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EFFECT OF ETHANOL CONCENTRATION ON RATES OF ETHANOL ELIMINATION IN NORMAL AND ALCOHOL-TREATED RATS *IN VIVO**

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Abstract—Ethanol was infused intravenously to yield in the blood concentrations between 30 and 40 mM (low dose) or 80 and 90 mM (high dose). Duplicate blood samples were taken every 30 min for gas chromatographic determination of ethanol. Elimination curves for both low and high does of ethanol were linear in normal rats until ethanol concentrations reached values of less than 5 mM. At the low and high doses, average rates of ethanol elimination were 179 ± 1 and 266 ± 13 μmoles/g/hr respectively. The stimulation of ethanol metabolism due to the high dose did not diminish as the concentration declined. At both doses in both normal and ethanol—pretreated rats, elimination rates were diminished over 80 per cent by prior treatment with 4-methylpyrazole. Pretreatment with aminotriazole produced a 20–25 per cent decrease in the rate at the high dose in normal rats and at both doses in ethanol-pretreated rats, but had no effect at the low dose in normal rats. From these data we conclude that a concentration effect of ethanol on rates of ethanol elimination, which has both an alcohol dehydrogenase—and a catalase–H_2O_2–dependent component, exists *in vivo*. Moreover, the adaptive increase in ethanol elimination due to chronic pretreatment with ethanol also involves both components. Pyruvate and ethanol pretreatment stimulated ethanol elimination at the low but not at the high dose of ethanol. It is further concluded that NADH reoxidation is rate-limiting for ethanol utilization at the low dose whereas the activity of alcohol dehydrogenase becomes limiting at the high dose and after pretreatment with ethanol in the fed state *in vivo*.

In recent years, ethanol oxidation by a number of *in vivo* preparations of liver, including slices [1], isolated cells [2, 3], and perfused organs [4, 5], has been shown to be accelerated at very high ethanol concentrations (e.g. above 60 mM ethanol). In contrast, some evidence suggests that the concentration effects of ethanol on ethanol elimination may not be present *in vivo*. First, rates of ethanol elimination have, in one study, been shown to be identical at high (50 mM) and low (10 mM) ethanol concentrations *in vivo* [6]. Second, it has been clearly established that the ethanol elimination curve *in vivo* is linear, not curvilinear, as would be expected if a concentration effect depended upon saturating enzyme systems [7–10]. On the other hand, if a high dose of ethanol causes a long-term response *in vivo* in addition to saturating ethanol-metabolizing systems, then linearity vs non-linearity of the elimination curve is not direct evidence against the presence of a concentration effect *in vivo*. Moreover, ethanol concentrations employed routinely *in vivo* do not fall within the range where the concentration effect was observed *in vitro*.

Ethanol is oxidized almost exclusively in the liver by two enzyme systems, alcohol dehydrogenase and catalase [5]. Alcohol dehydrogenase is saturated with ethanol at concentrations between 0.1 and 2.0 mM [11, 12]. Since ethanol levels necessary to produce the concentration effect *in vitro* are much higher, it might be concluded that alcohol dehydrogenase is not involved in the mechanism of this phenomenon. However, evidence suggests that the rate-limiting step in ethanol oxidation via alcohol dehydrogenase is the rate of reoxidation of NADH [13–15]. If the long-term response of ethanol postulated above is to accelerate the reoxidation of NADH, then alcohol dehydrogenase could be involved in the mechanism of the concentration effect.

Catalase–H_2O_2–dependent acetaldehyde production from ethanol is regulated by both substrate concentration and H_2O_2 supply [16, 17]. So, ethanol could produce a concentration effect involving catalase–H_2O_2 directly. Additionally, stimulation of H_2O_2 production by ethanol could also increase catalase–H_2O_2–dependent ethanol oxidation. In fact, unphysiologically high oxygen concentrations are routinely employed in the *in vitro* studies, and could stimulate catalase artifactually by promoting H_2O_2 generation.

These experiments were designed to examine (a) whether a concentration effect of ethanol on ethanol elimination exists *in vivo*, and (b) which enzymatic pathways are involved. Rates of ethanol elimination *in vivo* in ethanol concentration ranges of 20–40

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and 60–90 mM were compared. Selective inhibitors of the alcohol dehydrogenase (4-methylpyrazole) and catalase–H_2O_2 (aminotriazole) pathways have been employed in normal and ethanol-pretreated rats.

Preliminary accounts of this work have appeared elsewhere [18].

METHODS

Animal treatment. Well-fed, Sprague–Dawley female rats (200–300 g) were used in this study. When indicated, animals were pretreated with ethanol for 3–5 weeks with a diet of 25% w/v sucrose and 20% w/v ethanol as described by Porta *et al.* [19]. Control animals received 25% w/v sucrose, and both groups had free access to laboratory chow.

Surgical procedures. Rats were anesthetized lightly with ether (anesthesia grade) and secured on an animal board. An incision was made in the skin covering the left thigh area, and the femeral vein was cleared from other vessels and membranes. Two surgical ligatures were placed around the femoral vein but were not tied. Approximately 4 ml of either a 2.5 M (low dose; 2.5 g/kg) or a 5.0 M (high dose; 5.0 g/kg) solution of ethanol in normal saline was injected intravenously over a time period of 10–15 min. Subsequently, the two ligatures on the femoral vein were tied to prevent bleeding. The skin flap was closed surgically, and the animal was placed in a cage for subsequent blood sampling from the tail.

All drugs and inhibitors were administered intraperitoneally. Methylpyrazole was given 0.5 hr prior to the surgical procedure in a dose of 75 mg/kg of body weight [20]. Aminotriazole (1 g/kg) was given 2 hr prior to the surgery. Pyruvate was administered 0.5 or 3 hr after ethanol (see legends to Fig. 2 and Table 1) solution in a dose which was calculated to bring the animal's fluid volume (70 per cent of the body weight) to a final concentration of 10 mM.

Blood-sampling techniques. Duplicate blood samples were collected in heparinized 50-μl capillary pipets from the tip of the tail. The samples were then delivered into 25-ml Erlenmeyer flasks containing 1.0 ml of 25 mM thiourea and 0.6 N perchloric acid and immediately stoppered with a rubber septum.

Gas chromatographic determination of blood ethanol. The Erlenmeyer flasks referred to above were incubated in a water bath at 37° for at least 45 min. Subsequently, 1 ml of the vapor phase [21] was withdrawn through the rubber septum with a Hamilton gas-tight syringe and injected into a Hewlett-Packard model 5720A gas chromatograph equipped with a flame-ionization detector. The apparatus contained a 6 ft by $\frac{1}{8}$ in. Poropack Q

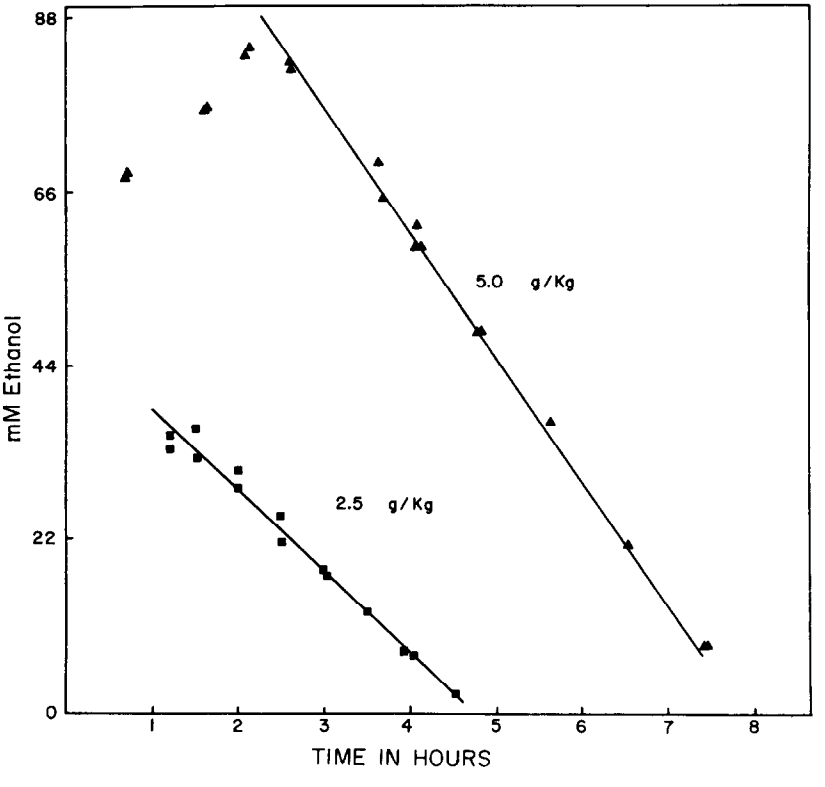

Fig. 1. *Blood ethanol concentration versus time in normal well-fed rats at two ethanol concentrations.* Rats were etherized prior to the infusion of ethanol into the femoral vein (see Methods). Animals were unrestrained throughout the rest of the experiment. Duplicate blood samples were collected every 30 min from the tip of the tail with heparinized 50-μl capillary pipets. Blood ethanol was determined by gas chromatography (see Methods). This figure depicts two typical rats, and is representative of data used to calculate values in Table 1. Key: ■, low dose, 2.5 g/kg; ▲, high dose, 5.0 g/kg.

column. Operating parameters were as follows: oven, 165°; detector, 300°; injection port, 252°; and carrier gas flow, 40 ml/min.

A peak corresponding to ethanol with a retention time of about 2.5 min was compared with ethanol standards.

RESULTS

Effect of ethanol concentration on rate of ethanol elimination in normal rats. After rats were treated with either 2.5 or 5.0 g/kg of ethanol, duplicate blood samples were taken from the tail at approximately 0.5-hr intervals over a 5–6 hr period. Typical results are shown in Fig. 1. With the 2.5 g/kg dose, ethanol concentrations in the blood reached 30–40 mM approximately 1 hr after dosing (Fig. 1). The concentration of ethanol in the blood subsequently declined linearly, and was undetectable after approximately 4–5 hr.

With the 5.0 g/kg dose, however, there was a lag of 2–3 hr before concentrations of 80–90 mM were reached. Subsequently, the concentration declined linearly but more rapidly than with the low dose and reached concentrations between 10 and 20 mM in 8 hr (Fig. 1).

With the 2.5 g/kg dose of ethanol, an average rate of ethanol elimination of 178.8 ± 1.0 μmoles/g liver (wet)/hr was observed (Table 1). The rates with the higher dose were approximately 50 per cent greater ($P < 0.001$), demonstrating that ethanol concentrations influenced the rate of ethanol elimination *in vivo*.

Inhibitor studies. Alkylpyrazoles such as 4-methylpyrazole have been shown to be potent competitive inhibitors of alcohol dehydrogenase [22, 23]. With the 2.5 and 5.0 g/kg doses of ethanol, ethanol elimination rates were decreased by 4-methylpyrazole to 31 and 58 μmoles/g of liver (wet)/hr respectively (Table 1). The methylpyrazole-insensitive rate obtained with the 5.0 g/kg dose was significantly greater ($P < 0.001$) than the rate obtained with the 2.5 g/kg dose (Table 1). With both doses of ethanol, a second addition of 4-methylpyrazole given 5–7 hr after the initial dosing period did not influence the rate (not shown). Thus, it was concluded that the dose of 4-methylpyrazole employed gave maximal inhibition under these conditions.

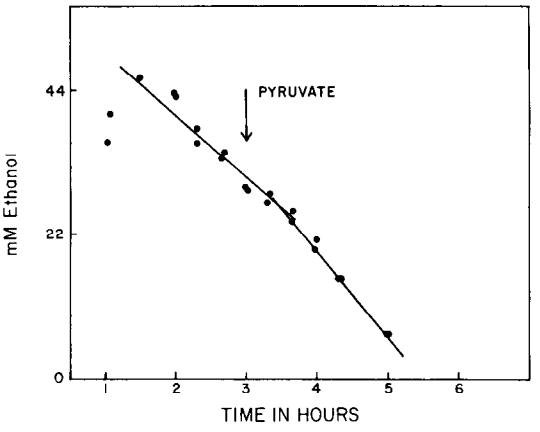

Fig. 2. *Effect of pyruvate on blood ethanol elimination after a low (2.5 g/kg) dose of ethanol.* Conditions are as in Fig. 1. Pyruvate was injected intraperitoneally in normal saline in a dose adjusted to bring the fluid volume (70 per cent of body weight) of the animal to 10 mM.

Previous work with the perfused liver has shown that aminotriazole pretreatment (1 g/kg) can abolish ethanol-induced perturbations in the steady state concentrations of catalase–H_2O_2 in approximately 90 min.* Therefore, aminotriazole was given 2 hr prior to the addition of ethanol. With the 2.5 g/kg dose of ethanol, a slight (10 per cent) decrease in the rate of ethanol elimination was observed; however, this was not statistically significant. However, with the 5.0 g/kg dose, a decrease of 24 per cent in the rate of ethanol elimination was observed ($P < 0.01$).

With both doses of ethanol, summation of the 4-methylpyrazole and aminotriazole-insensitive rates gave values which were similar to rates observed in the absence of inhibitors.

Effect of pyruvate. Pyruvate is a substrate for a highly active NAD^+-linked lactate dehydrogenase and stimulates ethanol metabolism by accelerating the rate of cofactor reoxidation [24].

In Fig. 2, an experiment is shown in which pyruvate was not injected until 3 hr after ethanol. Under these conditions, pyruvate addition uniformly accelerated

* N. Oshino, personal communication.

Table 1. Blood ethanol elimination *in vivo* in normal rats*

Treatment	Elimination rate (μmoles/g liver (wet)/hr)	
	Ethanol (2.5 g/kg)	Ethanol (5.0 g/kg)
None	178.8 ± 1.0†	266.3 ± 13.3
4-Methylpyrazole	31.2 ± 1.5	58.2 ± 1.9
Aminotriazole	157.8 ± 11.3	201.3 ± 12.4
Pyruvate	230.8 ± 12.6	245.0 ± 13.1

* Average data based on four or five rats in each group. Conditions were as in Fig. 1. 4-Methylprazole (75 mg/kg) was given 30 min and aminotriazole (1 g/kg) was given 2 hr before ethanol. Pyruvate was given 30 min before ethanol in a dose calculated to bring the fluid volume (70 per cent of body weight) of the animal to 10 mM. Both inhibitors were adminstered intraperitoneally.

† Mean ± S. E. M.

Table 2. Blood ethanol elimination *in vivo* in normal rats*

Treatment	Elimination rate (m-moles/kg body wt/hr) Ethanol (2.5 g/kg)	Ethanol (5.0 g/kg)
None	7.15 ± 0.15†	10.70 ± 0.53
4-Methylpyrazole	1.25 ± 0.06	2.33 ± 0.08
Aminotriazole	6.31 ± 0.45	8.05 ± 0.49

* Conditions were as in Table 1.
† Mean ± S. E. M.

Table 3. Blood ethanol elimination *in vivo* in ethanol-pretreated rats*

Treatment	Elimination rate (μmoles/g liver (wet)/hr) Ethanol (2.5 g/kg)	Ethanol (5.0 g/kg)
None	242.4 ± 11.1†	251.1 ± 14.2
4-Methylpyrazole	52.2 ± 2.4	49.1 ± 2.2
Aminotriazole	175.9 ± 9.3	188.7 ± 5.2
Pyruvate	233.3 ± 6.2	

* Conditions were as in Fig. 3 and Table 1. All data are averages from four to six rats in each group.
† Mean ± S. E. M.

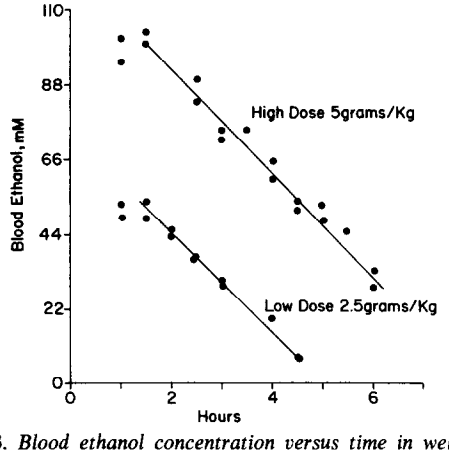

Fig. 3. *Blood ethanol concentration versus time in well-fed ethanol-pretreated rats at two ethanol concentrations.* Conditions are as in Fig. 1. Rats were maintained on an ethanol-containing diet for 3–5 weeks prior to the experiment [19].

the rate of ethanol elimination. Under slightly different conditions where pyruvate was administered 30 min after ethanol, a 31 per cent average increase in the rate of ethanol elimination was observed when compared with the control (Table 1) at the low dose. At the 5.0 g/kg dose, however, pyruvate had no effect.

When the data with the two doses of ethanol, the inhibitors and pyruvate were expressed per unit of body weight, essentially the same findings were observed (Table 2).

Effect of ethanol concentration on rate of ethanol elimination in ethanol-pretreated rats. Figure 3 depicts typical results obtained from ethanol-pretreated rats at low and high doses of ethanol. With both doses of ethanol, blood ethanol concentration vs time plots were linear. With the 2.5 g/kg dose of ethanol, elimination rates in ethanol-pretreated animals (Table 3 and Fig. 3) were 38 per cent greater than in normals (Table 1 and Fig. 1). However, there was no difference in rates at the 5.0 g/kg dose between normal and ethanol-pretreated animals (Tables 1 and 3). Moreover, the difference between rates of ethanol elimination at low and high doses of ethanol present in normal animals (Table 1 and Fig. 1) was absent in ethanol-treated animals (Table 3 and Fig. 3). Two important observations can be made from these comparisons. First, the concentration effect is absent in ethanol-treated animals. Second, the well-documented increase in ethanol metabolism due to chronic pretreatment with ethanol was only observed at the lower dose of ethanol.

With both doses of ethanol, 4-methylpyrazole inhibited rates of ethanol oxidation approximately 80 per cent (Table 3). The 4-methylpyrazole-insensitive rate of ethanol elimination was significantly greater ($P < 0.001$) in ethanol-pretreated rats than in normals at the low dose (Tables 1 and 3). However, in contrast to normal animals, this insensitive rate was independent of ethanol concentrations in ethanol-treated animals (Table 3).

With both doses of ethanol, aminotriazole decreased rates of ethanol oxidation approximately 25 per cent (Table 3) in ethanol-pretreated rats. At the low dose, aminotriazole treatment produced statistically significant ($P < 0.01$) decreases in ethanol elimination rates in ethanol-treated rats but not in controls (Tables 1 and 3). However, no dose effect could be detected in ethanol-treated rats with respect to aminotriazole-insensitive rates of ethanol elimination (Table 3).

In contrast to normal animals, pyruvate had no effect on the rate of ethanol metabolism in ethanol-pretreated rats at the low dose of ethanol (Table 3).

Qualitatively, comparisons of the data from ethanol-pretreated animals were similar when expressed per unit of body weight (Table 4).

Table 4. Blood ethanol elimination *in vivo* in ethanol-pretreated rats*

Treatment	Elimination rate (m-moles/kg body wt/hr)	
	Ethanol (2.5 g/kg)	Ethanol (5.0 g/kg)
None	10.67 ± 0.49†	11.05 ± 0.62
4-Methylpyrazole	2.29 ± 0.11	2.16 ± 0.10
Aminotriazole	7.74 ± 0.41	8.30 ± 0.03
Pyruvate	10.26 ± 0.48	

* Conditions were as in Table 3.
† Mean ± S. E. M.

DISCUSSION

Effect of ethanol concentration on rates of ethanol elimination in normal rats in vivo. An increase of ethanol elimination from the blood was observed with a high dose of ethanol (5.0 g/kg) with respect to a low dose (2.5 g/kg) in normal rats. These data demonstrated clearly that the concentration effect observed *in vitro* [1–5] also exists *in vivo* in normal rats (Table 1). Why has this effect previously gone unnoticed *in vivo*? The reasons may be two: (1) the ethanol concentrations employed and (2) the route of administration of ethanol. Previous studies have used ethanol concentrations only as high as 50 mM [6, 7], and the effects observed in this study may not be triggered until blood levels reach 80–90 mM. Also, previous studies have employed either intragastric or intraperitoneal routes of administration. Since ethanol is absorbed slowly by these routes, a long distribution time is observed [6]. This study employed intravenous injections of ethanol through which high blood ethanol levels were reached rapidly and distribution time was reduced.

If one assumes that the 4-methylpyrazole-sensitive rate is specific for alcohol dehydrogenase and, conversely, that the aminotriazole-sensitive rate is due to catalase–H_2O_2, separate alcohol dehydrogenase- and catalase–H_2O_2–dependent components of the concentration effect can be identified (Table 5). Employing these criteria, it can be seen (Table 5) that about 40–50 μmoles/g/hr of the increase in the rate of ethanol elimination is due to alcohol dehydrogeanse, and a component of similar magnitude is due to catalase–H_2O_2. The increase in the alcohol dehydrogenase-dependent rate plus the increase in the catalase–H_2O_2–dependent rate of ethanol oxidation equals the total increase due to ethanol concentration. Thus, since these two components account for the increase due to to concentration, it may be suggested that a separate microsomal ethanol-oxidizing system [25] is not operating at high ethanol concentrations.

The increase in the alcohol dehydrogenase-dependent and catalase-H_2O_2-dependent rates may be linked or independent. DeDuve [26] postulated that the NADH produced from alcohol dehydrogenase can stimulate hydrogen donor formation for oxidases in the peroxisomes and thereby facilitate H_2O_2 formation and catalase–H_2O_2-dependent ethanol oxidation. If this were the case, then 4-methylpyrazole would not only inhibit the alcohol dehydrogenase-dependent increase in ethanol utilization due to ethanol concentration, but would inhibit the increase in the catalase–H_2O_2-dependent rate as well. This was not found to be the case (Table 1); therefore, it is concluded that the two components of the concentration effect are independent.

The alcohol dehydrogenase-dependent increase in ethanol oxidation due to ethanol concentration was approximately 50 μmoles/g of liver (wet)/hr (Table 5). An increase in alcohol dehyrogenase-dependent ethanol metabolism can involve any of three mechanisms: (1) an increase in enzyme activity; (2) saturation of alcohol dehydrogenase with ethanol; or (3) an increase in the rate of reoxidation of NADH.

Table 5. Alcohol dehydrogenase-dependent and catalase –H_2O_2– dependent rates of ethanol elimination in normal and ethanol-pretreated rats*

	Elimination rate (μmoles/g liver (wet)/hr)			
	Normal rats		Ethanol-pretreated rats	
	Ethanol (2.5 g/kg)	Ethanol (5.0 g/kg)	Ethanol (2.5 g/kg)	Ethanol (5.0 g/kg)
Alcohol dehydrogenase-dependent	148 ± 13†	209 ± 2	190 ± 2	202 ± 2
Catalase–H_2O_2–dependent	21 ± 11	66 ± 12	67 ± 9	62 ± 5

* Data are from Tables 1 and 3. The alcohol dehydrogenase-dependent rate is the difference between the rate in the presence of 4-methylpyrazole and the rate in the absence of inhibitor. Similarly, the catalase–H_2O_2–dependent rate is the difference between the rate in the presence of aminotriazole and in the absence of inhibitor.
† Mean ± S. E. M.

Since the concentration effect occurs rapidly (<2 hr; Fig. 1), a change in enzyme levels under these conditions appears unlikely. Although the ethanol concentration is obviously increased, it is improbable that this stimulates ethanol metabolism directly, since the K_m of alcohol dehydrogenase for ethanol is around 2 mM [11, 12]. Furthermore, ethanol elimination rates were linear at both doses (Fig. 1). If the mechanism of this phenomenon involved saturation of enzyme, one would predict a slowing of the rate of elimination as the concentration decreased with time with the high dose into the range observed with the low dose. This possibility can be eliminated because of the linearity of the elimination rate observed with the high dose.

Considerable evidence exists [13] indicating that NADH reoxidation is the rate-limiting step in alcohol dehydrogenase-dependent ethanol oxidation. High ethanol concentrations could therefore stimulate NADH reoxidation in some unknown fashion. Since pyruvate, which is known to stimulate reoxidation of NADH, accelerated ethanol elimination at the low but not at the high dose of ethanol (Table 1), an increase in the rate of NADH reoxidation is the most likely explanation for the alcohol dehydrogenase-dependent component of the rate stimulated at high ethanol concentrations. How ethanol might be doing this will require further investigation.

The increase in the catalase–H_2O_2-dependent rate with high ethanol concentration was about 45 μmoles/g of liver (wet)/hr (Table 5). This rise could also be due to any of three mechanisms: (1) an increase in enzyme activity, (2) formation of more tertiary complex (catalase-H_2O_2–ethanol) at higher ethanol concentrations, or (3) an increase in the rate of H_2O_2 generation. Since the concentration effect occurs rapidly, a change in enzyme levels is unlikely under these conditions, as argued above for alcohol dehydrogenase. The present study does not, however, allow us to conclude whether either increased formation of the tertiary complex or enhanced H_2O_2 supply accounts for the catalase–H_2O_2-dependent portion of the stimulation of ethanol utilization due to high concentrations of ethanol.

It is possible that the concentration effect observed in many *in vitro* preparations of liver is an artifact, since high oxygen (95%) concentrations used *in vitro* could lead to high rates of H_2O_2 production and subsequent activation of catalase. However, the results presented here clearly demonstrate that a catalase–H_2O_2 portion (Table 5) of the concentration effect is present *in vivo* (i.e. at 21% O_2).

Effect of ethanol pretreatment on ethanol elimination in vivo *at low and high doses of ethanol.* An adaptive increase in ethanol metabolism was observed in ethanol-pretreated rats at the 2.5 g/kg dose (Tables 1 and 3), confirming similar observations in other laboratories [27, 28]. However, at the high dose of ethanol (5.0 g/kg), rates were similar to those observed with the same dose in the normal animal. Moreover, in contrast to the normal animal, rates of ethanol elimination in ethanol-pretreated rats were identical at both the low and high doses of ethanol.

It is intriguing that the stimulation of ethanol metabolism by ethanol concentration (Table 1) is the same order of magnitude as the stimulation observed by prior chronic pretreatment with ethanol. Moreover, since no concentration effect was observed in ethanol-pretreated animals, one may conclude that these processes are not additive. These facts lead logically to the speculation that the underlying mechanisms responsible for the adaptive increase and the concentration effect may be similar. This hypothesis is supported by the calculation that the alcohol dehydrogenase- and the catalase–H_2O_2-dependent components of both of these phenomona are of similar magnitude (e.g. 40–50 μmoles/g/hr) (Table 5).

Alcohol dehydrogenase activity has been shown in previous studies to increase, stay at the same level or decline with ethanol pretreatment [29, 30]. Therefore, an increase in enzyme activity to account for the adaptive increase in these studies to be unlikely.

On the other hand, considerable evidence has indicated that reoxidation of NADH for alcohol dehydrogenase is involved in the mechanism of the adaptive increase in liver slices [12] and perfused liver [15, 31]. Furthermore, the inhibitor studies (Table 5) presented in this report present clear evidence that the alcohol dehydrogenase pathway is partially responsible for an adaptive increase *in vivo* with ethanol concentrations in the 10–40 mM range.

While the data presented here allow little insight into the mechanism of the alcohol-dehydrogenase-dependent component of the adaptive increase, by elimination (see above) and based on previous work *in vitro* [15, 31], reoxidation of NADH at an accelerated rate is the most likely mechanism. This conclusion is identical with the conclusion reached above, namely that enhanced NADH reoxidation was responsible for the concentration effect. While hard evidence is still lacking, these conclusions do lend credence to the hypothesis that the adaptive increase and the concentration effect may operate via common mechanisms. Experiments to test this possibility are presently underway in our laboratory.

Previous studies have suggested that catalase–H_2O_2 plays a role in the mechanism of the adaptive increase in the perfused liver [15]. However, those studies were inconclusive. The data presented here show clearly that a catalase–H_2O_2-dependent component is involved in the adaptive increase *in vivo* (Table 5).

Rate limitation of alcohol dehydrogenase-dependent ethanol oxidation in vivo. There is considerable evidence indicating that increasing the rate of reoxidation of NADH will accelerate alcohol dehydrogenase-dependent ethanol metabolism in the fasted state [13, 14, 32]. However, the case for the fed state is not so clear. There are two schools of thought on what is rate-limiting in the fed state: (1) the activity of alcohol dehydrogenase, and (2) the rate of reoxidation of NADH.

Crow *et al.* [33] showed that the average of many published rates of ethanol metabolism was 65 per cent of the experimental maximal rate of alcohol dehydrogenase determined *in vitro*. From this comparison, they concluded that ethanol elimination can be limited by alcohol dehydrogenase. However, such extrapolations to *in vivo* rate limitations based on *in vitro* activity determinations should be regarded with caution.

Pyruvate accelerates the reoxidation of NADH

in the liver via the lactate dehydrogenase equilibrium. At the low dose of ethanol in normal well-fed rats, pyruvate accelerated the rate of ethanol elimination *in vivo* by about 31 per cent (Table 1), confirming similar experiments with the perfused liver [24]. Therefore, it is concluded that the rate of NADH reoxidation and not alcohol dehydrogenase activity is rate-limiting in well-fed rats *in vivo* which have received a moderate to low (2.5 g/kg) dose of ethanol.

In contrast, pyruvate failed to stimulate ethanol metabolism at the low dose in ethanol-pretreated animals or with the high dose in normal animals where ethanol utilization was already accelerated (Tables 1 and 3). Under these conditions it is concluded that the likely rate limitation is alcohol dehydrogenase. Therefore, the rate-limiting step in ethanol elimination in fed rats depends upon the dose of ethanol employed as well as the previous prior exposure of the animal to ethanol.

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