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IMPAIRMENT OF HEPATIC MICROSOMAL AND PLASMA ESTERASES OF THE RAT BY DISULFIRAM AND DIETHYLDITHIOCARBAMATE*

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Abstract—Twenty-four hr after oral administration (0·2 to 2·0 g/kg) of disulfiram (DS) to male rats, significant impairment of hepatic microsomal carboxylesterase and plasma carboxyl- and cholinesterase was observed. Plasma esterase activities returned to control values between 48 and 72 hr after a single oral dose of DS (2·0 g/kg), but microsomal carboxylesterase activity was still significantly lower in treated animals at both times. Daily administration of DS (0·1 or 0·4 g/kg) resulted in decreased microsomal carboxylesterase activity after 2 days. However, continued administration of DS for a total of 12 days did not produce further depression of microsomal esterase activity. Microsomal and plasma carboxylesterase activities were also decreased 24 hr after oral administration (1·0 to 2·0 g/kg) of sodium diethyldithiocarbamate (DDTC), the reduced metabolite of DS. Hepatic microsomal esterases that migrated rapidly toward the anode during polyacrylamide disc gel electrophoresis were the most sensitive to impairment by DS or DDTC. Esterase activity in the lung was also impaired after DS or DDTC administration, whereas esterases of the heart, kidney and testis were essentially unaffected. Incubation *in vitro* of liver microsomes with DS decreased microsomal carboxylesterase activity, while incubation with DDTC had little effect. Plasma carboxylesterase was inhibited *in vitro* to a greater extent than microsomal esterases by both DS and DDTC. Diethylamine and CS_2, the decomposition products of DDTC, were essentially inactive as esterase inhibitors *in vitro*.

Disulfiram (Antabuse, DS) has been used for over 20 yr in avoidance therapy for certain patients with chronic alcoholism. Ingestion of ethanol by a patient taking DS produces a wide range of unpleasant symptoms called the "Antabuse reaction." DS inhibits aldehyde dehydrogenase; thus when ethanol is ingested acetaldehyde accumulates. Therefore, it has been suggested that acetaldehyde accumulation is primarily responsible for the Antabuse reaction [1, 2]. However, since acetaldehyde administration does not duplicate all of the symptoms of this reaction, it has also been suggested that the chemical product of a direct ethanol–DS interaction may be responsible [3] or that ethanol may increase the toxicity of DS by inhibiting its metabolism [4].

Although attention has been focused primarily on the biochemical basis for the effectiveness of DS in alcohol avoidance therapy, other unrelated actions of clinical significance have been reported. Of particular significance is the observation that DS inhibits oxidative drug metabolism both *in vitro* and *in vivo* [5–7]. Studies in man have shown that DS administration prolongs antipyrine half-life [8] and may inhibit metabolism of diphenylhydantoin and warfarin [9, 10]. Recent experiments in our laboratory have been designed to characterize the effects of DS on oxidative and hydrolytic drug metabolism and the mechanisms by which it exerts these effects. Since plasma and tissue esterases are responsible for hydrolysis of many ester and amide drugs [11–13], one aspect of our work has been to study the effects of DS and its reduced metabolite, diethyldithiocarbamate (DDTC), on certain esterases of the rat. The results of these studies are presented in this report.

MATERIALS AND METHODS

Animals. Male Sprague–Dawley rats (200–300 g) purchased from Charles River Labs were used throughout the experiments. All animals had free access to Purina lab chow and water, except where noted.

Chemicals. Tetraethylthiuramdisulfide, butyrylthiocholine chloride and 5,5-dithiobisnitrobenzoate were purchased from Sigma Chemical Co. (St. Louis, Mo.). Sodium diethyldithiocarbamate trihydrate was obtained from Fisher Scientific Co. (Fair Lawn, N.J.), and indophenylacetate from Eastman Kodak Co. (Rochester, N.Y.).

Preparation of tissues. After decapitation, blood was collected in heparinized tubes and plasma was separated by centrifugation (800 *g*) for 15 min at 4°. Lungs, kidneys, hearts, testes and livers were homogenized in 4 vol. of 0·05 M Tris–HCl, pH 7·4, and centrifuged at 9000 *g* for 20 min at 4°. The 9000 *g* supernatant was kept on ice and used for determination of esterase activity in these tissues. Liver microsomes were prepared as previously described [5]. Protein content was determined by the biuret method [14].

Electrophoresis. Hepatic microsomal esterases were separated by polyacrylamide disc gel electrophoresis.

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The microsomal suspension was diluted with 0·02 M Tris–HCl (pH 7·4)–1·15% KCl containing 1% Triton X-100 to a final protein concentration of 3 mg/ml. Electrophoresis was performed according to the method of Ornstein [15] and Davis [16] as modified in our laboratory [17]. Each tube (0·5 cm i.d.) contained 1·0 ml of separating gel (5% acrylamide) above which was polymerized approximately 0·15 ml of stacking gel. Just prior to electrophoresis, 10 μl of the solubilized microsomal suspension was added to a small volume of stacking gel which was polymerized on top of the pre-existing stacking gel. The upper and lower bath buffers contained 0·038 M glycine and 0·005 M Tris HCl, pH 8·3. Gels were run at a constant current of 4 mA/tube for 45 min. Esterase activity was localized by immersing each gel in a solution of 1% α-naphthylacetate and Fast Blue RR, according to the method of Markert and Hunter [18]. After staining for 5 min, the gels were rinsed with distilled water and fixed in methanol water acetic acid (4:4:1) overnight and were rehydrated in 7% acetic acid for 1 day prior to scanning. The gels were scanned at 445 nm in a Gilford-2400 spectrophotometer equipped with a gel scanning attachment. The resulting peaks were integrated by a Hewlett–Packard 3373B integrator. The integrator was interfaced with a Hewlett–Packard 9810A calculator programmed to total the integrated area of the scan and to determine the per cent activity of each peak.

Determination of esterase activity. Carboxylesterase activity was determined at 25° using a colorimetric assay with indophenylacetate (IPA) as the substrate [17, 19]. The assay mixture contained enzyme (0·1 ml plasma or 0·05 ml microsomes, 1–2 mg protein/ml), 0·05 M Tris–HCl buffer, pH 8·2, and 0·05 ml of 0·02 M IPA in absolute ethanol (final substrate concentration, $3·3 \times 10^{-4}$ M) in a total volume of 3·0 ml. To determine tissue carboxylesterase activities, the following amounts of 9000 *g* supernatant were added to buffer and 0·05 ml of 0·02 M IPA in a total volume of 3·0 ml: heart, 0·1 ml; lung, 0·05 ml; liver, 0·01 ml; kidney, 0·02 ml; and testis, 0·05 ml. The specific activity of the enzyme was calculated from the absorbance change at 522 nm/min by means of a standard curve prepared with the hydrolysis product, sodium indophenol. Plasma cholinesterase activity was determined colorimetrically by measuring the rate of hydrolysis of 5×10^{-4} M butyrylthiocholine (BUTCH) at 412 nm [17, 20]. Specific activity was calculated with the molar extinction coefficient of 13,600 M^{-1} cm^{-1} for the 5-thio-2-nitrobenzoate ion released in the reaction.

Measurement of esterase inhibition in vitro. Plasma and hepatic microsomal suspensions prepared from five male rats were diluted to a final protein concentration of 5 mg/ml with 0·05 M Tris HCl, pH 8·2. Five ml of each sample was incubated at 37° for 20 min, either alone or in the presence of DS, DDTC, CS_2, diethylamine (DEA), or CS_2 + DEA. Aqueous solutions of DDTC (0·05 M), DEA (0·1 M) or CS_2 (0·038 M) were added to produce a final concentration of 0·1 or 0·5 mM. The water solubility of DS was not sufficient to prepare a concentrated aqueous solution; therefore, appropriate amounts of a 0·05 M DS solution in acetone were added to the incubations to produce a final DS concentration of 0·1 or 0·5 mM. The amount of acetone added (10–50 μl) had no effect on liver or plasma carboxylesterase activity. After incubation, carboxylesterase activity was determined in an aliquot of the incubate as described above. No significant differences in carboxylesterase activity were observed between incubated controls and unincubated plasma or microsomal samples.

Statistical methods. Significant differences between control and treated groups after DS or DDTC administration were determined by means of Student's *t*-test. Data obtained after incubation *in vitro* of DS, DDTC, CS_2, or DEA with microsomes or plasma were compared by means of a paired *t*-test.

RESULTS

Microsomal carboxylesterase activity was significantly reduced in male rats 24 hr after oral DS administration (Table 1). Activity was reduced from 67 to 46 per cent of control activity by doses of DS

Table 1. Microsomal and plasma esterase activity 24 hr after oral administration of DS or DDTC*

Compound administered	Dose (g/kg-[m-moles/kg])	Esterase activity		
		Microsomal carboxylesterase†	Plasma carboxylesterase‡	Plasma cholinesterase§
DS	Control	1·10 ± 0·05	13·75 ± 0·81	0·94 ± 0·05
	0·2-[0·7]	0·78 ± 0·07‖ (67)	10·88 ± 0·77¶ (79)	0·69 ± 0·04‖ (73)
	1·0-[3·4]	0·68 ± 0·04‖ (58)	7·53 ± 0·69‖ (54)	0·44 ± 0·03‖ (47)
	2·0-[6·7]	0·54 ± 0·04‖ (46)	7·98 ± 0·69 (58)	0·47 ± 0·07‖ (50)
DDTC	Control	0·99 ± 0·10	15·30 ± 0·45	0·85 ± 0·14
	0·2-[0·9]	1·00 ± 0·04 (101)	13·95 ± 0·59 (92)	0·92 ± 0·06 (109)
	1·0-[4·4]	0·78 ± 0·05 (71)	10·82 ± 0·93‖ (71)	0·93 ± 0·05 (110)
	2·0-[8·9]	0·49 ± 0·08‖ (50)	7·70 ± 0·54‖ (50)	0·63 ± 0·08 (74)

* After overnight starvation, male rats were given DS or DDTC by gavage in 2·3 ml of 0·5% carboxymethylcellulose (CMC). Control animals received CMC vehicle only. Values are the means of four animals ± S. E. M. Numbers in parentheses are percentage of control.

† μmoles IPA hydrolyzed/min/mg of microsomal protein.

‡ nmoles IPA hydrolyzed/min/mg of plasma protein.

§ nmoles BUTCH hydrolyzed/min/mg of plasma protein.

‖ Different from control ($P < 0·01$).

¶ Different from control ($P < 0·05$).

Table 2. Microsomal and plasma esterase activity 24 hr after i.p. administration of DS or DDTC*

Experimental group	Microsomal carboxylesterase†	Plasma carboxylesterase‡	Plasma cholinesterase§
Control	1·18 ± 0·07	11·35 ± 0·36	0·99 ± 0·06
DS	0·66 ± 0·05‖ (56)	7·21 ± 0·51‖ (64)	0·44 ± 0·02‖ (44)
Control	1·53 ± 0·05	12·61 ± 0·62	0·94 ± 0·06
DDTC	1·12 ± 0·05‖ (73)	13·12 ± 0·94 (104)	0·74 ± 0·07 (79)

* In separate experiments, male rats were given 0·4 g/kg (1·4 m-moles/kg) of DS or 0·4 g/kg (1·8 m-moles/kg) of DDTC by i.p. injection. Control animals were given CMC vehicle i.p. Values are the means of four animals ± S. E. M. Numbers in parentheses are percentage of control.

† μmoles IPA hydrolyzed/min/mg of microsomal protein.

‡ nmoles IPA hydrolyzed/min/mg of plasma protein.

§ nmoles BUTCH hydrolyzed/min/mg of plasma protein.

‖ Different from control ($P < 0·01$).

ranging from 0·2 to 2·0 g/kg. Plasma carboxyl- and cholinesterase activities were also reduced to approximately 75 or 50 per cent of control after 0·2 or 1·0 g/kg of DS. However, no further decrease in plasma esterase activities occurred when 2·0 g/kg of DS was given.

In contrast, 0·2 g/kg of DDTC had no effect on esterase activities and 1·0 g/kg of DDTC impaired only plasma carboxylesterase activity (Table 1). When DDTC was administered at the highest dose (2·0 g/kg), microsomal and plasma carboxylesterase activity was similar to activity recorded after 2·0 g/kg of DS (approximately 50 per cent of control). However, plasma cholinesterase impairment was not statistically significant even after 2·0 g/kg of DDTC. It should be noted that, although the doses of DS and DDTC were equal on a g/kg basis, on a molar basis approximately 30% more DDTC (trihydrate sodium salt) was given at each dosage level.

Since one explanation for the lower potency of DDTC as compared to DS could be that DDTC decomposes in the gut to CS_2 and diethylamine before it can be absorbed [4], the effects of DS and DDTC were also compared 24 hr after i.p. administration. As shown in Table 2, 0·4 g/kg (1·4m-moles/kg) of DS significantly impaired microsomal carboxylesterase and plasma carboxyl- and cholinesterase 24 hr after i.p. administration. On the other hand, 0·4 g/kg (1·8 m-moles/kg) of DDTC significantly decreased only microsomal carboxylesterase activity. These results appear to rule out destruction of DDTC in the gut as the only reason for the observed differences in oral effectiveness of these compounds.

Since, in clinical practice, DS is usually administered for several days or weeks, an experiment was conducted to determine if repeated administration of DS could lead to cumulative impairment of esterase activity. Groups of male rats were given either 0·1 or 0·4 g/kg of DS by gavage daily for 12 days; the effects of this treatment on microsomal carboxylesterase activity are presented in Fig. 1. Maximum inhibition was seen after 2 days for both doses, indicating that cumulative impairment of microsomal esterases beyond 2 days does not occur.

Plasma carboxyl- and cholinesterase activities were also determined in these experiments, but the results were not included in Fig. 1. Neither enzyme was affected in the group given 0·1 g/kg of DS, but both were significantly reduced to about 65 per cent of control levels after 2 days of administration of 0·4 g/kg. Unexpectedly, both plasma carboxyl- and cholinesterase activities returned to control levels by day 5, despite continued DS administration.

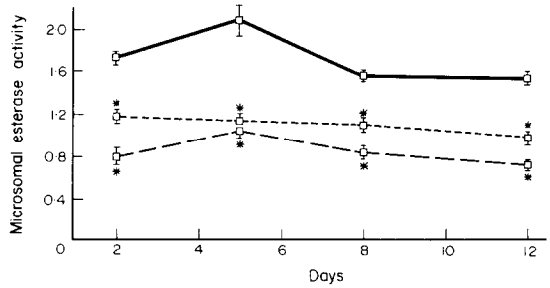

Fig. 1. Impairment of hepatic microsomal carboxylesterase activity during daily disulfiram administration. Male rats were divided into three groups and received either 0·1 or 0·4 g/kg of DS suspension or 0·5% carboxymethylcellulose vehicle by gavage. Animals were dosed daily; groups (N = 4) were sacrificed after selected intervals. Esterase activity is expressed as μmoles IPA hydrolyzed/min/mg of microsomal protein (mean ± S. E. M.). Control, vehicle only (——); 0·1 g/kg of DS (. . . . .); 0·4 g/kg of DS (– – –). An asterisk indicates different from control ($P < 0·05$).

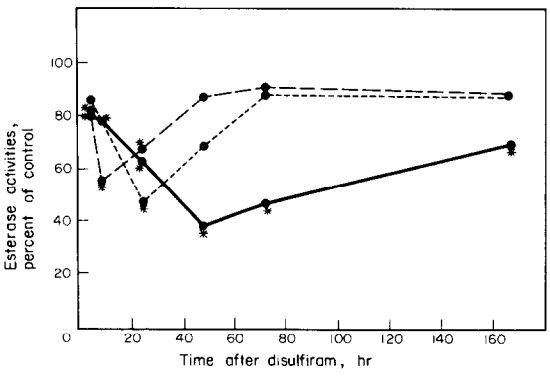

Fig. 2. Time course of esterase impairment after a single oral dose of disulfiram (2·0 g/kg). Male rats were starved overnight and received either DS suspension or 0·5% CMC vehicle by gavage. At the indicated times, control and treated animals (N = 4) were sacrificed and esterase activities were determined. Values are expressed as percentage of control activity for each measurement. Microsomal carboxylesterase ( ): plasma carboxylesterase (– – –); plasma cholinesterase (· · · · · ·). An asterisk indicates different from control ($P < 0·05$.

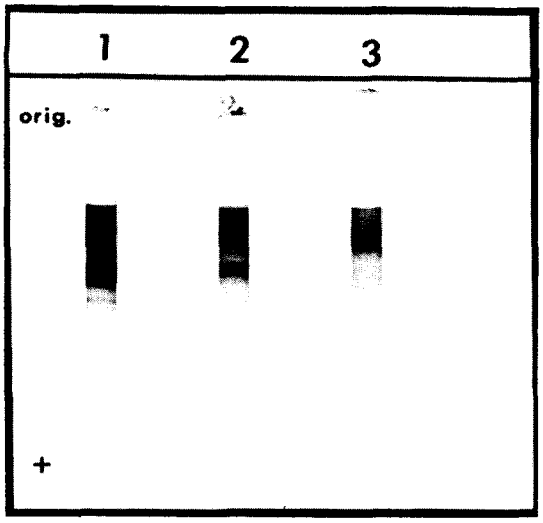

Fig. 3. Polyacrylamide disc gel electrophoresis of hepatic microsomal esterases. Gel 1, control; gel 2, DS, 0·1 g/kg p.o., for 12 days; gel 3, DS, 0·4 g/kg p. o., for 12 days. Microsomes from each group of four animals were pooled and diluted to equal protein concentrations for electrophoresis.

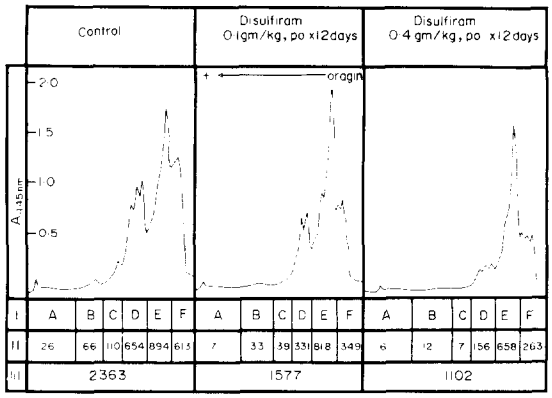

Fig. 4. Densitometric tracings of polyacrylamide gels from Fig. 3. Row I shows division of the pattern into six major areas of activity designated peaks A through F. Row II represents the integrated area of each individual peak. Row III is the total integrated area of each scan. The origin is to the right of each tracing and the anode is to the left.

A similar dissociation between liver and plasma esterases was seen when esterase activity was measured at selected intervals after a single dose of DS (Fig. 2). Maximal inhibition of plasma esterases occurred 8 24 hr after DS, but recovery was rapid and activity returned to control levels by 48–72 hr. In contrast, microsomal carboxylesterase impairment was maximal at 48 hr and activity was still below control levels 168 hr after DS administration. Twelve days (288 hr) after DS administration, microsomal carboxylesterase activity had also returned to control levels (data not shown).

The substrates used to measure "carboxylesterase" activity in the described assay procedure and on polyacrylamide gels (IPA and α-naphthylacetate respectively) are hydrolyzed by A- and B-esterases [19, 21, 22], both of which have been identified in rat liver homogenates [23] and in hepatic microsomes [24]. Previous studies have further demonstrated that microsomal A- and B-esterases can be separated and identified by electrophoresis and that A-esterases migrate more rapidly toward the anode during electrophoresis [24]. Therefore, microsomal esterases were separated and quantitated by disc gel electrophoresis to determine if DS pretreatment selectively impaired certain "isozymes," since this distinction could not be made by simply measuring IPA hydrolysis.

The esterase zymograms of control microsomes and of microsomes isolated from DS-treated rats are presented in Fig. 3. Daily DS administration (0·1 or 0·4 g/kg) resulted in greater impairment of the microsomal esterases that migrated more rapidly toward the anode. The gels pictured in Fig. 3 were scanned as described in Methods and the resulting peaks were divided into six major areas of activity designated peaks A through F (Fig. 4). Selective impairment of the esterases contained within peaks A–D was evident, since the percentage of control activity for these esterases was consistently lower than the corresponding percentage decreases in peaks E and F. Selective impairment of the esterases contained within peaks A–D was also observed 24 hr after single doses of DS or DDTC (2·0 g/kg).

Since esterases are found in most rat tissues [23], an experiment was carried out to determine if the activities of various extrahepatic esterases were also decreased after DS or DDTC administration. The results of this study are summarized in Table 3. Consistent with previous results (Tables 1 and 2), carboxylesterase activity in plasma and liver was significantly reduced 24 hr after DS (1·0 g/kg) administration, while this dose of DDTC had little effect. Carboxylesterase activity in the heart and testis was unaffected by DS or DDTC, and activity in the kidney was slightly decreased by DS (not statistically significant). In con-

Table 3. Tissue carboxylesterase activity* 24 hr after oral administration of DS or DDTC†

Experimental group	Tissue					
	Liver	Plasma	Kidney	Heart	Lung	Testis
Control	52·82 ± 2·02	0·68 ± 0·04	30·12 ± 1·85	1·07 ± 0·15	4·19 ± 0·25	4·79 ± 0·42
DS	28·86 ± 3·39‡ (55)	0·47 ± 0·03‡ (69)	22·98 ± 2·88 (76)	0·97 ± 0·09 (91)	2·82 ± 0·06‡ (67)	4·31 ± 0·23 (90)
DDTC	48·55 ± 3·44 (92)	0·66 ± 0·06 (97)	29·00 ± 1·78 (96)	1·04 ± 0·08 (97)	2·43 ± 0·14‡ (58)	4·46 ± 0·20 (93)

* μmoles IPA hydrolyzed/min/g of tissue or ml of plasma.

† After overnight starvation, male rats were given 1·0 g/kg (3·4 m-moles/kg) of DS or 1·0 g/kg (4·4 m-moles/kg) of DDTC by gavage. Control animals received CMC vehicle only. Values are the means of four animals ± S. E. M. Numbers in parentheses are percentage of control.

‡ Different from control ($P < 0·01$).

Table 4. Inhibition *in vitro* of microsomal and plasma carboxylesterases by DS, DDTC, carbon disulfide (CS_2) and diethylamine (DEA)*

Enzyme source	Concn of inhibitor (mM)	Carboxylesterase activity †‡					
		Control	DS	DDTC	CS_2	DEA	CS_2 + DEA
Microsomes	0·1	1·01 ± 0·10	0·89 ± 0·11§	0·99 ± 0·09	1·01 ± 0·10	1·01 ± 0·10	1·01 ± 0·11
Microsomes	0·5	1·06 ± 0·08	0·79 ± 0·10§	0·97 ± 0·10	1·00 ± 0·09	1·02 ± 0·09	0·99 ± 0·09
Plasma	0·1	11·83 ± 0·64	7·93 ± 0·52§	5·22 ± 0·37§	11·92 ± 0·82	11·86 ± 0·78	8·62 ± 0·88§

* Plasma or microsomes from male rats (5 mg protein/ml) were incubated at 37° for 20 min, either alone (controls) or in the presence of the indicated compounds. Values are the means of five animals ± S. E. M. Data were analyzed by a paired *t*-test.

† Microsomes, μmoles IPA hydrolyzed/min/mg.

‡ Plasma, nmoles IPA hydrolyzed/min/mg.

§ Different from control ($P < 0.01$).

‖ Different from control ($P < 0.05$).

trast, carboxylesterase activity in the lung was significantly impaired 24 hr after either DS or DDTC administration. In a separate experiment, neither acetylcholinesterase nor carboxylesterase activity in the brain was altered 24 hr after oral DS (2·0 g/kg) administration (data not shown).

In order to directly compare DS and DDTC as esterase inhibitors, carboxylesterase activity was measured in plasma and microsomes after incubation with these compounds *in vitro*. The acid decomposition products of DDTC (CS_2 and diethylamine) were also tested *in vitro* to evaluate the possibility that one of these compounds might contribute to esterase inhibition, since CS_2 has been shown to inhibit oxidative drug metabolism by hepatic microsomes [25]. The results of these experiments are summarized in Table 4. Carboxylesterase activity of microsomes incubated with 0·1 or 0·5 mM DS was decreased to 88 and 75 per cent of control activity respectively. On the other hand, the same concentrations of DDTC had little effect. Moreover, 0·1 or 0·5 mM CS_2 or diethylamine (DEA), either alone or in combination, did not alter microsomal carboxylesterase activity. Incubation of plasma with either 0·1 mM DS or DDTC significantly decreased plasma carboxylesterase activity to 67 and 44 per cent of control respectively. When 0·1 mM CS_2 or DEA was incubated with plasma, no alterations in plasma carboxylesterase activity were observed; however, a combination of 0·1 mM CS_2 and DEA decreased carboxylesterase activity to 73 per cent of control activity.

DISCUSSION

The administration of DS in doses used in previous drug metabolism studies [5–7] produced a significant decrease in microsomal carboxylesterase and plasma carboxyl- and cholinesterase. Although the physiological role of esterases and the effect of inhibition of these enzyme are unclear [11, 12], our results suggest that disulfiram administration may alter the metabolism and activity of the more than 200 pharmacologically active esters which may be hydrolyzed by plasma and tissue esterases [13]. Therefore, patients on disulfiram therapy or persons who are occupationally exposed to analogs of disulfiram in industry or agriculture [26] may show exaggerated responses to pharmacologically active esters or to other environmental esterase inhibitors such as organophosphate insecticides.

The extent to which hydrolysis of an ester or amide drug is impaired by DS treatment may depend on whether the drug is primarily hydrolyzed by plasma or microsomal esterases. Such considerations must be made, since plasma esterases recover more rapidly than the microsomal enzymes (Fig. 2) and are less sensitive to impairment by daily DS administration. Since previous studies indicate that plasma esterases are derived in part from the liver [27, 28], it does not appear likely that the return of plasma esterase activity to basal levels is due to synthesis of new enzyme, because microsomal esterase activity is still declining. A recovery of plasma esterase activity could occur, however, if membrane-bound esterases were released from microsomes into the plasma by the actions of DS. In this regard, Higashino *et al.* [29] have demonstrated that i.p. administration of CCl_4 to mice results in a loss of hepatic microsomal esterase that leads to higher levels of esterase activity in hepatocyte cytosol and in plasma. Release of microsomal esterase activity into plasma has also been demonstrated after exposure of rats to CCl_4 vapor [30]. Although we have no direct evidence that this is the case after DS administration, such a mechanism would explain the experimental observations presented in Fig. 2.

The return of plasma esterases to basal levels of activity could also reflect the disappearance of DS or an active metabolite from plasma. However, this possibility seems less likely since the absorption of the poorly soluble DS from the gut would still be occurring during the rise of serum esterase levels. Moreover, during continuous administration of DS, plasma esterases had returned to control levels at times when microsomal carboxylesterase activity was depressed (Fig. 1).

Electrophoretic analysis of microsomal esterases revealed that the more rapidly migrating esterases were impaired to the greatest extent by DS treatment (Figs. 3 and 4). It has been proposed that the microsomal esterases that migrate rapidly toward the anode are A-esterases, while the slowly migrating enzymes are B-esterases [24]. Although differences in the mechanism of catalysis between these two esterases are still not clear, it had been suggested that A-esterases contain an —SH group in their active site while B-esterases do not [31]. Since it has been proposed that DS inhibits enzymes such as hexokinase [32], aldehyde dehydrogenase [33, 34] and D-amino acid oxidase [35] by interacting with essential enzyme

—SH groups, the selective impairment of the rapidly migrating microsomal esterases (A-esterases) by DS may be the result of interactions between DS and active site thiol groups of these enzymes. The relatively weaker inhibition of the B-esterases by DS may result from interactions between DS and enzyme —SH groups on a portion of the molecule away from the active site.

Esterases exist in multiple forms not only in the liver but also in a wide range of other tissues that have been studied [23]. Therefore, the overall impairment of esterase activity in a given tissue after DS administration may depend on the relative populations of A- and B-esterases (or DS-sensitive vs DS-insensitive esterases) in the tissue. The heterogeneity of tissue esterase populations may account for the fact that liver, lung and plasma esterases were significantly impaired after DS administration while heart, kidney and testis esterases were not (Table 3). In addition, accumulation of DS or active metabolites in particular tissues could also contribute to the observed variation in sensitivity of tissue esterases to DS.

In the rat DS is rapidly reduced to the corresponding thiol, DDTC [4]. Twenty-four hr after oral DDTC administration, microsomal and plasma carboxylesterase activity was impaired but only at relatively high doses (Table 1). Since oral administration of DDTC may result in decomposition of a portion of the dose to CS_2 and diethylamine (DEA) in the gut [4], esterase activity was also determined 24 hr after i.p. administration of DS and DDTC (Table 2). After i.p. administration, DS also impaired esterase activity to a greater extent than DDTC, which may indicate that the presence of DS in the disulfide form is required for maximal esterase inhibition, as proposed for DS inhibition of several other enzymes [32–35]. However, even after i.p. administration of DS, absorption from the peritoneum is relatively slow. DDTC absorption is more rapid, and by 24 hr a significant portion of the administered dose may have already been conjugated with glucuronic acid or been otherwise metabolized or eliminated [4]. Therefore, different rates of absorption, biotransformation and excretion of DS and DDTC may contribute to the observed differences in esterase impairment 24 hr after administration. Moreover, i.p. administration of DS produces a serofibrinous inflammatory reaction, which may in some way contribute to enzyme inhibition or to the rate of recovery from the inhibition [5].

Comparisons between DS and DDTC are further complicated by the fact that DDTC can be reoxidized to DS by endogenous compounds such as cytochrome *c* and methemoglobin [32, 34]. Therefore, DS and DDTC were incubated with microsomes or plasma *in vitro* to compare their activity under conditions where metabolic interconversions between the oxidized and reduced compounds would not be a significant problem (Table 4). In contrast to the impairment seen after DS administration, incubation of control microsomes with DS or DDTC resulted in only small decreases in esterase activity. However, during these incubations, impairment was consistently greater in the presence of DS than DDTC. These observations may indicate that the disulfide is required for maximum impairment or, alternatively, that the more lipid-soluble disulfide may enter the microsomal membrane more easily than DDTC and therefore have better access to the membrane-bound esterases. In this regard, it had been shown that a solubilized microsomal amide-splitting enzyme was inhibited to a greater extent by SKF-525A (2-diethylaminoethyl 2,2-diphenylvalerate HCl) than the particulate enzyme from which it was derived [36]. Moreover, incubation of plasma with either 0·1 mM DS or DDTC produced approximately 50 per cent inhibition of plasma carboxylesterase activity (Table 4). The similar activity of DS and DDTC as inhibitors *in vitro* of plasma carboxylesterases may be due to the fact that the plasma enzymes are soluble and therefore equally accessible to DS and DDTC.

Although the studies *in vitro* did not define the nature of the interaction between DS or DDTC and carboxylesterases, they did demonstrate that esterase inhibition is not due to the metabolic products, CS_2 and diethylamine (DEA). Neither of these compounds, alone or in combination, had any effect on hepatic esterases *in vitro* (Table 4). Similarly, neither CS_2 nor DEA alone inhibited plasma carboxylesterases, but a combination of the two reduced activity to 73 per cent of control. However, an equimolar concentration of DDTC reduced activity to 44 per cent of control, which suggests that breakdown of DDTC to CS_2 and DEA is not responsible for esterase inhibition. We have also found that oral administration of CS_2 (0·25 or 0·50 ml/kg) to male rats had no effect on plasma or microsomal esterases (results not shown), further indicating that this metabolite of DS and DDTC is not responsible for the observed impairment of esterase activity. Inhibition by the CS_2–DEA mixture may be due to formation of DDTC when these compounds are combined. Such an effect had been reported with the fungicide dimethyldithiocarbamate (DMDTC), which decomposes to CS_2 and dimethylamine. A combination of CS_2 and dimethylamine was found to be many times more toxic to spores of *Monilia fructicola* than either compound alone, which implies that the breakdown of DMDTC may be reversible under appropriate conditions [26].

An interaction between DS and procaine (the ester of *p*-aminobenzoic acid and diethylaminoethanol) was observed when procaine HCl (230 mg/kg, i.p.) was given to control rats and to rats pretreated 24 hr previously with 2 g/kg of DS (unpublished observations). Although procaine produced convulsions in approximately 80 per cent of the rats in both groups, 60 per cent of the control animals recovered while all pretreated animals died. These preliminary observations suggest that DS does not alter the convulsive threshold to procaine but may increase its toxicity by impairing hydrolysis of the compound *in vivo*. Although procaine is hydrolyzed primarily by plasma esterases [12], the results reported herein suggest that the hydrolysis of drugs by hepatic microsomal esterases may also be impaired by DS treatment. Moreover, the degree of impairment may depend upon which microsomal esterases are responsible for hydrolysis of a specific substrate since electrophoretic data indicate that A-esterases appear to be particularly sensitive to DS inhibition. However, further studies are required to verify this hypothesis and to

identify the exact nature of the interactions between DS and individual microsomal esterases.

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