphetamine metabolism and caused hepatic necrosis, it did not significantly decrease the levels of hepatic cytochrome P-450 in the phenobarbital-pretreated animals when measured 48 hr after its administration. In addition, disulfiram decreased the levels of cytochrome P-450 and inhibited benzphetamine metabolism in the phenobarbital-pretreated animals to about the same degree as propylthiouracil, but did not cause hepatic necrosis. Also, propylthiouracil decreased cytochrome P-450 levels and inhibited benzphetamine metabolism to about the same degree in the phenobarbital-pretreated and 3-methylcholanthrene-treated rats but produced hepatic necrosis only in the phenobarbital-pretreated group. The reasons for the lack of a consistent correlation between the decrease in cytochrome P-450 levels, inhibition of benzphetamine metabolism and hepatic necrosis caused by the various thiono-sulfur compounds are unknown at this time. In addition, the reason for the increased susceptibility of the phenobarbital-pretreated animals to hepatic necrosis is also unknown.

With few exceptions, the various thiono-sulfur compounds examined in these studies consistently decreased the level of cytochrome P-450 and inhibited benzphetamine metabolism when preincubated with hepatic microsomes from the three treatment groups in the presence of NADPH. Just as consistently, the various thiono-sulfur compounds failed to decrease the level of cytochrome P-450 and inhibit benzphetamine metabolism when preincubated with microsomes in the absence of NADPH. Results similar to those reported here have recently been obtained *in vitro* using phenylthiourea and 1-naphthylisothiocyanate as substrates [3]. These results, as well as those from the experiments *in vivo*, imply that the decrease in the level of cytochrome P-450 and the inhibition of benzphetamine metabolism is the result of the interaction of an active metabolite or metabolites of these compounds with the macromolecules of the endoplasmic reticulum. These data also suggest that the hepatic necrosis is also the result of an interaction of an active metabolite or metabolites with liver macromolecules.

Based on the results of previous studies with parathion [1–3] and CS_2 [3, 6–8], it is attractive to postulate that the inhibitory effects of these thiono-sulfur-containing compounds, both *in vivo* and *in vitro*, may be the result of the mixed-function oxidase-catalyzed release and covalent binding of atomic sulfur. A

chemical mechanism for this type of reaction using CS_2, and parathion as substrates has recently been proposed [6, 16, 17].

It has been suggested that the decrease in cytochrome P-450 seen on incubation of phenylthiourea and 1-naphthylisothiocyanate with rat liver microsomes in the presence of NADPH was the result of conversion of cytochrome P-450 to cytochrome P-420 [3]. However, in the studies reported here, there was a roughly proportional decrease in both cytochrome P-450 and cytochrome P-420 in those experiments *in vivo* and *in vitro* where the thiono-sulfur-containing compounds had an effect on the level of this enzyme. Therefore, the decrease in cytochrome P-450, at least with the compounds examined in this study, is apparently not a result of conversion of cytochrome P-450 to cytochrome P-420.

A number of the compounds examined in this study have been used in the treatment of thyrotoxicosis in man. These include thiouracil, methylthiouracil, propylthiouracil and methimazole. Although not a frequent side-effect, each of these compounds has been reported to produce hepatic injury [18 21]. It is not clear from these reports whether the hepatic injury was a direct result of the action of these compounds or a hypersensitivity reaction. Nevertheless, the data from this study suggest that hepatic injury as a direct result of exposure to these compounds is certainly a possibility.

The compound disulfiram is used in the treatment of alcoholism. Disulfiram is metabolized in humans to both diethyldithiocarbamate and CS_2 [22]. However, to our knowledge, no hepatic injury in humans on exposure to disulfiram, diethyldithiocarbamate or CS_2 has been reported. However, an impairment of drug metabolism in rats by disulfiram, both *in vivo* and *in vitro*, has been previously reported [23]. As indicated in the introduction, there have been numerous reports of an impairment of drug metabolism and production of hepatic necrosis in rats exposed to CS_2.

Three of the compounds examined in this study have been reported to produce hepatomas in rats. These are thiourea [24], thioacetamide [24, 25] and 2-imidazolidinethione [26]. The compound 2-imidazolidinethione is occasionally found as a breakdown product of the ethylene-bis-dithiocarbamate fungicides. It is reasonable that the thiono-sulfur grouping is required for the carcinogenicity of at least thiourea and thioacetamide, since the corresponding oxygen analogs have not been reported to be carcinogenic. Whether these three compounds require metabolic activation to produce their carcinogenic effect is not yet known. Thioacetamide, which has been previously reported to inhibit the hepatic mixed-function oxidase enzymes and to decrease the levels of cytochrome P-450 when administered *in vivo* [27], proved to be somewhat anomolous in our studies *in vitro*. In contrast to the other thiono-sulfur compounds examined, thioacetamide decreased the levels of hepatic microsomal cytochrome P-450 and inhibited benzphetamine metabolism both in the presence and absence of NADPH. A recent report by Castro *et al.* [28] has suggested that the metabolism of thioacetamide by the hepatic mixed-function oxidase enzyme system is not the rate-limiting step in its activation to a necrogenic metabolite. However, the partial prevention of thioacetamide-induced necrosis by pretreatment with disulfiram [28] and the difference in the susceptibility of phenobarbital-pretreated animals as compared to untreated reported in this study imply that the mixed-function oxidase enzyme system is involved to some extent in activating thioacetamide to its necrogenic form. The fact that thioacetamide is rapidly metabolized by rat liver slices to acetamide [29], a compound which is not necrogenic, also suggests that the toxic effect of this compound may be the result of the mixed-function oxidase-catalyzed release and covalent binding of sulfur.

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