

Inhibition and Stimulation of Enflurane Metabolism in the Rat Following a Single Dose or Chronic Administration of Ethanol

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The effects of a single oral dose or chronic ingestion of ethanol on *in vivo* and *in vitro* metabolism of enflurane were studied in Fischer 344 rats. At various intervals after ethanol treatment, enflurane was administered ip and 1 h after enflurane administration fluoride and ethanol levels were measured in plasma and hepatic microsomes were prepared. The concentration of ethanol in plasma (\pm SE) was $0.138 \pm 0.035\%$ at 9 h after the single dose of ethanol and decreased almost to control levels by 17 h. The hepatic microsomal defluorination of enflurane was enhanced 3.5-fold and 6.3-fold at 9 and 25 h after the ethanol dose and returned to the control level by 33 h. *In vivo* defluorination was inhibited almost completely at 8 h after the ethanol dose, increased to 3.4 times the control level at 24 h, and decreased to the control level by 32 h. At 1 h after the end of chronic ethanol treatment, the concentration of ethanol in plasma was $0.254 \pm 0.018\%$, and it decreased to the control level by 9 h. Hepatic microsomal enflurane defluorinating activity was increased 10.5-fold at 1 h after the end of chronic treatment and decreased to the control level by 13 h. Immediately following chronic treatment, enflurane defluorination *in vivo* was almost totally inhibited. It increased to 9.3 times the control level at 4 h after chronic treatment was stopped and then decreased to nearly the control level at 12 h. Changes in hepatic microsomal enflurane defluorinating activity following ethanol administration were paralleled by changes in the amount of a microsomal protein that electrophoresed in the molecular weight region of the cytochrome P-450 isozymes. The results show that ethanol can produce a very rapid increase in hepatic microsomal enflurane defluorinating activity in rats and provide evidence that this increase results from the induction of a form of cytochrome P-450. Defluorination of enflurane *in vivo* can be inhibited or stimulated following treatment with ethanol, depending on the level of ethanol present when the enflurane is administered. (Key words: Anesthetic, volatile: enflurane. Biotransformation (drug): fluorometabolites. Enzymes: cytochrome P-450; induction; inhibition. Ions: fluoride. Liver: microsomes.

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ENFLURANE is metabolized oxidatively by cytochrome P-450, resulting secondarily in the release of inorganic fluoride, but the extent of metabolism is usually so small that the amount of fluoride that is released as the result of an ordinary clinical exposure poses no risk of nephrotoxicity.¹⁻⁴ Nevertheless, occasional individuals have plasma levels of inorganic fluoride following enflurane anesthesia that are very much higher than usual.^{2,5,6} Although conventional phenobarbital-type and polycyclic hydrocarbon-type inducers of cytochrome P-450 produce little or no enhancement of enflurane metabolism,^{3,4,7-11} chronic treatment of rats with isoniazid has been found to markedly increase the defluorination of enflurane by hepatic microsomes *in vitro*.^{12,13} Data suggest that isoniazid induces an isozyme of cytochrome P-450 that is different from those induced by phenobarbital or 3-methylcholanthrene.¹² It also has been shown that substantially greater than usual plasma levels of inorganic fluoride do occur in some patients who have been treated chronically with isoniazid.¹⁴ Because of earlier work¹⁵ that indicated that treatment of rats with ethanol induced the metabolism of a similar profile of substrates and caused a similar shift in microsomal spectral absorption maximum, as did treatment of rats with isoniazid,^{12,13} we initiated studies on the effects of treatment of rats with ethanol on enflurane defluorination. Since the *in vivo* metabolism of many drugs is inhibited during acute ethanol intoxication¹⁶ and since substances that inhibit drug metabolism *in vivo* can be washed out during the preparation of microsomes and their inhibitory effect lost, we examined the effects of treatment with ethanol on enflurane defluorination *in vivo* as well as *in vitro*. During the course of our studies it was reported by Van Dyke and by Rice *et al.* that chronic treatment of rats with ethanol results in substantial increases in the defluorination of enflurane by hepatic microsomes *in vitro*.^{10,11} The results of our studies show that increases caused by ethanol in enflurane defluorinating activity in hepatic microsomes occur rapidly, that changes in microsomal enflurane defluorinating activity are paralleled by changes in the concentration of a microsomal protein that electrophoreses in the molecular weight region of the cytochrome P-450 isozymes, and that, *in vivo*, when ethanol plasma levels are high, despite increased levels

of enflurane defluorinating enzyme activity in hepatic microsomes, there is marked inhibition of enflurane defluorination.

Materials and Methods

Male Fischer 344 rats (Charles River Laboratories, Cambridge, Massachusetts), 5–6 weeks old, were housed individually in plastic cages containing corncob bedding. They were given either Lieber-DeCarli formula control liquid diet or ethanol-containing liquid diet (Bio-Serv, Inc., Frenchtown, New Jersey) as the sole source of both food and water. The Lieber-DeCarli formula diets are nutritionally adequate diets.^{17,18} The ethanol-containing diet was identical to the control diet, except that ethanol isocalorically replaced part of the carbohydrate. The ethanol gradually was introduced into the diet over a 1-week period until it reached its final concentration of 36% of total calories (5 g ethanol/100 ml diet). For studies on the effects of chronic ethanol treatment, treated rats were given the ethanol-containing diet *ad libitum*. Control rats were given control diet in an amount equal to the average amount of the ethanol-containing diet that had been consumed by the treated rats during the prior 24 h. For studies of the effects of single dose ethanol treatment and of the effects of isoniazid treatment, both treated and control rats were fed control liquid diet. They were given the same amount of diet that had been given on the corresponding day of feeding to the control rats from the study of chronic ethanol treatment. When used for enflurane metabolism determinations, all rats had been fed either control or ethanol-containing liquid diet for 23–25 days.

For the study of the effects of chronic ethanol treatment, the ethanol-containing diet was removed from the cages of treated rats at various intervals of time from 0 to 30 h before the rats were administered enflurane and was replaced with control diet. The rats were fed the control diet at the mean rate ($40 \text{ ml} \cdot \text{rat}^{-1} \cdot 24 \text{ h}^{-1}$) that the group had consumed the ethanol-containing diet on the 23rd day this was fed. Rats were administered enflurane (Ohio Medical Products, Madison, Wisconsin) ($0.6 \mu\text{l/g}$, ip). This dose of enflurane rendered the rats slightly sedated and occasionally slightly ataxic and it produced in control animals plasma levels of fluoride comparable to those that would be seen following an ordinary clinical exposure to this drug.^{2,4,6,14} The administration of an inhalation anesthetic by intraperitoneal injection permits precise quantification of the dose and was found by Fry *et al.* in studies of methoxyflurane metabolism in mice to lead to more consistent data than administration by inhalation.¹⁹ Following enflurane administration the rats were permitted no further food.

For the study of the effects of treatment with a single dose of ethanol, rats were administered by gastric intubation ethanol (7 g/kg) as a 50% solution in water or an equivalent volume of water. The dose of ethanol used rendered the rats moderately sedated for a few hours and often slightly ataxic for a brief period of time. At various intervals of time, from 8 to 48 h after they received ethanol, the treated rats and, at 16 h after they received water, the control rats were administered enflurane ($0.6 \mu\text{l/g}$, ip). Following enflurane administration, the rats were permitted no further food.

For the study of the effects of treatment with isoniazid, isoniazid (0.06 g/100 ml) was added to the rats' diet after they had been fed the control liquid diet for 11 days. After 12 days of isoniazid treatment, the isoniazid was removed from the diet. Eighteen hours later, the rats were administered enflurane ($0.6 \mu\text{l/g}$, ip). Following enflurane administration, the rats were permitted no further food.

One hour after they were dosed with enflurane, the rats were anesthetized with diethyl ether. After laparotomy of the rat, blood was obtained by vena caval puncture; and it was heparinized and centrifuged, and the plasma was used immediately for determinations of ethanol and fluoride content. As soon as the withdrawal of blood was completed, livers were excised and microsomes immediately were prepared from them as described previously.²⁰ The microsomes were stored at -90°C until used for measurements of protein content, cytochrome P-450 content, enflurane defluorinating activity, and for sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Preliminary experiments established that the enzymes measured in microsomes were stable under the conditions of freezing and storage.

Ethanol concentration in plasma was determined by use of the Ethyl Alcohol Diagnostic Kit (Sigma Chemical Co., St. Louis, Missouri).

Fluoride concentration in plasma was determined using an ion specific electrode (Orion Research Inc., Cambridge, Massachusetts) as described by Fry and Taves.²¹ Standard curves were prepared by adding known amounts of fluoride to plasma from rats fed the control diet for 23 days. Preliminary experiments established that these were identical to standard curves prepared in plasma from rats fed the ethanol-containing diet for 23 days.

The protein concentration in rat liver microsomes was determined by the method of Lowry *et al.*, using bovine serum albumin as a standard.²²

Cytochrome P-450 content in rat liver microsomes was determined spectrally from the carbon monoxide difference spectrum of the reduced hemoprotein by the method of Omura and Sato.²³

The rate of enflurane defluorination by rat liver

TABLE 1. Effect of Chronic Ethanol Ingestion on Hepatic Microsomal Cytochrome P-450 Content and Enflurane Defluorination

Treatment	Group			Ethanol Plasma Concentration at Time of Hepatectomy‡ (per cent)	Hepatic Microsomal Cytochrome P-450 Content (nmol · mg protein ⁻¹)	Enflurane Defluorination			
	Interval after Removal of Ethanol from Diet		N*			In Vitro		In Vivo	
	Hours to Enflurane Administration	Hours to Hepatectomy				nmol · mg protein ⁻¹ · min ⁻¹	Ratio to Control	nmol · ml plasma ⁻¹	Ratio to Control
Control	NA†	NA†	6	0.003 ± 0.001	0.57 ± 0.08	0.064 ± 0.007	—	9.64 ± 1.79	—
Ethanol	0	1	6	0.254 ± 0.018	0.94 ± 0.16§	0.674 ± 0.084¶	10.53	1.38 ± 0.36	0.14
	1	2	6	0.131 ± 0.025	0.91 ± 0.11§	0.456 ± 0.066¶	7.13	1.24 ± 0.30	0.13
	2	3	6	0.064 ± 0.014	0.94 ± 0.05§	0.480 ± 0.048¶	7.50	44.02 ± 30.63	4.57
	4	5	6	0.053 ± 0.018	0.66 ± 0.03	0.184 ± 0.021	2.88	89.48 ± 9.94§	9.29
	8	9	6	0.004 ± 0.001	0.63 ± 0.08	0.124 ± 0.011	1.94	69.74 ± 8.21¶	7.23
	12	13	6	—	0.50 ± 0.03	0.057 ± 0.008	0.89	21.77 ± 3.63	2.26
	18	19	6	—	0.54 ± 0.06	0.022 ± 0.004	0.34	16.76 ± 1.86	1.74
	24	25	6	0.004 ± 0.001	0.57 ± 0.06	0.031 ± 0.012	0.48	5.47 ± 1.21	0.57
	30	31	6	—	0.50 ± 0.06	0.056 ± 0.007	0.88	14.28 ± 2.07	1.48

Values are mean ± SE
* Number of rats.
† Not applicable.

‡ Determined in separate groups of four to six rats.
§ Significantly different from control: §P < 0.05; ¶P < 0.01.

microsomes was determined by incubation of 10 µl of enflurane with 5 mg of microsomal protein as described by Rice *et al.*^{11,13} Inorganic fluoride released during the 30-min incubation was measured with an ion-specific electrode as described above. Incubations containing heat-inactivated microsomes were used as blanks. Standard curves were prepared by adding known amounts of fluoride to blanks. Preliminary experiments established that the administration of enflurane (0.6 µl/g, ip) to rats 1 h prior to preparation of microsomes did not influence either the levels of fluoride in blanks or the rates of enflurane defluorination.

Sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis of microsomal protein was carried out according to the procedure of Laemmli.²⁴ Isozymes of hepatic cytochrome P-450 from control rats and from rats treated with isosafrole or with Aroclor® 1254, which had been previously isolated, purified, and characterized, were used as standards.^{25,26}

When multiple comparisons were made to a single group, results were analyzed by using analysis of variance followed by Dunnett's Method with *t* statistics appropriately weighted for the number of comparisons that were made. All other data were analyzed using the unpaired Student's *t* test. Differences in mean values were considered significant if *P* values were less than 0.05. The significance of changes that occurred in *in vitro* enflurane defluorinating activity, in *in vivo* enflurane defluorination following the return of plasma ethanol concentrations to control level, and in *in vivo* enflurane defluorination following isoniazid treatment were evaluated by a one-tailed test, since the direction of these changes has been postulated. The significance of all other changes was evaluated by a two-tailed test.

Results

CHRONIC TREATMENT WITH ETHANOL

There was no difference in the gain of body weight between the control rats and the ethanol-treated rats. At the end of the treatment period, the control rats weighed 126.7 ± 2.1 g (mean ± SE) and the ethanol-treated rats weighed 123.4 ± 1.6 g. There was a significantly greater increase in liver weight in the ethanol-treated rats. The ratio of liver weight to body weight was 0.036 ± 0.001 for the control rats and 0.044 ± 0.001 for the ethanol-treated rats (*P* < 0.001). During the last week of treatment, ethanol consumption was 14–16 g · kg⁻¹ · day⁻¹.

The plasma level of ethanol, measured in separate groups of rats that had been treated identically to those used for the drug metabolism studies, was 0.254 ± 0.018% at 1 h after discontinuation of ethanol and had decreased to 0.053 ± 0.018% at 5 h after the discontinuation of ethanol (table 1). Ethanol levels no longer were increased at 9 h after its discontinuation.

Chronic treatment of rats with ethanol increased the liver microsomal cytochrome P-450 content, but the concentration of this hemoprotein decreased rapidly after discontinuation of the ethanol (table 1). At 1, 2, and 3 h after removal of ethanol from the rats' diet, the microsomal content of cytochrome P-450 was about 60% greater than in control rats. The concentration of cytochrome P-450 in liver microsomes from ethanol-treated rats was nearly back to the control level at 5 h. The CO-reduced difference spectral absorption maximum was shifted from 450 nm to 450.5 nm in microsomes from some but not the majority of ethanol-treated rats.

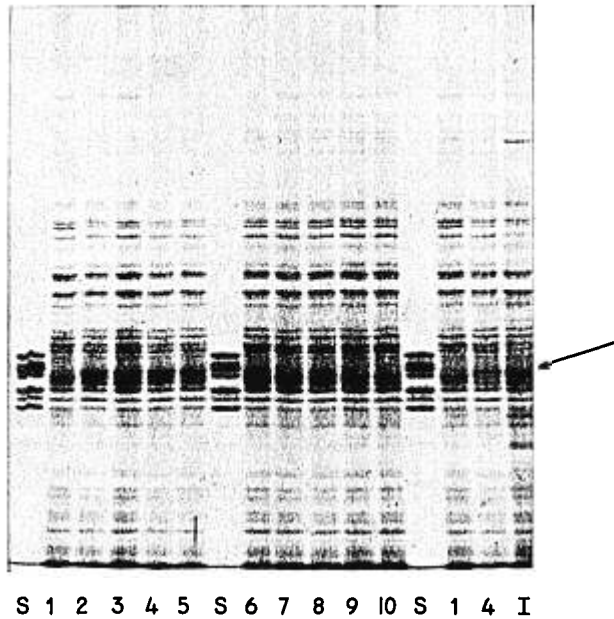


FIG. 1. Sodium dodecyl sulfate-polyacrylamide slab gel profile of hepatic microsomes from rats treated chronically with ethanol or isoniazid. A mixture of purified isozymes of cytochrome P-450 and epoxide hydrolase (0.2 μ g each) was placed in wells marked S. These standards with their minimum molecular weights are from top to bottom: P-450c (56,000); P-450e (52,500); P-450b (52,000); P-450f (51,000); P-450g (50,000); EH (49,000); P-450a (48,000). Other wells contained microsomal protein (10 μ g) from typical rats fed as follows: control diet—well 1; or ethanol containing diet terminated at 1 h—well 2, 2 h—well 3, 3 h—well 4, 5 h—well 5, 9 h—well 6, 13 h—well 7, 19 h—well 8, 25 h—well 9, 31 h—well 10 before death; or isoniazid in control diet—well I. Arrow indicates location of protein band induced by ethanol or isoniazid.

Following removal of ethanol from the rats' diet, enflurane-defluorinating activity *in vivo* initially was inhibited very markedly (table 1). Rats administered enflurane at 2 h after discontinuation of ethanol had a mean level of enflurane defluorinating activity *in vivo*

that was 4.6-fold greater than control animals. There was, however, marked variability in *in vivo* enflurane defluorinating capacity in the rats at this time. Plasma fluoride levels ranged from 0.63 to 194.74 nmol/ml, 1/16 to 20 times the control level, at 1 h after the administration of enflurane. The greatest enhancement of mean *in vivo* defluorinating capacity, a ninefold increase, occurred 4 h after the discontinuation of ethanol. By 12 h after the discontinuation of ethanol, the *in vivo* defluorination of enflurane had decreased to nearly the control level.

Enflurane-defluorinating activity in liver microsomes was increased 10.5-fold in rats killed at 1 h after ethanol was removed from their diet (table 1). *In vitro* defluorination of enflurane was about sevenfold higher than control values at 2 and 3 h after the discontinuation of ethanol. By 13 h after the discontinuation of ethanol, the microsomal defluorination of enflurane had decreased to the control level.

The chronic administration of ethanol resulted in the appearance of a protein band with an electrophoretic mobility on sodium dodecyl sulfate-polyacrylamide gels between that of cytochrome P-450 isozyme b and that of cytochrome P-450 isozyme f (fig. 1). The ethanol-induced protein had a calculated minimum molecular weight of approximately 51,500 daltons.^{25,26} The protein band was most intense when microsomes were used from rats killed at 1 or 2 h after removal of ethanol from their diet. As the time following removal of ethanol from the diet increased, the intensity of the band progressively decreased. By 13 h after discontinuation of ethanol, the band was not detectable.

TREATMENT WITH SINGLE DOSE OF ETHANOL

The plasma level of ethanol at 9 h after a single 7 g/kg oral dose was $0.138 \pm 0.035\%$ and by 17 h had fallen to $0.009 \pm 0.002\%$ (table 2). The ethanol that

TABLE 2. Effect of a Single Oral Dose of Ethanol on Hepatic Microsomal P-450 Content and Enflurane Defluorination

Treatment	Group			Ethanol Plasma Concentration at Time of Hepatectomy (per cent)	Hepatic Microsomal Cytochrome P-450 Content (nmol·mg protein ⁻¹)	Enflurane Defluorination			
	Interval after Ethanol Dose		N*			<i>In Vitro</i>		<i>In Vivo</i>	
	Hours to Enflurane Administration	Hours to Hepatectomy				nmol·mg protein ⁻¹ ·min ⁻¹	Ratio to Control	nmol·ml plasma ⁻¹	Ratio to Control
Control	NA†	NA†	4	0.02 ± 0.000	0.57 ± 0.01	0.030 ± 0.002	—	13.29 ± 1.57	—
Ethanol	8	9	5	0.138 ± 0.035	0.53 ± 0.03	$0.106 \pm 0.016\ddagger$	3.53	<0.50	<0.04
	16	17	5	0.009 ± 0.002	0.50 ± 0.03	$0.103 \pm 0.007\ddagger$	3.43	$44.35 \pm 18.14\§$	3.34
	24	25	5	0.006 ± 0.002	0.46 ± 0.03	$0.187 \pm 0.062\ddagger$	6.30	$45.62 \pm 11.53\§$	3.43
	32	33	5	0.005 ± 0.001	0.47 ± 0.05	0.023 ± 0.006	0.77	10.11 ± 1.28	0.76
	40	41	5	0.005 ± 0.000	0.44 ± 0.05	0.023 ± 0.001	0.77	8.16 ± 1.69	0.61
	48	49	5	0.004 ± 0.001	0.41 ± 0.04	0.030 ± 0.003	1.00	8.48 ± 1.08	0.64

Values are mean \pm SE.
* Number of rats.

† Not applicable.
‡ Significantly different from control: $\ddagger P < 0.01$; $\§ P < 0.05$.

was administered produced no significant change in microsomal cytochrome P-450 content (table 2). The CO-reduced difference spectral absorption maximum was shifted from 450 nm to 450.5 nm in microsomes from some but not the majority of ethanol-treated rats.

The administration of a single dose of ethanol produced a biphasic effect on enflurane defluorination activity *in vivo* (table 2). At 8 h after the dose of ethanol, marked inhibition was observed and there was little or no *in vivo* enflurane defluorination. However, at 16 h after ethanol administration, *in vivo* enflurane defluorination had increased to 3.3 times the control level. At 24 h after ethanol administration, *in vivo* enflurane defluorination was still markedly increased, but at 32 h it had decreased to the control level.

The *in vitro* defluorination of enflurane by rat liver microsomes was increased about 3.5-fold in rats killed at 9 or 17 h after the dose of ethanol (table 2). *In vitro* defluorination of enflurane was about 6.3-fold higher than control values at 25 h after ethanol administration and at 33 h had decreased to the control level.

The administration of a single dose of ethanol resulted in the appearance of a protein band on sodium dodecyl sulfate-polyacrylamide gels with an electrophoretic mobility identical to that of the protein band produced by chronic treatment of rats with ethanol (fig. 2). The band was observed in microsomes from rats killed 9, 17, and 25 h after the dose of ethanol. At 33 h after the dose of ethanol, the band was no longer detectable.

TREATMENT WITH ISONIAZID

Treatment of rats with isoniazid for 12 days enhanced enflurane defluorination *in vivo*. Plasma fluoride levels increased threefold from 9.00 ± 1.11 nmol/ml at 1 h after the dose of enflurane in the control rats (N = 5) to 27.34 ± 2.88 nmol/ml at 1 h after the dose of enflurane in the isoniazid-treated rats (N = 5) ($P < 0.005$).

The administration of isoniazid resulted in the appearance of a protein band on sodium dodecyl sulfate-polyacrylamide gels with an electrophoretic mobility identical to that of the protein band produced by chronic treatment of rats with ethanol and to that of the protein band produced by treatment of rats with a single dose of ethanol (fig. 1).

Discussion

The results of studies described here confirm previous work that showed that the chronic administration of ethanol to rats enhances defluorination of enflurane by hepatic microsomes *in vitro*^{10,11} and that this increase in microsomal defluorinating activity decreases rapidly fol-

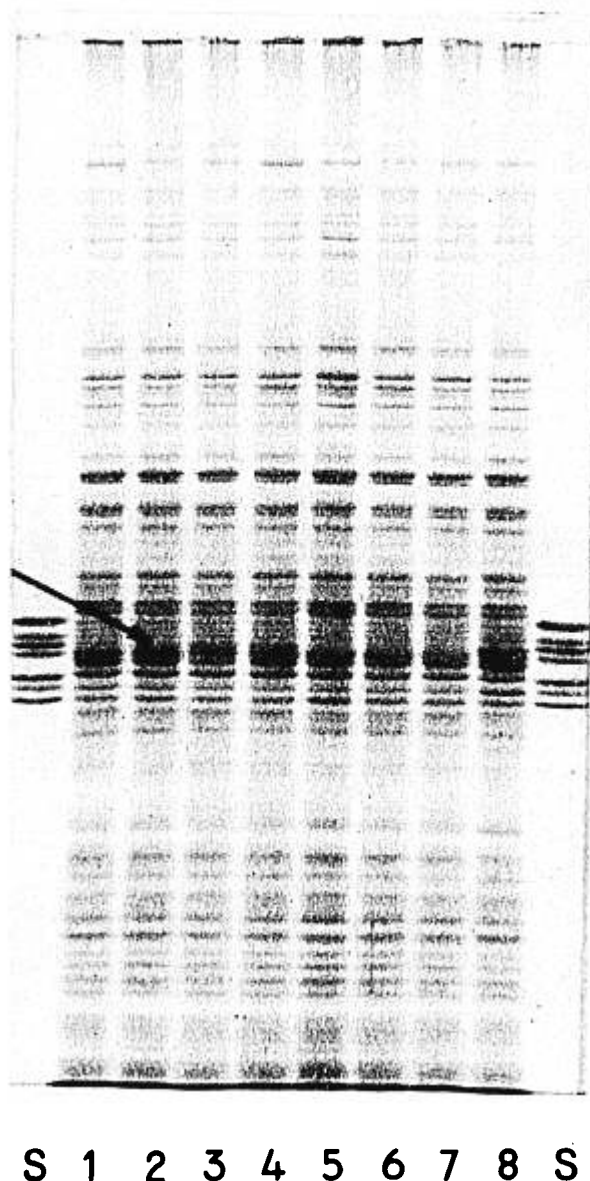


FIG. 2. Sodium dodecyl sulfate-polyacrylamide slab gel profile of hepatic microsomes from rats treated with a single dose of ethanol. A mixture of purified isozymes of cytochrome P-450 and epoxide hydrolase (0.2 μ g each) was placed in wells marked S. These standards with their minimum molecular weights are from top to bottom: P-450c (56,000); P-450e (52,500); P-450b (52,000); P-450f (51,000); P-450g (50,000); EH (49,000); P-450a (48,000). Wells 1-7 contained microsomal protein (10 μ g) from typical rats dosed with water—well 1; or ethanol at 9 h—well 2, 17 h—well 3, 25 h—well 4, 33 h—well 5, 41 h—well 6, 49 h—well 7 before death. Well 8 contained microsomal protein (10 μ g) from a typical rat chronically fed ethanol-containing diet until 3 h before death. Arrow indicates location of protein band induced by ethanol.

lowing the termination of ethanol treatment and is gone in less than 24 h.¹⁰ The results further show that, despite the markedly increased capacity of hepatic microsomes to defluorinate enflurane *in vitro* following the

termination of chronic ethanol treatment of rats, there is initially, when ethanol plasma levels are high, almost total inhibition of enflurane defluorination *in vivo*. This inhibition is followed, when ethanol levels have decreased, by an enhanced capacity to defluorinate enflurane *in vivo*. This enhanced capacity then rapidly disappears.

The inhibition of enflurane defluorination *in vivo* that is associated with high levels of ethanol appears to be caused by a direct inhibitory effect of ethanol on microsomal enflurane defluorination. We have found that the addition to microsomal reaction mixtures of ethanol, in concentrations comparable to those that we found in plasma immediately following the termination of the chronic treatment of rats with ethanol, produced very marked inhibition of enflurane defluorination (unpublished studies). That enflurane defluorination by microsomes from rats with high plasma levels of ethanol is not markedly inhibited is the consequence of the removal of ethanol from microsomes by the procedures used in their preparation.

If the enhancement of enflurane defluorinating activity following chronic ethanol treatment is due to induction of an isozyme of cytochrome P-450, and if this isozyme has as rapid a rate of degradation as would be required for the enhanced activity to be gone in less than 24 h, then the induction of this isozyme should be very rapid, since the time for a protein undergoing induction to reach one-half of its final steady state level theoretically is equal to the half-life of the protein.^{27,28} We have demonstrated that the administration to rats of a single dose of ethanol can produce a rapid marked increase in hepatic microsomal enflurane defluorinating activity *in vitro*. This increase in activity disappears at a rapid rate comparable to that seen following the discontinuation of chronic ethanol administration. It is accompanied when ethanol levels have diminished sufficiently by an increase in enflurane defluorinating capacity *in vivo*. Increases in microsomal metabolism, similar in both rapidity of onset and shortness of duration to that which we found for enflurane, have been reported to occur for aniline and several other substrates following the administration of a single dose of ethanol to rats.²⁹⁻³² Because we examined enflurane defluorinating activity only at 8-h intervals following the treatment of rats with a single dose of ethanol, our data do not establish the time at which the increase in microsomal enflurane defluorinating activity initially became detectable. For the same reason, our data do not establish the time or magnitude of peak microsomal enflurane defluorinating activity.

Prior studies, using sodium dodecyl sulfate-polyacrylamide gel electrophoresis, a technique that separates microsomal proteins on the basis of minimal molecular

weight, have shown that chronic treatment of rats with ethanol results in the formation of one or more microsomal proteins of appropriate molecular weight to be isozymes of cytochrome P-450.^{26,33,34} However, the very short duration of enhanced microsomal enflurane defluorinating activity following the termination of chronic ethanol treatment has caused Van Dyke to question whether the enhanced activity is due to the synthesis of a new isozyme of cytochrome P-450 or to some other mechanism.¹⁰ The results of our studies using sodium dodecyl sulfate-polyacrylamide gel electrophoresis strongly suggest that the increase in microsomal enflurane defluorinating activity that occurs following treatment of rats with ethanol results from the synthesis of a rapidly formed and rapidly degraded isozyme of cytochrome P-450. Our data show that two substances, ethanol and isoniazid, which cause an increase in microsomal enflurane defluorinating activity, also both cause the formation of a microsomal protein of the same minimum molecular weight, that this minimum molecular weight is appropriate for an isozyme of cytochrome P-450, and that in microsomes of ethanol-treated rats the concentration of protein of this minimum molecular weight varies in parallel with microsomal enflurane defluorinating activity. We believe that these data provide strong evidence that the increase in microsomal enflurane defluorinating activity in ethanol-treated rats is the result of induction of an isozyme of cytochrome P-450.

Our study shows an increase in total microsomal cytochrome P-450 content following chronic ethanol administration but not following the administration of a single dose of ethanol. Lack of a measurable increase in total microsomal cytochrome P-450 content does not preclude the enhanced metabolism of a substrate being the result of induction of an isozyme of cytochrome P-450, since this can occur if the enhanced metabolism results from the synthesis of a small amount, relative to the total quantity of cytochrome P-450 present, of an isozyme with a very high capacity to metabolize the substrate or if the increase in cytochrome P-450 resulting from the synthesis of the isozyme is offset by repressed synthesis or enhanced degradation of one or more other isozymes.

Our study shows a shift in the CO-reduced difference spectral absorption maximum to 450.5 nm in microsomes from some of the rats that had been treated chronically with ethanol and in microsomes from some of the rats that had been treated with a single dose of ethanol. Chronic treatment of rats with isoniazid has been reported to produce a shift in microsomal spectral absorption maximum to 451 nm.¹² Whether induction of an isozyme of cytochrome P-450 results in a shift in microsomal spectral absorption maximum depends on the spectral absorption peak of the induced isozyme, the

amount of the isozyme that is synthesized relative to the amount of other isozymes present, and whether the inducer stimulates or represses the synthesis or degradation of other isozymes. Therefore, it is possible for two substances to induce the same isozyme of cytochrome P-450 but, because they have different effects on other isozymes, to have different effects on the microsomal spectral absorption maximum.

Experimental design in studies of the effects of chronic ethanol administration on drug metabolism is confounded by ethanol having a caloric content. As a result, it is inevitable that there will be a difference between treated and control animals in caloric intake or in intake of one or more nutrients. In the present study we maintained isocaloric conditions by feeding the treated rats a diet identical to that fed to the control rats, except that ethanol isocalorically replaced part of the carbohydrate, and by restricting the amount of diet consumed by the control rats to the amount ingested by the treated rats. This experimental design has two consequences of possible concern. First, the control rats are fasted for part of each day, since they find their diet more palatable than do the ethanol-fed rats and finish their daily ration before the next feeding. Starvation for a day or more has been found to alter hepatic microsomal drug metabolism in the rat.³⁵⁻³⁷ However, we found in preliminary studies (data not shown), that despite a difference in weight gain, there was no significant difference in enflurane-defluorinating activity, *in vitro* or *in vivo*, among groups of rats fed 1) the control diet restricted to the daily rate at which rats consumed the ethanol-containing diet, 2) the control diet *ad libitum*, or 3) Purina rat chow *ad libitum*. A second concern is that rats fed the ethanol-containing diet receive less carbohydrate than do rats fed the control diet. It has been found that the inductive effect of ethanol on hepatic microsomal enzyme activity can be enhanced to some extent by a diet low in carbohydrate.³⁸⁻⁴⁰ It is possible that the magnitude of the increase in microsomal enflurane-defluorinating activity produced by chronic ethanol administration in our study has been influenced by the low carbohydrate content of the ethanol-containing diet. However, in our study on the effect of a single dose of ethanol on microsomal enflurane-defluorinating activity both treated and control animals were fed the control diet.

If ethanol-induced changes in enflurane defluorination comparable to those that we observed in rats occur in humans, then our data suggest that the interval of time between cessation of ethanol ingestion and administration of enflurane will determine whether the defluorination of enflurane is inhibited, stimulated, or unchanged. It is possible that an ordinary clinical exposure to enflurane following ethanol ingestion will result in some individuals in levels of fluoride in plasma in the nephrotoxic range.⁴¹

Plasma levels of fluoride following enflurane anesthesia decrease at such a rapid rate that renal dysfunction should it occur is likely to be transient.^{2,14,41}

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