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ETHANOL METABOLISM IN *PEROMYSCUS* GENETICALLY DEFICIENT IN ALCOHOL DEHYDROGENASE

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Abstract—Ethanol metabolism was compared in two strains of the deermouse, *Peromyscus maniculatus*. Animals of the Adh^N/Adh^N strain, which lack liver alcohol dehydrogenase (ADH) activity, eliminated ethanol at a signficantly slower rate ($P < 0.0005$) than those of the Adh^F/Adh^F strain, which have normal liver ADH activity. However, a comparison of the blood ethanol elimination rate (BEER) in the two strains indicated that, at high blood ethanol concentrations, non-ADH mediated pathways may account for as much as two-thirds of normal ethanol elimination in this species. Chronic ethanol consumption induced an elevated BEER in Adh^F/Adh^F mice but not in Adh^N/Adh^N mice. This strain difference in response to ethanol feeding suggests that increases in BEER are mediated primarily via the ADH pathway. A microsomal ethanol-oxidizing system (MEOS), independent of ADH and catalase, was shown to exist in microsomal preparations from both strains of *P. maniculatus*. MEOS activity of naive Adh^N/Adh^N mice was 2.3-fold higher than that of naive Adh^F/Adh^F animals. Both strains had a 3-fold greater MEOS activity following chronic ethanol consumption. Contrary to similar investigations in ethanol-fed rats, the alteration in MEOS activity was not accompanied by significant changes in cytochrome P-450, NADPH-cytochrome *c* reductase or phospholipid. Most importantly, the elevated *in vitro* MEOS activity of ethanol-fed Adh^N/Adh^N mice had no significant effect upon BEER. These results suggest caution in attaching physiological significance to the simultaneous, ethanol-induced increase of the *in vitro* MEOS and of BEER in experimental animals with normal liver ADH activities.

The mammalian liver is responsible for 95 per cent of blood alcohol elimination. The metabolic pathways utilized by the liver for alcohol oxidation have been subject to extensive investigation in the past 10 years. It is generally recognized that, at low blood ethanol concentrations (<0.2%), at least 80 per cent of alcohol oxidation proceeds via a two-step pathway in which the initial reaction is catalyzed by alcohol dehydrogenase (ADH, alcohol: NAD oxidoreductase, EC 1.1.1.1). However, at high blood ethanol concentrations, only 50 per cent of *in vivo* alcohol oxidation can be suppressed by the potent ADH inhibitor pyrazole or its alkyl derivatives.

Pyrazole-insensitive ethanol oxidation has been attributed to a peroxidative activity of catalase and/or a microsomal ethanol-oxidizing system (MEOS) which bears some relation to the microsomal drug-metabolizing system. There is little doubt that at least some portion of *in vivo* alcohol oxidation is suppressed by the catalase inhibitors sodium azide and aminotriazole. *In vitro* microsomal ethanol-oxidizing activity is also partially suppressed by these inhibitors. However, there is a sodium azide and aminotriazole insensitive ethanol-oxidizing activity in microsomes which has been attributed to ADH contamination [1] or to a distinct MEOS [2]. Still other workers have shown that the endoplasmic reticulum does not participate in ethanol oxidation *in vivo* [3].

Teschke *et al.* [4, 5] have reported the separation of MEOS activity from ADH, catalase, and NADPH oxidase activities by DEAE cellulose chromatography. They have reconstituted MEOS-like activity from partially purified cytochrome P-450, NADPH-cytochrome *c* reductase and L-α-dioleoyl lecithin [2]. The isolation of the MEOS and its reconstitution from components of the microsomal electron transport pathway have been confirmed by Miva *et al.* [6]. Chronic ethanol feeding of rats [2] was also shown to induce quantitative and qualitative alterations in the cytochrome P-450 of hepatic microsomes, in addition to stimulating an increase in the overall blood ethanol elimination rate. The elevation of the ethanol clearance rate in response to chronic ethanol consumption or metabolic tolerance is a well-established phenomenon [7–9].

These studies of mammalian alcohol metabolism have been hampered by the inflexibility of experimental animals which require the use of inhibitors to study specific aspects of ethanol elimination. A unique animal model has been developed recently which circumvents the requirement for pyrazole to study *in vivo* non-ADH mediated alcohol oxidation. Two deermouse strains which are genetically defined for liver ADH variants have been derived from laboratory populations of *Peromyscus maniculatus*. The Adh^F/Adh^F strain has a normal liver ADH activity, while the Adh^N/Adh^N strain has no liver ADH activity which could be detected on zymograms or in spectrophotometric assays of the reverse reaction [10]. Moreover, Adh^N/Adh^N animals exhibit no antigenically crossreacting material in immunochemical tests employing monospecific anti-ADH antisera [11]. These strains of *P. maniculatus* were used in the present work to examine the relative importance of non-ADH mediated pathways in ethanol metabolism and to confirm the existence of an *in vitro* MEOS activity independent of catalase and ADH.

EXPERIMENTAL

Genetic stocks. Animal stocks used in the current studies were recently derived from laboratory populations of *P. maniculatus* maintained by Dr. Wallace Dawson, Mammalian Genetics Laboratory, University of South Carolina, and have the following genotypes for liver alcohol dehydrogenase: Adh^F/Adh^F and Adh^N/Adh^N [12].

Ethanol-induced sleep time. Ethanol-induced sleep times were determined by the technique of Belknap *et al.* [13]. All tests were performed between 9:00 a.m. and 3:00 p.m. and utilized animals 30- to 40-days-old. Deermice of both strains were injected intraperitoneally in the lower abdomen with 0.015 ml/g body weight of 30% ethanol in isotonic saline. The time of initial injection was noted. Each animal was restrained until the righting response was lost (<1 min) and then placed upside down in a V-shaped trough. Sleep time was defined as the length of time required, following ethanol injection, for an animal to regain the righting response, measured as the ability to right three times in 30 sec.

Ethanol elimination rate. The blood ethanol elimination rate (BEER) was determined for each strain of *P. maniculatus* which had been maintained on water (control) or an ethanol drinking regime. Ethanol consumption was initiated in 1- to 2-month-old animals and was continued for 6 weeks. The ethanol-feeding regime consisted of 10% ethanol in drinking water along with solid food (Wayne Lab Blox) available *ad lib.* until 24 hr prior to BEER measurement.

All animals were maintained on a 16:8 light versus dark cycle, and blood ethanol elimination rates were determined between 9:00 a.m. and 3:00 p.m. For these tests, animals (2.5- to 3.5-months-old) were injected intraperitoneally with a hypnotic dose of 30% ethanol in isotonic saline, as described above. Preliminary experiments showed that maximum blood ethanol concentrations were reached 30 min after injection; the linear decrease had begun by 1 hr post-injection. At two of four selected time intervals after injection (0.5, 2.5, 4.0 and 6.0 hr), blood (20 μl) was drawn from the suborbital sinus of each animal, added to 0.5 ml of 400 mM perchloric acid in a plastic vial, capped tightly, and refrigerated at 4° until all samples were collected. The vials were then centrifuged at 13,000 *g* for 20 min and assayed for ethanol by an enzymatic technique [14]. BEER was calculated as the change in blood ethanol content per hour over the portion of each elimination curve above 200 mg ethanol/100 ml blood, where clearance is essentially a linear function.

MEOS assay. Microsomes of naive and ethanol-fed animals from both *P. maniculatus* strains were prepared by homogenizing freshly excised liver 1:3 (w/v) in 50 mM sodium phosphate–0.15 M potassium chloride (pH 7.5), and centrifuging for 30 min at 10,000 *g*. The resulting supernatant fraction was layered on isotonic sucrose and the microsomal fraction pelleted by centrifugation at 105,000 *g* for 30 min. The microsomes were washed free of sucrose, and then resuspended in 0.1 M potassium phosphate (pH 7.5). MEOS was assayed as described by Lieber and DeCarli [7] in 30 ml Warburg flasks with rubber stoppers, using 1–3 mg of microsomal protein per flask. Except where otherwise indicated, the alcohol substrate was at 50 mM, and 0.1 mM sodium azide was added to each assay. Reactions were stopped by the addition of 0.5 ml of 70% trichloroacetic acid (TCA) from the sidearm. Aldehyde produced during the reaction was trapped in the center well with 0.4 ml of 15 mM semicarbazide–HCl in 0.1 M potassium phosphate (pH 7.4) by diffusion overnight at 23°. The contents of the center well were added to 1.6 ml of distilled water and absorbance was read at 224 nm. This value was converted to nmoles aldehyde, using standard curves generated for acetaldehyde and propionaldehyde over a range of 225–1350 nmoles/flask.

Other assays. ADH activity was measured in the reverse reaction as described previously [10]. Catalase was assayed by the method of Luck [15], with one unit equal to one ΔA_{240}/min at 23°. NADPH-cytochrome *c* reductase was assayed as by Masters *et al.* [16], with one unit defined as one ΔA_{550}/min at 23°. NADPH-oxidizing activity was measured by following the decrease in absorbance at 340 nm [17]. Cytochrome P-450 was quantified by the method of Omura and Sato [18], and phospholipid as described by Dittmer and Wells [19]. Protein content was determined by the method of Lowry *et al.* [20] using bovine serum albumin as a standard.

Statistical analysis. The average (±standard deviation) was determined for each experimental condition. The significance of the difference between an experimental and control value was assessed by Student's paired *t*-test.

RESULTS

Chronic ethanol-induced sleep times were measured in mice of both ADH genotypes. Following injection of a weight-specific hypnotic dose of 30% ethanol, Adh^N/Adh^N animals required a significantly

Table 1. Individual blood ethanol elimination rates for naive and ethanol-fed individuals of both *P. maniculatus* strains*

Regimen	Adh^F/Adh^F	Adh^N/Adh
Naive	94.9 ± 24.9 (32)	63.9 ± 19.5 (44)
Ethanol-fed	114.3 ± 26.9 (43)	59.5 ± 18.8 (38)

* Rates are expressed as the change in blood ethanol content (mg ethanol/100 ml of blood) per hour for the time interval during which blood ethanol content remained greater than 200 mg ethanol/100 ml of blood. No significant difference was found between the blood ethanol elimination rates of male and of female *P. maniculatus* at these high blood ethanol concentrations. Values given are the average of pooled male and female blood ethanol elimination rates ± one standard deviation. The number of individual determinations is given in parentheses. In comparing the two genotypes, whether naive or ethanol-fed, the values are significantly different ($P < 0.0005$). Ethanol feeding does not significantly alter the blood ethanol elimination rate in Adh^N/Adh^N animals, but does in Adh^F/Adh^F animals ($P < 0.005$).

Table 2. ADH, catalase and MEOS activities in naive and ethanol-fed *P. maniculatus**

	ADH (units/mg liver protein)	Catalase (units/mg liver protein)	Catalase (units/mg liver microsomal protein)	MEOS (propanol + azide) (units/mg liver microsomal protein)	MEOS (propanol + azide) (units/0.25 g liver)
Adh^F/Adh^F (naive)	0.22 ± 0.07	5.75 ± 1.54	0.38 ± 0.14	8.46 ± 3.10	19.12 ± 7.03
Adh^F/Adh^F (ethanol-fed)	0.28 ± 0.12	8.08 ± 2.09	0.38 ± 0.10	25.46 ± 6.60	64.22 ± 11.19
Adh^N/Adh^N (naive)	0	8.02 ± 3.52	0.46 ± 0.31	19.35 ± 5.38	69.33 ± 21.42
Adh^N/Adh^N (ethanol-fed)	0	7.63 ± 4.00	0.55 ± 0.27	55.57 ± 7.53	230.98 ± 90.29

* No significant difference was observed between males and females for these activities. Values presented here are the averages ± S.D. of six to ten animals. Ethanol feeding does not significantly alter ADH activity in Adh^F/Adh^F animals, nor is catalase activity significantly different in any of the four groups of animals. MEOS activity is significantly different ($P < 0.0005$) between naive Adh^N/Adh^N and Adh^F/Adh^F animals. Ethanol feeding significantly alters MEOS values in both strains ($P < 0.0005$).

longer time than Adh^F/Adh^F mice to regain the righting response. Adh^N/Adh^N sleep times averaged 138.2 (±9.4 S.E.) min (N = 48); Adh^F/Adh^F sleep times were 76.1 (±3.8 S.E.) min (N = 89). These values are significantly different ($P < 0.0005$).

This difference in ethanol-induced sleep time may represent a real difference in the ethanol elimination rate or result from differential nervous system sensitivity to ethanol. Therefore, blood ethanol content was measured directly at several points following administration of the same hypnotic ethanol dose. Calculated blood ethanol elimination rate (BEER) values are given in Table 1 for mice of both strains which were either naive or chronically fed 10% ethanol for 6 weeks prior to these tests. Both groups of Adh^N/Adh^N mice showed significantly lower BEERs when compared to Adh^F/Adh^F animals on the same drinking regime ($P < 0.005$). Liver ADH-negative mice eliminated ethanol at 67.3 per cent of the rate in Adh^F/Adh^F animals when naive blood ethanol elimination rates were compared. Ethanol feeding failed to increase BEER in Adh^N/Adh^N mice, while the same regime induced a small (20.4 per cent) but significant ($P < 0.005$) increase in Adh^F/Adh^F BEER. Ethanol-fed Adh^N/Adh^N animals eliminated ethanol at about one-half the rate found in Adh^F/Adh^F mice.

Activities of ADH, catalase and MEOS in naive and ethanol-fed mice of both strains are given in Table 2. As expected, Adh^N/Adh^N mice on both control and ethanol diets had no liver ADH activity. Ethanol-feeding did not significantly alter ADH activity in Adh^F/Adh^F animals. Catalase activities in crude liver 30,000 *g* supernatant fractions were not significantly different for any of the four experimental groups. No significant difference was detected in the level of catalase contamination of microsomal preparations from animals of either strain on control and ethanol diets. However, MEOS activities in Table 2, which are values with propanol substrate in the presence of 0.1 mM sodium azide, show striking differences among the four groups when the activities are expressed per mg of microsomal protein. MEOS activity of naive Adh^N/Adh^N mice was at least 2-fold more than in Adh^F/Adh^F animals. Ethanol consumption induced approximately a 3-fold increase in MEOS of both strains, such that the activity of ethanol-fed Adh^N/Adh^N mice remained at least 2-fold more than in Adh^F/Adh^F animals on the same regime. Similar changes are seen in MEOS activity expressed per 0.25 g liver wet weight.

The relationship of MEOS activity with propanol in the presence of sodium azide to other assay conditions is demonstrated in Table 3 for microsomes of both *P. maniculatus* strains. MEOS has much higher activity with ethanol than with propanol, yet while ethanol-oxidizing activity is markedly depressed in the presence of azide, the propanol oxidation rate remains virtually unchanged in the presence of this catalase inhibitor. These results are not surprising, because catalase, which contaminates most preparations of microsomes, has a marked substrate specificity for methanol and ethanol over longer chain alcohols. Rat liver catalase-H_2O_2 oxidizes propanol at only 1.5 per cent the rate at which

Table 3. Relative activities of the liver microsomal ethanol-oxidizing system of 4- to 6-month-old females from two strain of *P. maniculatus* under four assay conditions*

Assay condition	(nmoles aldehyde/min/mg microsomal protein) Adh^F/Adh^F	Adh^N/Adh^N	(nmoles aldehyde/min/ 0.25 g liver) Adh^F/Adh^F	Adh^N/Adh^N
Ethanol (50 mM)	55.8 ± 8.9 (3)	88.5 ± 17.9 (2)	122.4 ± 12.0 (3)	263.0 ± 32.5 (2)
Propanol (50 mM)	14.6 ± 4.3 (5)	23.7 ± 3.4 (4)	26.8 ± 5.9 (5)	68.0 ± 4.9 (4)
Ethanol (50 mM) + sodium azide (0.1 mM)	23.1 ± 2.5 (5)	74.0 ± 14.1 (4)	51.0 ± 1.4 (5)	212.5 ± 30.0 (4)
Propanol (50 mM) + sodium azide (0.1 mM)	11.8 ± 1.2 (4)	18.1 ± 4.3 (4)	30.5 ± 2.1 (4)	52.0 ± 15.0 (4)

* There is no significant difference between MEOS activity with propanol and propanol plus azide. MEOS (propanol plus azide) activity in the two genotypes is significantly different ($P < 0.005$). Numbers in parentheses are the number of determinations.

it oxidizes ethanol, and ethanol oxidation by catalase is maximally inhibited by 0.1 mM azide [21]. The use of propanol as substrate in the presence of azide assures a reliable measure of MEOS activity independent of catalase peroxidation.

Measurements of several microsomal components which have been implicated in MEOS and shown to increase in rats following chronic ethanol administration are presented in Table 4. Cytochrome P-450, NADPH-cytochrome *c* reductase and phospholipid were not significantly different for any of the *P. maniculatus* control or ethanol-fed groups. Further, the NADPH-oxidizing activity of microsomes, which may provide rate-limiting quantities of hydrogen peroxide for catalase peroxidation of alcohols, was actually significantly lower ($P < 0.05$) in hepatic microsomes of ethanol-fed Adh^N/Adh^N mice than in any of the other groups, despite a much higher MEOS activity. Thus, these large increases in MEOS activity cannot be attributed to quantitative alterations in major components of the microsomal electron transport pathway, nor to changes in the catalase or ADH-mediated ethanol oxidation rate. And, in Adh^N/Adh^N mice, elevated MEOS activity was not accompanied by increased BEER.

DISCUSSION

The Adh^N/Adh^N strain of *P. maniculatus* provides a unique animal model for studies of non-ADH mediated alcohol oxidation in mammals. These animals have been used in this report to study several parameters of ADH and non-ADH mediated ethanol metabolism. A significant finding of the current study is that ethanol consumption, under the conditions imposed, does not alter BEER in Adh^N/Adh^N animals but does increase BEER in Adh^F/Adh^F animals. The drinking regime of the present studies, which provides ethanol in drinking water for *ad lib.* consumption, results in a 20.4 per cent increase of BEER in Adh^F/Adh^F mice. Korvula and Lindros [8] reported an 11–19 per cent BEER increase with chronic ethanol consumption in rats on a completely liquid diet with isocaloric substitution of other carbohydrates for ethanol in the control diet. However, Lieber and DeCarli [7] and Tobon and Mezey [9] have reported larger increases in BEER following chronic ethanol consumption by rats on liquid diets.

These results suggest that ethanol consumption may induce an increase only in the ADH pathway

Table 4. Measurements of selected microsomal components of naive and ethanol-fed *P. maniculatus* from Table 2*

	NADPH-cytochrome *c* reductase (units/mg microsomal protein)	Cytochrome P-450 (nmoles/mg microsomal protein)	Phospholipid (μmoles/mg microsomal protein)	NADPH-oxidizing activity (ΔA_{340}/5 min/mg microsomal protein)
Adh^F/Adh^F (naive)	1.65 ± 0.57	0.34 ± 0.18	3.00 ± 0.69	0.54 ± 0.28
Adh^F/Adh^F (ethanol-fed)	1.32 ± 0.21	0.42 ± 0.26	2.95 ± 0.43	0.48 ± 0.18
Adh^N/Adh^N (naive)	1.40 ± 0.28	0.31 ± 0.11	2.52 ± 0.74	0.30 ± 0.06
Adh^N/Adh^N (ethanol-fed)	2.12 ± 0.56	0.26 ± 0.05	2.56 ± 0.30	0.21 ± 0.03

* The value for NADPH oxidase in ethanol-fed Adh^N/Adh^N mice is significantly different from the naive Adh^F/Adh^F values ($P < 0.1$) and from ethanol-fed Adh^F/Adh^F ($P < 0.05$) and naive Adh^N/Adh^N ($P < 0.05$). All other component values are not significantly different in the four groups of animals. Values are average ± S.D. of six to ten animals.

of alcohol oxidation or that the contribution of non-ADH pathways to *in vivo* ethanol elimination may be maximally elevated in naive Adh^N/Adh^N mice, but not in their Adh^F/Adh^F counterparts. That the adaptive increase in ethanol utilization requires alcohol dehydrogenase activity is supported by the work of Thurman *et al.* [22–24] on liver slices which demonstrated that the adaptive increase was inhibited by 4-methylpyrazole, an inhibitor of alcohol dehydrogenase activity. However, ethanol-fed Adh^F/Adh^F animals have no higher ADH levels than controls, confirming the work of Tobon and Mezey [9] and Korvula and Lindros [8] but conflicting with other reports (e.g. Ref. 25). Ethanol oxidation via the ADH pathway is normally modulated not by ADH activity, but rather by the reoxidation rate of NADH, and by the rate of electron flux in the mitochondrial respiratory chain. In support of this hypothesis are several experimental observations. Uncouplers of oxidative phosphorylation, such as dinitrophenol, increase the rate of mitochondrial oxidation and these agents are known to increase the rate of ethanol metabolism in liver slices [26]. Chronic ethanol treatment not only increases the rate of ethanol metabolism but also increases the rate of oxygen utilization by the liver *in vitro* [27, 28]. An increased utilization of adenosine triphosphate (ATP) by the $Na^+ + K^+$-activated ATPase system and the resulting drop in ATP/ADP + P_i ratio appear to be responsible for the increased oxygen consumption [28, 29] and ethanol metabolism [30] in livers of animals chronically treated with ethanol. In addition, ouabain, an inhibitor of the $Na^+ + K^+$-activated ATPase, can block completely the increased ethanol metabolism stimulated by chronic ethanol treatment [30]. The observation that ethanol consumption increases BEER in Adh^F/Adh^F mice, but not in Adh^N/Adh^N animals strongly suggests that the hypermetabolic state of mitochondria in hepatocytes increases *in vivo* ethanol elimination primarily by the ADH pathway, and exerts no effect on *in vivo* ethanol clearance when ADH is absent.

However, Cederbaum *et al.* [31] report that after pyrazole treatment, ethanol-fed rats still have ethanol clearance rates higher than controls. They calculate that 40 per cent of the BEER increase is not blocked by this ADH inhibitor. If the inhibitor were completely effective in blocking ADH mediated ethanol oxidation, their study suggests that ethanol consumption would increase *in vivo* ethanol elimination by ADH and alternate pathways. In this case, an explanation must be offered for the unchanged BEER in ethanol-fed Adh^N/Adh^N mice which completely lack ADH activity but do possess these alternate pathways for ethanol oxidation. It is possible that some rate-limiting component of non-ADH mediated alcohol clearance is maximally elevated in naive Adh^N/Adh^N animals, but not in naive Adh^F/Adh^F mice. Supporting this contention are the measurements of hepatic MEOS in naive *P. maniculatus* of both strains, where the propanol-oxidizing activity is 2.3-fold higher per mg of microsomal protein in Adh^N/Adh^N than in Adh^F/Adh^F mice. However, ethanol feeding further increases *in vitro* hepatic MEOS of both strains approximately 3-fold, but this alteration is not reflected in Adh^N/Adh^N BEER. *In vivo* MEOS may be further rate-limited by a second factor which is present in excess in *in vitro* MEOS assay.

Since a definite pathway for MEOS has not been established, the nature of possible *in vivo* rate-limiting factors is not known. The observation that cytochrome P-450, NADPH-cytochrome *c* reductase and phospholipid increase in hepatic microsomes of ethanol-fed rats [32, 33] has not been confirmed in *P. maniculatus* in the present work, despite elevation of *in vitro* MEOS in ethanol-fed Adh^N/Adh^N animals to levels at least 2- to 3-fold higher than previously reported in the literature. It is unlikely, then, that any of these components of the drug-metabolizing system limit the hepatic MEOS. In fact, Cederbaum *et al.* [34] have demonstrated dissociation of MEOS from drug metabolism by the use of potent hydroxyl radical scavengers which inhibit alcohol oxidation without affecting aniline hydroxylase or aminopyrine demethylase activity. This suggests that rate-limiting factors which may be explored further with MEOS activity *in vitro* and *in vivo* are the rate of generation of hydroxyl radicals by the microsomal electron transport pathway and the supply of NADPH for oxidation by this pathway.

An interesting aspect of MEOS activity with ethanol is that azide inhibits the Adh^F/Adh^F animals by 50 per cent, whereas microsomes from Adh^N/Adh^N animals retain more than 80 per cent of their ethanol-oxidizing activity in the presence of this catalase inhibitor (Table 3). Microsomes of Adh^F/Adh^F animals do not contain significantly elevated catalase (Table 2). These results suggest that a greater proportion of ethanol oxidation due to noncatalase-mediated oxidation is found in microsomes of Adh^N/Adh^N animals than in microsomes of Adh^F/Adh^F animals. Although ethanol elimination in Adh^N/Adh^N animals in the presence of azide is 3-fold greater than in Adh^F/Adh^F animals, the increase in propanol oxidation is less than 2-fold. This suggests that some portion of the elevated MEOS activity in Adh^N/Adh^N animals may operate specifically, or at least preferentially, on ethanol and not on propanol. This result requires further experimentation for clarification.

These studies in *P. maniculatus* demonstrate that, at high blood ethanol concentrations, non-ADH mediated pathways may account for as much as two-thirds of the elimination of ethanol. The presence of catalase and ADH-independent MEOS activity *in vitro* is not accompanied by significant quantitative alterations in the major components of the microsomal electron transport pathway. Further, when ethanol-fed, both strains of *P. maniculatus* have increased *in vitro* MEOS activities, but BEER increases only in the Adh^F/Adh^F strain and remains unchanged in ADH-negative mice. This result strongly suggests that BEER increases, following chronic ethanol consumption, are mediated primarily via the ADH pathway. However, the higher MEOS activity in naive Adh^N/Adh^N mice than in their Adh^F/Adh^F counterparts indicates that non-ADH mediated ethanol elimination may already be induced in the ADH-negative strain. Further, ethanol-induced MEOS increases might not be reflected in Adh^N/Adh^N BEER due to other, non-

inducible factors which may be rate limiting for MEOS *in vivo*. It is evident, though, that simultaneous increases in BEER and *in vitro* MEOS activity cannot be taken as evidence in favor of a contribution of non-ADH mediated pathways to the induction of ethanol clearance rates by ethanol consumption.

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