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FURTHER INVESTIGATIONS OF THE METABOLISM OF FLUROXENE AND THE DEGRADATION OF CYTOCHROMES P-450 *IN VITRO**

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Abstract—The effects of inhibitors and of inducing agents for cytochromes P-450 on the fluroxene mediated destruction of cytochromes P-450 were investigated with hepatic microsomes from male rats *in vitro* and compared with the metabolism of fluroxene (2,2,2-trifluoroethyl vinyl ether) to 2,2,2-trifluoroethanol under similar conditions. The fluroxene mediated destruction of cytochromes P-450 and the metabolism of fluroxene are fully inhibited under totally anaerobic conditions. Carbon monoxide, SKF 525A and metyrapone fully inhibit the fluroxene mediated destruction of cytochromes P-450 and partially inhibit the metabolism of fluroxene to trifluoroethanol in microsomes from phenobarbital pretreated rats. The K_m values for the destruction of cytochromes P-450 by fluroxene *in vitro* were calculated as 0.8, 3.3 and 1.5 mM for microsomes from phenobarbital induced, 3-methylcholanthrene induced and uninduced animals, respectively. V_{max} values for 3-methylcholanthrene and phenobarbital induced microsomes (approximately 0.5 nmol cytochromes P-450 destroyed/mg microsomal protein/7 min) are elevated compared to uninduced microsomes (0.2 nmol cytochromes P-450 destroyed/mg microsomal protein/10 min). The K_m value for the metabolism of fluroxene to trifluoroethanol in control microsomes of approximately 1.0 mM is unchanged following induction, and V_{max} for the production of trifluoroethanol is increased relative to controls only in phenobarbital induced microsomes. It is concluded that the fluroxene mediated destruction of cytochromes P-450 appears to involve both cytochrome P-448 and cytochrome P-450 whereas the production of trifluoroethanol from fluroxene is catalyzed by cytochrome P-450 but not by cytochrome P-448.

Fluroxene (2,2,2-trifluoroethyl vinyl ether), a volatile anaesthetic agent, is known to be metabolized by the hepatic cytochrome P-450 dependent drug metabolizing enzyme system *in vivo* and *in vitro*§. Elevated levels of hepatic cytochrome P-450, but not cytochrome P-448, are accompanied by increased metabolism of fluroxene *in vitro* and enhanced toxicity of fluroxene anaesthesia *in vivo* [1, 2].

Following fluroxene anaesthesia of animals induced for elevated levels of type P-450 cytochromes with phenobarbital or 3-methylcholanthrene, a marked decrease in the levels of hepatic microsomal total type P-450 cytochromes was observed [2]. The fluroxene mediated degradation of hepatic cytochromes P-450 was mimicked *in vitro* in the presence of hepatic microsomes, fluroxene and NADPH [3]. Greater destruction of type P-450 cytochromes was observed with microsomes from phenobarbital or 3-methylcholanthrene pretreated rats with initially elevated levels of type P-450 cytochromes than with microsomes from control rats. The degradation appears to be specific for type P-450 cytochromes since the levels of other microsomal enzymes such as cytochrome b_5 or NADPH-cytochrome *c* reductase are not affected. The observed decrease in the levels of hepatic microsomal cytochromes P-450 seen in the presence of fluroxene appears to reflect chemical alteration of the heme moiety of cytochromes P-450 [3]. Based on the observation that 2,2,2-trifluoroethyl ethyl ether—the saturated analogue of fluroxene—does not destroy cytochromes P-450 *in vivo* or *in vitro*, it was proposed that the vinyl portion of the fluroxene molecule is responsible for the fluroxene mediated destruction of hepatic cytochromes P-450 [2, 3].

The mechanism of the fluroxene mediated destruction of cytochromes P-450 is currently being investigated in our laboratories. Reported herein are the effects of various inhibitors, reducing agents and inducing agents for cytochromes P-450 on the fluroxene mediated destruction of cytochromes P-450 and on the metabolism of fluroxene to 2,2,2-trifluoroethanol.

MATERIALS AND METHODS

Materials. Materials were obtained as follows: cylinders of pure gases, Afrox Ltd.; sodium succinate and EDTA, British Drug Houses Ltd. Reduced glutathione (Sigma Chemicals) was assayed by the method

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§ In this manuscript the terminology "type P-450 cytochromes" or "cytochromes P-450" indicates the heterogeneous group of cytochromes including cytochrome P-450 and cytochrome P-448 (P_1-450). The terms "cytochrome P-450" or "cytochrome P-448" designate the specific enzyme indicated, except in phrases such as "the cytochrome P-450 dependent drug metabolizing pathway".

of Stadtman [4] and found to be over 90% reduced. Allyl-*iso*-propyl-acetamide and SKF 525A (β-diethylaminoethyl-2,2-diphenylvalerate) were generous gifts from Hoffmann-La Roche, Inc., Nutley, New Jersey, and from Smith, Klein & French, Ltd., respectively. Metyrapone [2-methyl-1,2-bis(3-pyridyl)-1-propane] was provided as a gift by Ciba-Geigy Ltd., Basle, Switzerland. Electron transport particles were prepared from fresh beef heart by Method 2 of Crane *et al.* [5]. The electron transport particles were stored at 4° and their activity in the presence of succinate was assayed by measuring oxygen consumption with a Clark type oxygen electrode. Male Wistar rats weighing between 180 and 230 g were used for all experiments. Animals were induced with phenobarbital and 3-methylcholanthrene or treated with allyl-*iso*-propylacetamide as described earlier [2, 3]. Hepatic microsomes were prepared by gel filtration according to the method of Tangen *et al.* [6] as described earlier [3].

Preparation of reaction mixtures. For investigations of the destruction of microsomal proteins and the metabolism of fluroxene *in vitro*, reaction samples contained microsomes (2 mg protein/ml 0.02 M Tris–HCl buffer, pH 7.4), 0.2 mM EDTA, NADPH generating system (0.4 mM NADP, 7.5 mM glucose-6-phosphate, 0.5 units per ml glucose-6-phosphate dehydrogenase, 5 mM $MgCl_2$, 1 mM nicotinamide) and fluroxene or other compounds as specified. References for spectral work contained only microsomes and were incubated as for samples. For most studies, reaction samples and references were contained in vials of appropriate size so that the air space in the vial was approximately 10–15 per cent of the total volume once the vial was stoppered with a serum cap. To prepare reaction samples, microsomal suspensions were equilibrated at 30°, fluroxene was added below the level of the microsomal suspension and the mixture was stoppered and vortex mixed for 30 sec. Any further additions were made through the serum cap of the stoppered vial. The reaction was then initiated by the addition (through the serum cap) of a solution of EDTA plus NADPH generating system. Samples were assayed for total type P-450 cytochromes and the production of 2,2,2-trifluoroethanol at time zero and following incubation with shaking in a Galenkampstat shaking bath equilibrated at 30°. At the end of the incubation period of 7 min (unless otherwise indicated) for destruction with induced microsomes and 10 min for destruction with uninduced microsomes and for metabolism with all types of microsomes, samples were removed immediately for assay or the reaction was quenched by bubbling with CO for 30 sec and assayed shortly thereafter. For experiments under different atmospheric conditions, oxygen or a mixture of CO–O_2 (80:20, v/v) was bubbled through the microsomal suspension for 30 sec immediately prior to the addition of fluroxene.

Anaerobic experiments. For anaerobic experiments the *modus operandi* was as for the preparation of reaction mixtures described above with the following modifications. A mixture of microsomes (2 mg protein/ml, final concentration) and electron transport particles (0.4 mg protein/ml, final concentration) in 0.02 M Tris–HCl buffer, pH 7.4, was deoxygenated for 15 min by repeated flushing with deoxygenated nitrogen and evacuation by aspiration. This treatment resulted in a decrease in the oxygen content of the suspension to approximately 15 per cent that of air saturated microsomes as measured by oxygen electrode. The suspension was then transferred to a nitrogen bag (Instruments for Research and Industry, Cheltenham, Pennsylvania) which was continually flushed with nitrogen or argon. Succinate was added to the suspension, and 3.0 ml aliquots were placed in 4 ml cuvettes with teflon stoppers. Solutions of the remaining reagents which had been previously deoxygenated by bubbling with oxygen-free nitrogen for 15 min were added to the microsomal suspension as described for the aerobic experiments. The cuvettes were stoppered with teflon caps and, where necessary, vortex mixing was performed in the nitrogen bag. Cuvettes were then removed from the nitrogen bag and incubated in air without shaking at 30°.

Assay by oxygen electrode of samples so prepared, containing microsomes, electron transport particles and succinate indicated in every case that the concentration of oxygen was effectively zero and was not decreased further following addition of the oxygen scavanger sodium dithionite. The *O*-demethylation of *p*-nitroanisole that occurred with a first order rate constant of 0.2 min^{-1} aerobically did not proceed measurably over 10 min under the anaerobic experimental conditions described above, which further indicates that the experimental conditions were indeed totally anaerobic. The activity of the electron transport particles under the conditions of the anaerobic experiments was 0.05–0.10 μmol O_2 consumed per min.

Assays. The levels of cytochromes P-450, cytochrome b_5 and heme were determined according to the method of Omura and Sato [7]. NADPH-cytochrome *c* reductase activity was measured as described by Omura and Takesue [8].

Table 1. Effects of inhibitors on the fluroxene mediated destruction of type P-450 cytochromes and on the metabolism of fluroxene in phenobarbital induced hepatic microsomes *in vitro*

Conditions*	Destruction cyts P-450†‡	TFE formed (nmol/min/mg microsomal protein)
—	16%	9.7 ± 1.0
O_2	18%	8.9 ± 1.1
Anaerobic	0%	<0.01
CO–O_2(80:20)	0%	3.5 ± 0.1
50 μM SKF 525A	17%	6.8 ± 1.7
330 μM SKF 525A	0%	4.1 ± 0.4
50 μM metyrapone	0%	2.4 ± 0.4

* Incubation mixture contained microsomal suspension (2 mg protein/ml 0.02 M Tris–HCl buffer, ph 7.4), 0.2 mM EDTA, 6 mM fluroxene and NADPH generating system at 30°.

† S.D. < ±0.05 nmol cytochromes P-450/mg microsomal protein. Initial levels (approximately 2.5 nmol/mg microsomal protein) were determined at time zero on samples identical to those incubated for 10 min.

‡ Abbreviations used are TFE, 2,2,2-trifluoroethanol; cyts P-450, type P-450 cytochromes.

2,2,2-Trifluoroethanol was quantitated by gas liquid chromatography using a 6 mm × 2 m copper column containing 10% di-*iso*-decylphthalate on acid washed Chromosorb P (Applied Scientific Labs., Inc.) in a Beckman GC-M gas chromatograph as described by Gion *et al* [9]. The identification of trifluoroethanol was achieved by mass spectrometry.

Microsomal lipid peroxidation was monitored by measuring malonaldehyde production according to the method of Ernster and Nordenbrand [10]. All incubation mixtures for this assay contained EDTA.

Binding studies. Studies of the binding of fluroxene were performed as described earlier [1]. The inhibitor SKF 525A when present was at identical concentrations in the sample and reference cuvettes.

All other materials and experimental procedures are as described earlier [1, 3].

Calculations. K_m and V_{max} values were calculated from computerized Hanes plots of [fluroxene]/v versus [fluroxene]. Initial rates (v) for the destruction of cytochromes P-450 and the production of 2,2,2-trifluoroethanol were monitored over the time periods indicated, during which the respective reactions were linear to a good approximation.

RESULTS

The effects of inhibitors on the metabolism of fluroxene and on the fluroxene mediated destruction of cytochromes P-450 in vitro. The effects of inhibitors on the fluroxene mediated destruction of cytochromes P-450 and on the metabolism of fluroxene in hepatic microsomes from phenobarbital induced rats are presented in Table 1. The destruction of cytochromes P-450 and the conversion of fluroxene to trifluoroethanol seen in the presence of fluroxene, NADPH and hepatic microsomes is completely abolished when the reaction is performed under anaerobic conditions. Carbon monoxide, metyrapone and SKF 525A (330 μM) fully inhibit the destruction of cytochromes P-450 and decrease the metabolism of fluroxene by approximately 65 per cent. At low concentrations (50 μM), SKF 525A does not measurably inhibit the degradation of cytochromes P-450 but does partially (*ca.* 30 per cent) inhibit the production of trifluoroethanol in phenobarbital induced microsomes (Table 1).

Table 2. Effect of SKF 525A on the binding of fluroxene to cytochromes P-450 in phenobarbital induced hepatic microsomes *in vitro*

SKF 525A (μM)	K_s (mM)	ΔA_{max} (absorbance)
0	1.4 ± 1.0	0.090 ± 0.008
50	1.5 ± 0.2	0.096 ± 0.002
100	1.2 ± 0.5	0.064 ± 0.003
330	0.7 ± 0.4	0.042 ± 0.007

As reported earlier, SKF 525A at this concentration (50 μM) does not inhibit the binding of fluroxene to hepatic microsomal cytochrome P-450 [1], but does so at higher concentrations (Table 2). The inhibition of the binding of fluroxene to cytochrome P-450 in phenobarbital microsomes by SKF 525A ($K_i \cong 270$ μM) is of a noncompetitive or mixed type. Since fluroxene binds preferentially to cytochrome P-450 [1] and SKF 525A at low concentrations has been reported not to interact with this cytochrome [11], it would appear that at the higher concentrations of SKF 525A utilized herein, this inhibitor binds to more than one type P-450 cytochrome.

Table 3. The abilities of oxidants and reductants to support the fluroxene mediated destruction of cytochromes P-450 and the metabolism of fluroxene in phenobarbital induced hepatic microsomes *in vitro*

Additions*	Fluroxene (30 mM)	Cytochromes P-450¶ (nmol/mg microsomal protein)		TFE formed† (nmol/mg microsomal protein)	
		0 min	10 min	0 min	10 min
None	+	2.9	2.9	nil	nil
NADPH generating system	+	2.9	2.4	nil	102 ± 7
NADH (0.6 mM)	+	2.7	2.7	nil	15 ± 5
$Na_2S_2O_4$ (10 mM)	−‖	1.8	1.3	—	—
	+‖	1.8	1.3	nil	nil
Ascorbate (10 mM)	−	2.1	2.1	—	—
	+	2.0	2.0	nil	nil
HIO_4 (7.5 mM)	−	2.6	2.1	—	—
	+	2.5	2.0	2.8	17 ± 2
	+§	—	—	9	34
H_2O_2 (10 mM)	−	2.4	1.8	—	—
	+	2.4	2.4	2.9	7
	+§	—	—	128	92

* Incubation mixture contained microsomes (2 mg protein/ml) and 0.2 mM EDTA in 0.02 M Tris–HCl, pH 7.4, 30°. Abbreviation used is TFE, 2,2,2-trifluoroethanol.

¶ S.D. ± 0.1 nmol/mg microsomal protein.

† Limit of measurement is less than 0.1 nmol TFE/mg microsomal protein.

‖ Microsomes appeared to precipitate during incubation.

§ Microsomes omitted from incubation mixture.

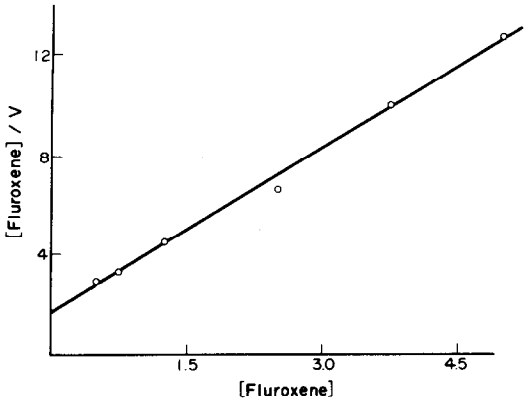

Fig. 1. Hanes plot of the fluroxene mediated destruction of cytochromes P-450 as a function of the concentration of fluroxene in microsomes from phenobarbital induced rats. Fluroxene concentration, mM; rate, nmol cytochromes P-450/mg microsomal protein/7 min. Microsomes (2 mg protein/ml 0.02 M Tris–HCl buffer, pH 7.4), 0.2 mM EDTA, NADPH generating system and fluroxene incubated at 30°.

The abilities of oxidants and reductants to support the fluroxene mediated destruction of cytochromes P-450 *and the metabolism of fluroxene by hepatic microsomes* in vitro. The reductants sodium dithionite and ascorbate support neither the fluroxene mediated destruction of cytochromes P-450 nor the metabolism of fluroxene *in vitro* (Table 3). NADH supports the production of trifluoroethanol to 15% of the extent seen with NADPH, but does not measurably support the fluroxene mediated destruction of cytochromes P-450.

Periodic acid and hydrogen peroxide, compounds that have been reported to support the hydroxylation of steroids by partially purified hepatic microsomal cytochromes P-450 [12], do not apparently support the cytochrome P-450 dependent metabolism of fluroxene or the fluroxene mediated destruction of cytochromes P-450 *in vitro*. It appears that these reagents are utilized in side reactions with hepatic microsomal protein and in the non-specific degradation of fluroxene.

K_m *and* V_{max} *values for the conversion of fluroxene to 2,2,2-trifluoroethanol and for the fluroxene mediated destruction of cytochromes* P-450 in vitro. Hanes plots of the destruction of cytochromes P-450 and the production of trifluoroethanol as a function of the concentration of fluroxene are linear for all types of induction (see e.g. Fig. 1), permitting calculation of K_m and V_{max} parameters. The effects of inducers of cytochrome P-450 and cytochrome P-448 on the K_m and V_{max} parameters for both of these reactions are presented in Table 4. The K_m values for the fluroxene mediated destruction of cytochromes P-450 vary in differently induced microsomes. The V_{max} values for this reaction are increased relative to controls by 3-methylcholanthrene and phenobarbital induction. The K_m values for the metabolism of fluroxene to 2,2,2-trifluoroethanol are unchanged by induction, and V_{max} is elevated relative to controls only in phenobarbital induced microsomes. Similar results were obtained by monitoring the metabolism of fluroxene by NADPH consumption [1].

In microsomes from 3-methylcholanthrene—allyl-*iso*-propylacetamide pretreated rats the levels of cytochromes P-450 in isolated hepatic microsomes were decreased by 0.6 nmol of cytochromes P-450 relative to microsomes from 3-methylcholanthrene pretreated animals (Table 4). In comparison to 3-methylcholanthrene induced microsomes, the K_m value for the metabolism of fluroxene was unchanged and V_{max} for this reaction was decreased by approximately 50 per cent. The extent of destruction of type P-450 cytochromes by fluroxene was 0.44/1.25 and 0.51/2.09 nmol/mg microsomal protein/10 min in microsomes from 3-methylcholanthrene—allyl-*iso*-propylacetamide pretreated and 3-methylcholanthrene induced animals, respectively.

The effects of induction on the production of 2,2,2-trifluoroethanol in comparison to the fluroxene mediated destruction of cytochromes P-450. The effects of inducing agents for cytochromes P-450 and P-448 on the production of trifluoroethanol and on the fluroxene mediated destruction of cytochromes P-450 are presented in Fig. 2. With microsomes from rats induced with 3-methylcholanthrene and phenobarbital, respectively, the ratios of the amount of trifluoroeth-

Table 4. The effects of induction of hepatic microsomal type P-450 cytochromes on the fluroxene mediated destruction of cytochromes P-450 and on the metabolism of fluroxene *in vitro*

		Destruction of cyts P-450		Metabolism of fluroxene (Production of TFE)	
Induction*	Cyts P-450 (nmol/mg microsomal protein)	K_m (mM)	V_{max} (nmol cyts P-450/mg microsomal protein/7 min)	K_m§ (mM)	V_{max}¶ (nmol TFE/min/mg microsomal protein
None	1.2	1.5 ± 0.1	0.19 ± 0.02†	1.4	3.7
PB	2.7	0.8 ± 0.4	0.45 ± 0.21	0.7	14.2
MC	2.1	3.3 ± 1.4	0.48 ± 0.16	1.3	4.8
MC/AIA	1.4	—	—	1.2	2.1

* Abbreviations used are cyts P-450, cytochromes P-450; MC, 3-methylcholanthrene; PB, phenobarbital; TFE, 2,2,2-trifluoroethanol; AIA, allyl-*iso*-propylacetamide.

§ S.D. ± 0.3 mM.

¶ S.D. ± 0.7 nmol TFE/min/mg microsomal protein.

† nmol cyts P-450/mg microsomal protein/10 min.

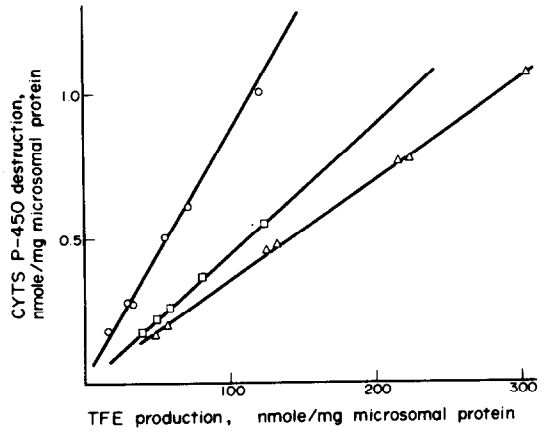

Fig. 2. The destruction of cytochromes P-450 versus the production of 2,2,2-trifluoroethanol. Microsomes from uninduced (□), phenobarbital (△) and 3-methylcholanthrene (○) induced rats (2 mg protein/ml 0.02 M Tris–HCl buffer, pH 7.4), 0.2 mM EDTA, NADPH generating system and 30 mM fluroxene incubated at 30°. Points in the graph were generated as a function of time (ca. 5, 7, 10, 15, 20, 30, 60 min). Boosters of NADPH generating system minus $MgCl_2$ and nicotinamide were added every eighth minute. Both assays were performed on identical reaction mixtures.

anol produced to the amount of cytochromes P-450 degraded was 107 and 296 respectively and the ratio observed with control microsomes was 226.

Lipid peroxidation. Malonaldehyde production is not appreciably enhanced after 30 min incubation in the presence of fluroxene and/or NADPH generating system relative to appropriate controls, indicating that lipid peroxidation is not involved in the fluroxene mediated destruction of cytochromes P-450 *in vitro*.

The effect of fluroxene on the levels of exogenous heme. The presence of fluroxene in a reaction mixture containing phenobarbital induced microsomes, NADPH generating system, EDTA and added hematin (*ca.* 25 μM) did not appreciably affect the total heme content of the mixture after 30 min incubation at 30°. The total heme content decreased from 26.8 ± 0.1 μM to 20.0 ± 0.1 μM and from 23.2 ± 0.7 μM to 17.5 ± 1.0 μM after 30 min incubation in the absence and presence of fluroxene, respectively. The data corresponds to a heme content of 75% after 30 min incubation in the absence or the presence of fluroxene. These figures have been corrected for the destruction of the heme moiety of cytochromes P-450 (*ca.* 1 μM) seen in the presence of fluroxene.

DISCUSSION

We have attempted to clarify the role of cytochromes P-450 in the fluroxene mediated destruction of cytochromes P-450 *in vitro*. In order to ascertain if the metabolism of fluroxene is required for the destruction reaction, we have compared the effects of various experimental conditions on the fluroxene mediated destruction of cytochromes P-450 and on the cytochrome P-450 dependent conversion of fluroxene to 2,2,2-trifluoroethanol. The results reported herein support an essential role for cytochrome P-450 in the metabolism of fluroxene to 2,2,2-trifluoroethanol and suggest that the fluroxene mediated destruction of cytochromes P-450 also requires the metabolism of fluroxene by the cytochrome P-450 dependent pathway.

The requirement of the destruction reaction for NADPH, fluroxene, oxygen and hepatic microsomes and the enhancement of the destruction reaction following induction of elevated levels of type P-450 cytochromes (data reported herein and [3]) are consistent with an essential role for cytochromes P-450 in the fluroxene mediated destruction of cytochromes P-450. The inhibition of the fluroxene mediated destruction of cytochromes P-450 by recognized inhibitors of cytochrome P-450 dependent reactions such as carbon monoxide, SKF 525A and metyrapone (Table 1) provides further support for the involvement of the cytochrome P-450 pathway in the fluroxene mediated destruction of cytochromes P-450 *in vitro*.

Although the fluroxene mediated destruction of cytochromes P-450 and the conversion of fluroxene to trifluoroethanol both require the binding and metabolism of fluroxene by the cytochrome P-450 dependent drug metabolizing system, there appears to be no fixed ratio between the extent of the cytochrome P-450 dependent conversion of fluroxene to trifluoroethanol and the extent of the fluroxene mediated destruction of cytochromes P-450 in microsomes induced for different type P-450 cytochromes (Fig. 2).

One possible explanation of this phenomenon is that different type P-450 cytochromes are associated with the destruction and metabolism reactions. Considerable evidence has accumulated suggesting that this is the case. The metabolism of fluroxene appears to be catalyzed predominantly by cytochrome P-450, and not greatly by cytochrome P-448 [1]. In contrast, the fluroxene mediated destruction of cytochromes P-450 appears to involve both cytochrome P-450 and cytochrome P-448. Lack of variation in K_m, and enhancement of V_{max} only in phenobarbital induced microsomes in studies of the metabolism of fluroxene by trifluoroethanol production (Table 4) or NADPH consumption [1] indicate that cytochrome P-450, but not cytochrome P-448, catalyzes the metabolism of fluroxene. This is further supported by the observation that allyl-*iso*-propylacetamide, a compound reported to decrease the levels of cytochrome P-450 but not cytochrome P-448 [13], markedly decreases the metabolism of fluroxene to trifluoroethanol (Table 4).

The observed variation in the K_m values for the destruction reaction with induction of different type P-450 cytochromes (Table 4) indicates that more than one type P-450 cytochrome may be involved in the destruction reaction. The involvement of cytochrome P-448 in the fluroxene mediated destruction of cytochromes P-450 is suggested by the lack of effect of allyl-*iso*-propylacetamide on the destruction reaction in microsomes from 3-methylcholanthrene induced rats (Table 4), whereas an essential role for cytochrome P-450 is indicated by the inhibition of the destruction reaction in phenobarbital induced microsomes by metyrapone, a compound proposed to be a specific inhibitor of cytochrome P-450 [11]. The elevation of V_{max} values for the fluroxene mediated destruction of cytochromes P-450 by either 3-methyl-

cholanthrene or phenobarbital induction (Table 4) further suggests that cytochrome P-448 and cytochrome P-450 may be involved in the destruction reaction in microsomes induced for elevated levels of these enzymes. It is envisaged that different type P-450 cytochromes may be involved in distinct aspects of the fluroxene mediated degradation of cytochromes P-450. One type of cytochromes P-450 may catalyze the conversion of fluroxene to a reactive species, whereas the same or another type of cytochromes P-450 may be degraded by metabolically activated fluroxene.

The destruction of cytochromes P-450 by a medically used compound such as fluroxene provides the first example of a novel mechanism for effecting drug-drug interactions. It has been proposed that a single drug can directly affect the metabolism of another drug by the three mechanisms of induction, inhibition and competition [14]. In view of the results presented herein and earlier documenting the ability of fluroxene to destroy hepatic cytochromes P-450 *in vivo* and *in vitro* [2, 3], it would appear that a fourth possible mechanism, involving the destruction of the drug metabolizing enzymes should be added to this list. We are continuing to investigate the mechanism of destruction of cytochromes P-450 by fluroxene and other compounds, in the hope that these studies will aid in elucidating the complexities of drug-drug interactions in animals and man *in vivo*.

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