Inhibition of microsomal oxidation of alcohols and of hydroxyl-radical-scavenging agents by the iron-chelating agent desferrioxamine

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Rat liver microsomes (microsomal fractions) catalyse the oxidation of straight-chain aliphatic alcohols and of hydroxyl-radical-scavenging agents during NADPH-dependent electron transfer. The iron-chelating agent desferrioxamine, which blocks the generation of hydroxyl radicals in other systems, was found to inhibit the following microsomal reactions: production of formaldehyde from either dimethyl sulphoxide or 2-methylpropan-2-ol (*t*-butylalcohol); generation of ethylene from 4-oxothiomethylbutyric acid; release of $^{14}\text{CO}_2$ from [$1\text{-}^{14}\text{C}$]benzoate; production of acetaldehyde from ethanol or butanal (butyraldehyde) from butan-1-ol. Desferrioxamine also blocked the increase in the oxidation of all these substrates produced by the addition of iron–EDTA to the microsomes. Desferrioxamine had no effect on a typical mixed-function-oxidase activity, the *N*-demethylation of aminopyrine, nor on the peroxidatic activity of catalase/ H_2O_2 with ethanol. H_2O_2 appears to be the precursor of the oxidizing radical responsible for the oxidation of the alcohols and the other hydroxyl-radical scavengers. Chelation of microsomal iron by desferrioxamine most likely decreases the generation of hydroxyl radicals, which results in an inhibition of the oxidation of the alcohols and the hydroxyl-radical scavengers. Whereas desferrioxamine inhibited the oxidation of 2-methylpropan-2-ol, dimethyl sulphoxide, 4-oxothiomethylbutyrate and benzoate by more than 90%, the oxidation of ethanol and butanol could not be decreased by more than 45–60%. Higher concentrations of desferrioxamine were required to block the metabolism of the primary alcohols than to inhibit the metabolism of the other substrates. The desferrioxamine-insensitive rate of oxidation of ethanol was not inhibited by competitive hydroxyl-radical scavengers. These results suggest that primary alcohols may be oxidized by two pathways in microsomes, one dependent on the interaction of the alcohols with hydroxyl radicals (desferrioxamine-sensitive), the other which appears to be independent of these radicals (desferrioxamine-insensitive).

Desferrioxamine is an iron-chelating agent that is used clinically to treat cases of iron poisoning. This compound is of special interest to researchers in the oxygen-radical field because production of hydroxyl radicals ($\text{OH}^\bullet$) in biological systems is catalysed by iron (McCord & Day, 1978; Halliwell, 1978*a,b*), and therefore may be prevented by potent iron-chelating agents. Desferrioxamine has been shown to prevent the degradation of hyaluronic acid by ascorbate (Wong *et al.*, 1981) or by a superoxide-radical ($\text{O}_2^{\bullet-}$)-generating system (Gutteridge *et al.*, 1979) or the breakdown of deoxyribose

catalysed by xanthine plus xanthine oxidase plus iron salts (Halliwell & Gutteridge, 1981). The bactericidal activity of an acetaldehyde/xanthine oxidase/EDTA system was prevented by desferrioxamine (Rosen & Klebanoff, 1981), as was lipid peroxidation initiated by the addition of iron–EDTA to liposomes and the aromatic hydroxylation of salicylic acid (Gutteridge *et al.*, 1979). The autoxidation of dopamine (3,4-dihydroxyphenethylamine) or noradrenaline (norepinephrine) (Heikkilä & Cabbat, 1981), as well as the formation of $\text{OH}^\bullet$ during the autoxidation of 6-hydroxydopamine (Floyd & Wiseman, 1979) were also prevented by the addition of desferrioxamine. These examples point to the use of desferrioxamine as an effective agent to evaluate the role of iron in catalysing the production of oxygen radicals in various systems.

Abbreviations used: $\text{OH}^\bullet$, hydroxyl radical or a species with the oxidizing power of the hydroxyl radical; Me_2SO , dimethyl sulphoxide; OTBA, 2-oxo-4-thiomethylbutyric acid.

Previous experiments have shown that the oxidation of ethanol by microsomes was inhibited by competitive $\text{OH}^\bullet$ -scavenging agents (Cederbaum *et al.*, 1978, 1979). These agents had no effect on microsomal drug metabolism or NADPH-dependent electron transfer. The microsomes catalysed the NADPH-dependent oxidation of classical $\text{OH}^\bullet$ -scavenging agents such as OTBA or 3-(methylthio)propionaldehyde (methional) (Cohen & Cederbaum, 1979, 1980), Me_2SO (Klein *et al.*, 1981), 2-methylpropan-2-ol (t-butyl alcohol) (Cederbaum & Cohen, 1980) and benzoate (Winston & Cederbaum, 1982). These studies led to the suggestion that microsomes produce a powerful oxidant, with properties similar to that of $\text{OH}^\bullet$, during electron transfer from NADPH. This oxidant was responsible, at least in part, for the oxidation of alcohols by microsomes. The addition of iron-EDTA resulted in an increase in the oxidation of ethanol, butan-1-ol and OTBA by the microsomes (Cederbaum *et al.*, 1980). Experiments were therefore carried out with desferrioxamine to evaluate a role for 'microsomal iron' in catalysing the oxidation of alcohols and $\text{OH}^\bullet$ -scavenging agents by rat liver microsomes, and to determine if this compound would prove to be an effective inhibitor of these oxidation reactions.

Materials and methods

Rat liver microsomes were prepared from male Sprague-Dawley rats as previously described (Cederbaum *et al.*, 1978). The microsomes were washed once and resuspended in 125 mM-KCl. Protein was determined by the method of Lowry *et al.* (1951). The water used for all solutions, as well as the phosphate, pyrophosphate and KCl solutions were routinely passed through columns containing chelex-100 resin to remove extraneous iron.

The basic reaction mixture used in all experiments consisted of 100 mM-potassium phosphate buffer, pH 7.4, 10 mM-sodium pyrophosphate, 10 mM- MgCl_2 , 0.1 mM-EDTA, 1 mM- NaN_3 , 0.3 mM-NADP⁺, 10 mM-glucose 6-phosphate, 7 units of glucose 6-phosphate dehydrogenase, 3–5 mg of microsomal protein, and substrate, in a final volume of 3.0 ml. Six different substrates were used, namely 51 mM-ethanol, 35 mM-butan-1-ol, 35 mM-2-methylpropan-2-ol, 33 mM- Me_2SO , 10 mM-OTBA or 10 mM-benzoic acid [which contained trace amounts of [*carboxy*- ^{14}C]benzoate (final specific radioactivity 5.5 $\mu\text{Ci}/\text{mmol}$)]. Previous experiments demonstrated that the microsomes oxidized Me_2SO and 2-methylpropan-2-ol to formaldehyde (Klein *et al.*, 1981; Cederbaum & Cohen, 1980); ethylene was produced from OTBA (Cohen & Cederbaum, 1980); $^{14}\text{CO}_2$ was produced from [^{14}C]benzoate (Winston & Cederbaum, 1982); ethanol and butan-1-ol

were oxidized to acetaldehyde and butanal (1-butyraldehyde) respectively (Cederbaum *et al.*, 1978; Teschke *et al.*, 1975). Reaction periods were either 15 min (ethanol, butan-1-ol or OTBA) or 30 min (Me_2SO , 2-methylpropan-2-ol or benzoate). All reactions were initiated with the NADPH-generating system, and incubations were carried out at 37°C in a Dubnoff shaker. The reactions were terminated with either trichloroacetic acid (ethanol, butan-1-ol, Me_2SO or 2-methylpropan-2-ol) added to a final concentration of 4.5% (w/v) or by HClO_4 (OTBA or benzoate) added to a final concentration of 7% (w/v). The production of formaldehyde from Me_2SO of 2-methylpropan-2-ol was assayed by allowing 1.5 ml portions of the supernatant obtained after centrifugation to react with 1.5 ml of Nash (1953) reagent (acetylacetone/excess ammonium salts). The production of acetaldehyde or butanal from ethanol or butan-1-ol was determined by trapping the aldehydes in centre-well flasks containing 0.6 ml of 15 mM-semicarbazide hydrochloride in the centre well. After an overnight diffusion period at room temperature, the absorbance of the aldehyde-semicarbazone complex was determined at 224 nm. For the OTBA experiments, reactions were carried out in flasks sealed with serum caps. Reactions were terminated by injecting HClO_4 through the cap, and ethylene was determined by head-space gas chromatography (Cohen & Cederbaum, 1980). For the benzoate experiments, reactions were carried out in flasks sealed with stoppers containing hanging plastic centre wells. Reactions were terminated by injecting HClO_4 through the cap. Hyamine hydroxide (0.3 ml) was injected into the centre well and $^{14}\text{CO}_2$ was allowed to collect in the centre well for 45 min. The centre well was removed, placed into a vial containing Econofluor scintillation fluid, and radioactivity was counted in a Beckman model LS-9000 liquid-scintillation counter with automatic quench correction. For all substrates, all values were corrected for zero-time controls which contained the acid added before the NADPH-generating system.

The *N*-demethylation of aminopyrine was assayed as a routine mixed-function-oxidase activity. Aminopyrine (10 mM) was used in place of the other substrates. The reaction period was 10 min and formaldehyde production was assayed by the method of Nash (1953) on 1.5 ml portions of the trichloroacetic acid supernatant fraction.

All values are means $\pm$ S.E.M. Statistical analysis was performed by Student's *t* test. The number of experiments is indicated in the Table legends.

All materials were of the highest grade available. Desferrioxamine (desferal mesylate or desferrioxamine monomethanesulphonate) was from CIBA Pharmaceutical Company, Summit, NJ, U.S.A.; OTBA and Me_2SO were from Sigma Chemical Co.,

St. Louis, MO, U.S.A. 2-Methylpropan-2-ol and butan-1-ol were from Fisher Scientific Co., Fairlawn, NJ, U.S.A.; [$1\text{-}^{14}\text{C}$]benzoic acid was from New England Nuclear Corp., Boston, MA, U.S.A. NADP⁺, glucose 6-phosphate and glucose 6-phosphate dehydrogenase were from Boehringer Mannheim, Indianapolis, IN, U.S.A., Chelex-100 resin (200–400 mesh) was from Bio-Rad Laboratories, Richmond, CA, U.S.A.

Results

Effect of desferrioxamine on microsomal oxidation of OH[•]-scavenging agents

Initial experiments concerned the effects of various concentrations of desferrioxamine on ethylene production from OTBA and of $^{14}\text{CO}_2$ production from [$1\text{-}^{14}\text{C}$]benzoate. Desferrioxamine was a very potent inhibitor of the microsomal oxidation

of both OTBA and benzoate (Table 1). Strong inhibition was observed at desferrioxamine concentrations of $3.3\text{ }\mu\text{M}$, and near-complete inhibition was found at desferrioxamine concentrations of 0.1 mM (Table 1).

Formaldehyde was shown to be a major product of the interaction of either Me_2SO or 2-methylpropan-2-ol with $\text{OH}^\bullet$ generated by model $\text{OH}^\bullet$ -generating systems as well as microsomes (Klein *et al.*, 1980, 1981; Cederbaum & Cohen, 1980). Table 2 shows that desferrioxamine was a potent inhibitor of formaldehyde production from these two substrates. To rule out the possibility that the inhibition of oxidation of $\text{OH}^\bullet$ scavengers by desferrioxamine may be due to interference with microsomal electron transfer or toxic effects on NADPH:cytochrome *P*-450 reductase or cytochrome *P*-450, or non-specific effects on the microsomes, the effect of desferrioxamine on formaldehyde production from aminopyrine was determined.

Table 1. *Effect of desferrioxamine on the production of ethylene from OTBA and of $^{14}\text{CO}_2$ from [$1\text{-}^{14}\text{C}$]benzoate by microsomes*

The production of ethylene from OTBA or of $^{14}\text{CO}_2$ from [$1\text{-}^{14}\text{C}$]benzoate was assayed as described in the Materials and methods section, in the presence of the indicated concentrations of desferrioxamine. Results are from five (benzoate) or six (OTBA) experiments. Levels of significance: * $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$.

[Desferrioxamine] (μM)	Production of ethylene from OTBA		Production of CO_2 from benzoate	
	Rate (nmol/min per mg of microsomal protein)	Effect of desferrioxamine (%)	Rate (nmol/min per mg of microsomal protein)	Effect of desferrioxamine (%)
0	2.58 ± 0.56	—	1.40 ± 0.12	—
3.3	1.03 ± 0.20	−60*	0.48 ± 0.21	−66†
33	0.71 ± 0.29	−72†	0.21 ± 0.17	−85‡
100	0.51 ± 0.14	−80†	0.11 ± 0.08	−92‡
250	0.38 ± 0.11	−85†	0.08 ± 0.08	−94‡
500	0.22 ± 0.13	−91†	0	−100‡

Table 2. *Effect of desferrioxamine on the production of formaldehyde from either aminopyrine, dimethyl sulphoxide or 2-methylpropan-2-ol by microsomes*

The production of formaldehyde from 10 mM-aminopyrine, 33 mM-dimethyl sulphoxide or 35 mM-2-methylpropan-2-ol was assayed as described in the Materials and methods section. Results are from three (aminopyrine), six (dimethyl sulphoxide) or five (2-methylpropan-2-ol) experiments. Rate refers to nmol of formaldehyde produced/min per mg of microsomal protein. Levels of significance: * $P < 0.05$; † $P < 0.001$; ‡ $P < 0.01$.

[Desferrioxamine] (μM)	Rate of formaldehyde production and effect of desferrioxamine					
	Dimethyl sulphoxide		2-Methylpropan-2-ol		Aminopyrine	
	Rate	Effect (%)	Rate	Effect (%)	Rate	Effect (%)
0	3.46 ± 0.40	—	1.47 ± 0.10	—	8.50 ± 0.87	—
3.3	1.47 ± 0.45	−58*	0.21 ± 0.07	−86‡	—	—
33	0.68 ± 0.32	−80†	0.14 ± 0.04	−90†	8.61 ± 1.96	+1
100	0.38 ± 0.20	−89†	0.15 ± 0.04	−90†	8.17 ± 1.46	−4
250	0.21 ± 0.07	−94†	0.16 ± 0.04	−89†	7.90 ± 0.66	−7
500	0.16 ± 0.03	−95†	0.15 ± 0.04	−90†	6.81 ± 1.68	−20

Concentrations of desferrioxamine that nearly completely inhibited the production of formaldehyde from Me_2SO or 2-methylpropan-2-ol had no effect on the production of formaldehyde from aminopyrine (Table 2). These results indicate that the potent inhibition by desferrioxamine of the metabolism of $\text{OH}^\bullet$ -scavenging agents was not associated with effects on the microsomal mixed-function-oxidase system.

Effect of desferrioxamine on microsomal oxidation of ethanol and butan-1-ol

Previous experiments indicated that $\text{OH}^\bullet$ played a role in the molecular mechanism by which microsomes oxidize primary aliphatic alcohols such as ethanol or butan-1-ol (Cederbaum *et al.*, 1978). Inhibition of $\text{OH}^\bullet$ production should therefore also result in an inhibition of microsomal oxidation of ethanol and butan-1-ol. Table 3 shows that concentrations of desferrioxamine that inhibited the oxidation of $\text{OH}^\bullet$ scavengers also inhibited the oxidation of ethanol and butan-1-ol. Two interesting points emerge when comparing the effects of desferrioxamine on the oxidation of primary alcohols and other $\text{OH}^\bullet$ scavengers. First, desferrioxamine was more inhibitory against the latter than the former group of substrates (e.g. $3.3\text{ }\mu\text{M}$ -desferrioxamine blocked the oxidation of ethanol and butan-1-ol by 33%, whereas the oxidation of $\text{OH}^\bullet$ scavengers was inhibited by 60–86%; Tables 1, 2 and 3). Second, even at very high concentrations of desferrioxamine (when metabolism of scavengers was inhibited >90%), a significant amount of oxidation of ethanol and butan-1-ol continued, i.e., inhibition of the oxidation of the primary alcohols by desferrioxamine did not exceed 45–60%.

Catalase is present in isolated microsomes as a contaminant. Since ethanol (but not butan-1-ol) can be oxidized via the peroxidatic activity of catalase, azide was routinely added in these experiments to inhibit the activity of catalase. When azide is omitted, microsomal oxidation of ethanol reflects, in part, the peroxidatic oxidation of ethanol by catalase (Thurman *et al.*, 1972). The latter pathway depends on NADPH-mediated microsomal electron transfer to provide the H_2O_2 required as a cofactor for catalase. Table 3 shows that desferrioxamine did not significantly inhibit the oxidation of ethanol in the absence of azide. These results, coupled to the lack of effect on aminopyrine demethylation, suggest some selectivity in the effects of desferrioxamine on the oxidation of the alcohols and $\text{OH}^\bullet$ -scavenging agents.

Effect of $\text{OH}^\bullet$ -scavenging agents on the desferrioxamine-insensitive rate of microsomal oxidation of ethanol

Microsomal oxidation of ethanol in the absence of desferrioxamine is sensitive to inhibition by competitive $\text{OH}^\bullet$ -scavenging agents. For example, ethanol oxidation was inhibited by 38 or 50% by 33 mM- or 67 mM-OTBA respectively; by 39 or 50% by 33 mM- or 67 mM-benzoate respectively; and by 58 or 70% by 47 mM- or 94 mM- Me_2SO respectively (Cohen & Cederbaum, 1980; Cederbaum *et al.*, 1978). To determine if the pathway of ethanol oxidation that persists in the presence of desferrioxamine is mediated by $\text{OH}^\bullet$, the effect of the above competitive scavengers was studied. Neither OTBA nor benzoate inhibited the desferrioxamine-insensitive rate of microsomal oxidation of ethanol (Table 4). Some inhibition was found with Me_2SO (Table 4).

Table 3. *Effect of desferrioxamine on the oxidation of ethanol and butan-1-ol by rat liver microsomes*
The oxidation of ethanol (in the absence and presence of 1.0 mM-azide) or butan-1-ol to acetaldehyde or butanal was assayed as described in the Materials and methods section. Results are from six (ethanol + azide), three (ethanol – azide) or five (butan-1-ol + azide) experiments. Rate refers to nmol of aldehyde produced/min per mg of microsomal protein. Levels of significance: * $P = 0.081$; † $P < 0.001$; ‡ $P < 0.05$.

[Desferrioxamine] (μM)	Rate of alcohol oxidation and effect of desferrioxamine					
	Ethanol plus azide		Ethanol minus azide		Butan-1-ol plus azide	
	Rate	Effect (%)	Rate	Effect (%)	Rate	Effect (%)
0	6.41 ± 0.65	—	6.78 ± 0.57	—	4.05 ± 0.97	—
3.3	4.38 ± 0.52	–32*	7.20 ± 0.57	+6	2.72 ± 0.71	–33
33	3.01 ± 0.25	–53†	6.26 ± 0.68	–8	2.66 ± 0.64	–34‡
100	2.79 ± 0.28	–56†	5.64 ± 0.65	–17	2.23 ± 0.49	–45‡
250	2.87 ± 0.26	–55†	5.59 ± 0.81	–18	2.34 ± 0.61	–42‡
500	2.42 ± 0.34	–62†	5.57 ± 0.83	–18	2.12 ± 0.53	–48‡

Effect of desferrioxamine on the stimulation by iron-EDTA of microsomal oxidation of OH[•]-scavenging agents

The addition of iron-EDTA to rat liver microsomes was shown to increase the oxidation of ethanol and propan-2-ol (Cederbaum *et al.*, 1980,

Table 4. *Effect of competitive OH[•] scavengers on the desferrioxamine-insensitive rate of ethanol oxidation by microsomes*

The oxidation of ethanol was carried out in the presence of 0.33 mM-desferrioxamine in all cases. This rate of ethanol oxidation is referred to as the desferrioxamine-insensitive rate of ethanol oxidation. Results are from three experiments. **P* < 0.05.

Addition	Concn. (mM)	Ethanol oxidation (nmol/min per mg of microsomal protein)	Effect of addition (%)
Control	—	2.06 ± 0.23	—
Me ₂ SO	47	1.65 ± 0.01	-20
	94	1.35 ± 0.02	-34*
OTBA	10	2.25 ± 0.11	+9
	33	2.48 ± 0.06	+20
	67	2.56 ± 0.09	+24
Benzoate	33	2.46 ± 0.06	+19
	67	2.65 ± 0.24	+29

1981). As Table 5 shows, the increase in microsomal oxidation of ethanol was accompanied by an increase in the oxidation of other OH[•] scavengers such as OTBA, Me₂SO and 2-methylpropan-2-ol (see the legend to Table 5). Desferrioxamine inhibited the oxidation of ethanol, 2-methylpropan-2-ol, Me₂SO and OTBA in the absence, as well as in the presence, of iron-EDTA (Table 5). In general, the percentage inhibition by desferrioxamine was similar in the absence of iron-EDTA and in the presence of a lower concentration (8 μM) of iron-EDTA (see columns 3 and 4 of Table 5). However, the inhibition by desferrioxamine, at least at lower concentrations, was less in the presence of 50 μM-iron-EDTA (see columns 3 and 5 of Table 5), i.e., the ability of desferrioxamine to inhibit microsomal oxidation of ethanol and other OH[•]-scavenging agents could be attenuated by the addition of external iron. This further suggests that the inhibition by desferrioxamine of microsomal oxidation of alcohols and OH[•] scavengers, in the absence of added iron, reflects chelation of endogenous microsomal iron.

Discussion

The ability of microsomes to oxidize ethanol represents a minor pathway of ethanol oxidation.

Table 5. *Effect of desferrioxamine on microsomal oxidation of ethanol and OH[•] scavengers in the absence and in the presence of iron-EDTA*

The oxidation of ethanol, 2-methylpropan-2-ol, Me₂SO or OTBA was assayed as described in the Materials and methods section in the presence of the indicated concentrations of desferrioxamine. Experiments were carried out in the absence of iron-EDTA or in the presence of either 8 μM-iron-16 μM-EDTA or 50 μM-iron-100 μM-EDTA. Control rates (nmol/min per mg of microsomal protein) in the absence of desferrioxamine were as follows: ethanol, 5.24, 11.62 and 18.64 in the presence of 0, 8 or 50 μM-iron-EDTA respectively; 2-methylpropan-2-ol, 1.5, 5.07 and 8.91 in the presence of 0, 8 or 50 μM-iron-EDTA respectively; Me₂SO, 5.2, 11.3 and 14.9 in the presence of 0, 8 or 50 μM-iron-EDTA respectively; OTBA, 3.25, 13.84 and 25.9 in the presence of 0, 8 or 50 μM-iron-EDTA respectively. Results are from two experiments.

Substrate	[Desferrioxamine] (μM)	Concn. of iron-EDTA (μM) ...	Percentage inhibition by desferrioxamine		
			0	8	50
Ethanol	3.3		36	31	4
	33		43	55	10
	100		56	68	44
	500		54	72	54
2-methylpropan-2-ol	3.3		97	58	+1
	33		100	92	39
	100		100	95	66
	500		100	98	87
Me ₂ SO	3.3		77	41	5
	33		81	82	13
	100		95	93	42
	500		98	96	76
OTBA	3.3		52	64	7
	33		62	72	12
	100		73	78	69
	500		82	87	83

This pathway assumes greater significance after chronic consumption of ethanol (Lieber & DeCarli, 1970, 1972). Rat liver microsomes can catalyse the oxidation of several OH[•]-scavenging agents during NADPH-dependent electron transport (Cohen & Cederbaum, 1979, 1980; Cederbaum & Cohen, 1980; Klein *et al.*, 1981). A role for an oxidant with properties similar to OH[•] in the molecular mechanism of ethanol oxidation by microsomes has been suggested (Cederbaum *et al.*, 1978, 1979). Since azide stimulates the oxidation of OH[•] scavengers such as OTBA (Cohen & Cederbaum, 1980), Me₂SO (Klein *et al.*, 1981) and 2-methylpropan-2-ol (Cederbaum & Cohen, 1980), as well as the oxidation of alcohols that do not serve as effective substrates for the peroxidatic activity of catalase, e.g., butan-1-ol and propan-2-ol (Cederbaum *et al.*, 1978, 1981), a role for H₂O₂ as the precursor of OH[•] in microsomal systems has been suggested. H₂O₂ may produce OH[•] in the presence of iron via the Fenton reaction:

In view of the Chelex treatment of most solutions, possible sources of microsomal iron that may participate in the Fenton reaction are adventitious iron in the microsomes or haem protein such as cytochrome *P*-450. The present results demonstrate that desferrioxamine, which has been found to be an effective inhibitor of the production of OH[•] by several systems (e.g. xanthine plus xanthine oxidase plus iron, autoxidation of catecholamines) also is an effective inhibitor of the NADPH-dependent oxidation of OH[•] scavengers and alcohols by microsomes. However desferrioxamine does not inhibit microsomal drug metabolism at concentrations that strongly inhibit the oxidation of the OH[•] scavengers (Table 2). These results disassociate the ability of the microsomes to catalyse drug metabolism from the oxidation of the OH[•] scavengers, in agreement with previous results demonstrating that typical OH[•] scavengers such as benzoate, mannitol or Me₂SO do not inhibit microsomal oxidation of aminopyrine or aniline (Cederbaum *et al.*, 1978).

In view of the potent inhibition by desferrioxamine, iron appears to play an important role in the generation of OH[•] by microsomes. The disassociation between the metabolism of the scavengers and typical drug substrates suggests either that cytochrome *P*450 does not play a significant role in the generation of OH[•] or that different forms of cytochrome *P*-450 participate in the two reactions. Preliminary experiments indicate that NADH does not support microsomal oxidation of OH[•] scavengers, indicating little or no role for cytochrome *b*₅ in catalysing the production of OH[•] by microsomes. These results therefore suggest that other sources of microsomal iron, e.g. adventitious

iron, are the likely catalyst of the Fenton reaction. Reduction of this iron to the ferrous state may occur either via microsomal electron transport itself or via O₂^{-•} generated during microsomal electron transport. Superoxide is known to be produced during microsomal electron transfer (Aust *et al.*, 1972; Prough & Masters, 1973; Strobel & Coon, 1971; Dybing *et al.*, 1976). The addition of superoxide dismutase does not inhibit the oxidation of ethanol or of propan-2-ol by the microsomes (Cederbaum *et al.*, 1978, 1980, 1981); however, the added superoxide dismutase may not penetrate to the active site of O₂^{-•} generation within the microsomes. More recent experiments with the iron chelator diethylenetriaminepenta-acetic acid, which blocks the iron-catalysed Haber-Weiss reaction (Buettner *et al.*, 1978; Halliwell, 1978a) by preventing reduction of ferric chelates by O₂^{-•} (Cohen & Sinet, 1982), has shown inhibition of microsomal oxidation of OH[•] scavengers and alcohols by this chelator (A. I. Cederbaum & G. Cohen, unpublished work). In any event, the potent inhibition by desferrioxamine suggests that microsomal iron reacts with H₂O₂ (generated by microsomal electron transfer) to produce the oxidant responsible for the oxidation of the OH[•] scavengers and of alcohols by the microsomes.

It is noteworthy that desferrioxamine appears to inhibit the oxidation of typical OH[•] scavengers such as OTBA, Me₂SO, benzoate and 2-methylpropan-2-ol to a greater extent than the oxidation of primary aliphatic alcohols such as ethanol and butan-1-ol (Tables 1, 2 and 3). Even high concentrations of desferrioxamine do not suppress the microsomal oxidation of ethanol or butan-1-ol by more than 50–60%. Differences in the sensitivity to desferrioxamine may reflect the possibility that, whereas primary aliphatic alcohols react with OH[•] by a chain mechanism (one OH[•] can result in the oxidation of more than one alcohol), other OH[•] scavengers such as 2-methylpropan-2-ol react with OH[•] only by a non-chain-reaction mechanism (Merz & Waters, 1949). In other experiments we have observed that the oxidation of ethanol is as sensitive to inhibition by desferrioxamine as is the oxidation of 2-methylpropan-2-ol when OH[•] is generated by a xanthine/xanthine oxidase/iron system (A. I. Cederbaum & E. Dicker, unpublished work). This appears to rule out chain versus non-chain mechanisms as an explanation for the different sensitivities to desferrioxamine. Another possibility is that, in microsomes, primary aliphatic alcohols are oxidized by OH⁻-dependent and OH[•]-independent pathways (catalase is ruled out, since azide is usually present to inhibit this enzyme; butan-1-ol is not an effective substrate for the peroxidatic activity of catalase). In agreement with this possibility is the finding that the desferrioxamine-insensitive rate of microsomal oxi-

dation of ethanol or butan-1-ol is not inhibited by the competitive $\text{OH}^\bullet$ scavengers OTBA and benzoate. This suggests that $\text{OH}^\bullet$ does not play a significant role in oxidizing ethanol or butan-1-ol in the presence of desferrioxamine. Some inhibition of the desferrioxamine-insensitive rate is found in the presence of Me_2SO . However, this inhibition may not reflect interaction with $\text{OH}^\bullet$, since high concentrations (47–94 mM) of Me_2SO are required and the effect of Me_2SO in the presence of desferrioxamine (20–34% inhibition) is much less than the effect in the absence of desferrioxamine (58–70% inhibition). Recently, Me_2SO was found to bind to cytochrome *P*-450 LM3a with a K_s of 56 mM (Morgan & Coon, 1982). This suggests the possibility that the inhibition of the desferrioxamine-insensitive rate of microsomal oxidation of ethanol or butan-1-ol by Me_2SO may be due to interactions at the cytochrome *P*-450 binding site, and not scavenging of $\text{OH}^\bullet$.

In summary, these results indicate that desferrioxamine is a valuable agent to block the generation of $\text{OH}^\bullet$ by microsomes. The inhibition by desferrioxamine suggests that iron in the microsomes catalyses the production of $\text{OH}^\bullet$. Since desferrioxamine does not inhibit the activity of aminopyrine demethylase, the pathway of $\text{OH}^\bullet$ generation can be disassociated from the classical mixed-function oxidase system. If cytochrome *P*-450 catalyses production of $\text{OH}^\bullet$, different isoenzymes or forms of the cytochrome are required for $\text{OH}^\bullet$ generation and for aminopyrine demethylase activity. Alternatively, adventitious iron in the microsomes catalyses the production of $\text{OH}^\bullet$. If the latter, the possibility that there may be a physiological equivalent to iron–EDTA in the intact cell remains to be determined. Most cellular iron is usually sequestered as rather inert protein complexes, e.g., ferritin. Conditions such as induction of haem oxygenase activity or alcohol liver disease (Prieto *et al.*, 1975) result in iron overload of the liver. Clearly, when iron is available, the microsomes have the potential to generate reactive oxidants such as $\text{OH}^\bullet$, which may result in oxidative damage to the liver.

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