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ETHANOL METABOLISM *IN VIVO* BY THE MICROSOMAL ETHANOL-OXIDIZING SYSTEM IN DEERMICE LACKING ALCOHOL DEHYDROGENASE (ADH)

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Abstract—To assess the importance of non-ADH ethanol metabolism, ADH-negative and ADH-positive deermice were fed liquid diets containing ethanol or isocaloric carbohydrate for 2–4 weeks. Blood ethanol disappearance rate increased significantly after chronic ethanol feeding in both strains. Although at low ethanol concentrations (between 5 and 10 mM) there was no significant difference between ethanol-fed and pair-fed control animals, at high ethanol concentrations (between 40 and 70 mM) blood ethanol elimination rates were increased significantly after chronic ethanol feeding in both ADH-positive and ADH-negative animals. There was no significant effect of the catalase inhibitor 3-amino-1,2,4-triazole on the ethanol elimination rates in both strains. Whereas catalase and ADH activities were not altered after chronic ethanol treatment, the activity of the microsomal ethanol-oxidizing system (MEOS) was enhanced three to four times in both strains, and microsomal cytochrome P-450 content was also increased significantly. When MEOS activity was expressed per cytochrome P-450 content, it was higher in ADH-negative than in ADH-positive animals, and it increased after ethanol administration. When microsomal proteins were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, ethanol-fed animals had a distinct band which reflected the increase in microsomal cytochrome P-450 content and seemed to reflect a unique form of cytochrome P-450 induced by ethanol. Thus, despite the absence of the ADH pathway, a large amount of ethanol was metabolized by MEOS in ADH-negative deermice; this was associated with increased blood ethanol elimination rates, enhanced MEOS activity, and quantitative and qualitative changes of cytochrome P-450.

It is generally agreed that the liver is the main organ for oxidation of ethanol *in vivo* and that ethanol metabolism proceeds primarily via alcohol dehydrogenase (ADH), which is located in the liver supernatant fraction (cytosol). Almost 2 decades ago, Iseri *et al.* [1] reported that, in rats, ethanol feeding results in a proliferation of the smooth endoplasmic reticulum (SER). This observation raised the possibility that ethanol may also be metabolized by the hepatic microsomes. A microsomal system capable of ethanol oxidation was described [2], but its rate was very low and it was sensitive to catalase inhibitors; it was concluded by Ziegler [3] that this system is clearly different from the usual microsomal drug-metabolizing systems and involves the H_2O_2-mediated ethanol peroxidation by catalase. However, Lieber and DeCarli [4] described a system in hepatic microsomes that oxidizes ethanol to acetaldehyde in the presence of reduced nicotinamide adenine dinucleotide (NADPH) and oxygen, at a rate ten times higher than that reported by Orme-Johnson and Ziegler [2]. Furthermore, unlike the system of Orme-Johnson and Ziegler, this microsomal ethanol-oxidizing system (MEOS) of Lieber and DeCarli [5] also oxidizes higher alcohols, such as butanol, which are not substrates for catalase. These observations suggested the existence of a new and distinct ethanol-oxidizing system, but the mechanism of this reaction is still the subject of controversy [5, 6].

Lieber and DeCarli [5] reported that the MEOS can be distinguished from ADH by its subcellular localization (cytosol for ADH, microsomes for this system), its pH optimum (physiological pH vs pH 10–11 for ADH), its cofactor requirement (NADPH vs NAD^+ for ADH), and its requirement for oxygen and inhibition by CO, properties commonly found among microsomal drug-metabolizing enzymes. In 1972 Teschke *et al.* [7] and in 1973 Mezey *et al.* [8] reported the separation of MEOS from ADH and catalase using DEAE cellulose column chromatography. Ohnishi and Lieber [9] then reconstituted the MEOS using cytochrome P-450, NADPH–cytochrome *c* reductase and a synthetic phospholipid; they showed that the partially purified cytochrome P-450 from ethanol-fed rats was more active for ethanol oxidation than the control preparations. Miwa *et al.* [10] also reported the oxidation of ethanol by a catalase- and ADH-free reconstituted system containing purified P-450 from phenobarbital-

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treated rats, and a unique ethanol induced form has now been purified in the rabbit [11].

Although the existence of MEOS as an ethanol-oxidizing system distinct from catalase and ADH is now established *in vitro*, its role *in vivo* is still controversial, because thus far studies of this question relied largely on experiments with inhibitors. However, recently a new model for the assessment of non-ADH pathways became avaliable, when Burnett and Felder [12] described a strain of deermice, *Peromyscus maniculatus*, lacking ADH activity genetically. Burnett and Felder reported that, despite the absence of ADH, these animals consume ethanol when administered in drinking water. The amounts consumed, however, were relatively small. As with other animal species, deermice have an aversion for ethanol when given in drinking water. We succeeded in overcoming this natural aversion for ethanol by administering ethanol as part of a totally liquid diet similar to the one we have been using for the last 20 years in rats and baboons. Under these donditions, deermice were found to ingest large amounts of ethanol, even though they lacked ADH. We report here that under these conditions ethanol metabolism in deermice was strikingly increased, in association with unchanged or even decreased catalase and ADH activity but a rise in MEOS activity, which illustrates the *in vivo* importance of MEOS as a non-ADH ethanol-metabolizing pathway.

MATERIALS AND METHODS

Materials. The chemicals and enzymes were purchased from the following companies: NAD (Grade III), NADP monosodium salt, DL-isocitric acid trisodium salt, isocitric dehydrogenase (Type 1), Coomassie Brilliant Blue R and bovine albumin (Fraction V) from the Sigma Chemical Co., St. Louis, MO; perchloric acid (70%), hydrogen peroxide (30%) and sodium azide from the Fisher Scientific Co., Fair Lawn, NJ; sodium dithionite and 3-amino-1,2,4-triazole from the Aldrich Chemical Co., Milwaukee, WI; ethanol dehydrated from Publicker Industries Co., Linfield, PA; acrylamide and Bis from Bio-Rad Laboratories, Richmond, CA; 2-mercaptoethanol from the Eastman Kodak Co., Rochester, NY; and sodium dodecyl sulfate (SDS) from BDH Chemical Ltd., Poole, England.

Animals and diets. Animals used in the present studies had the following genotype for the liver alcohol dehydrogenase (ADH): ADH^F/ADH^F (ADH-positive) and ADH^N/ADH^N (ADH-Negative). The ADH-positive strain had a normal liver ADH activity while the ADH-negative strain had no detectable ADH activity on zymograms or in spectrophotometric assays and, moreover, exhibited no antigenically cross-reacting material in immunochemical tests employing monospecific anti-ADH antisera [12–14]. At first the animals were fed chow and tap water *ad lib.* until the liquid diet was started, at which time they were housed in individual plastic cages with Aspen shavings. They were pair-fed nutritionally adquate liquid diets containing ethanol and isocaloric carbohydrates [15]. The liquid diets were purchased from the Bio-Serv Co., Frenchtown, NJ. Ethanol concentration was increased gradually from 1% (w/v) to 3–5%. The lights of the animal room were switched on and off at 7:00 a.m. and 7:00 p.m. automatically.

Ethanol elimination study. To study rates of blood ethanol elimination, we applied to the deermouse [16] the air bubble technique that Lester *et al.* [17] had developed in the rat for the measurement of blood ethanol disappearance. Ethanol (3 g/kg body weight) was injected intraperitoneally, and then an air bubble was made on the back of the animal by injecting air subcutaneously. Every 30 min a 0.5 or 1.0 ml sample from the air sac was drawn and analyzed for ethanol content by head-space gas–liquid chromatography [18]. An excellent correlation between blood and air-sac ethanol concentration had been documented before [16]. Rates of ethanol disappearance were calculated per kg of body weight, using a body water to total-weight ratio of 0.7, a factor which was experimentally verified in this species. When indicated, ADH-positive ethanol-fed and chow-fed deermice were pretreated with 3-amino-1,2,4-triazole (aminotriazole) (1 g/kg body weight i.p., in physiological saline) 6 hr before the ethanol elimination study. A week prior to this aminotriazole pretreatment study some animals were subjected to the same blood ethanol elimination studies after injection of physiological saline solution as a control.

Daily urinary excretion of ethanol in ethanol-treated animals was measured by collecting urine in metabolic cages. The ethanol output was calculated from daily urinary volume and the ethanol concentration of the urine. Respiratory losses of ethanol were calculated from the total amounts of ethanol in the air of sealed oxygen containing flasks in which the animals were maintained for 5 min (with blood ethanol levels between 40 and 70 mM).

Enzyme assays. Animals were killed by cervical dislocation immediately after the blood elimination study or on the following morning. Their livers were excised, and 5% homogenates were prepared with 1.15% KCl solution. The following procedures were carried out at 0–4°: the liver homogenate was centrifuged at 10,000 *g* for 20 min. The supernatant fraction was centrifuged at 105,000 *g* for 1 hr. The resulting supernatant fraction (cytosol) was used as enzyme source for ADH assay, whereas the pellet was resuspended and washed in 1.15% KCl solution; microsomal pellets were obtained by spinning down this suspension at 105,000 *g* for another 1 hr. Microsomes were suspended in 0.1 M phosphate buffer (pH 7.4) and stored at −80°.

Cytochrome P-450 contents were measured in total homogenates and microsomes according to Matsubara *et al.* [19] and Omura and Sato [20] respectively. The preparative losses of microsomes were corrected for according to the method of Greim [21]. Cytosolic ADH activity was determined by the method of Bonnichsen and Brink [22], modified as follows: final ethanol concentration was 50 mM, and 3 mg NAD was used per cuvette. Catalase activity was measured according to the method of Lück [23], as modified by Cohen *et al.* [24]; the homogenate was incubated with 100 mM ethanol for 30 min in order to stabilize catalase in its active form. MEOS activity was determined by the method of Ohnishi and Lieber [18] using head-space gas–liquid chromatography.

The apparent K_m of MEOS was determined in ADH-positive and ADH-negative naive animals, by constructing Lineweaver–Burk plots.

SDS-polyacrylamide gel electrophoresis. Solubilized microsomal proteins of deermice were analyzed by electrophoresis in a sodium dodecyl sulfate (SDS) containing polyacrylamide gel system according to the method of Laemmli [25]. In this study, in addition to the ethanol-fed and the corresponding control animals, chow-fed male deermice of ADH-positive and -negative strains were also used. They were divided into three groups: untreated, phenobarbital-treated (80 mg/kg body wt, i.p., in physiological saline, 3 days) and 3-methylcholanthrene-treated (25 mg/kg body wt, i.p., in corn oil, 3 days). The microsomal samples were suspended in 25 mM Tris/HCl (pH 6.8) containing 1% SDS and 5% 2-mercaptoethanol, treated at 100° for 2 min, and cooled to room temperature. The separating gel (1.5 mm thick) contained 7.5% acrylamide, 0.375 M Tris/HCl (pH 8.8) and 0.1% SDS. The stacking gel contained 3% acrylamide, 0.125 M Tris/HCl (pH 6.8), and 0.1% SDS. Twelve micrograms of protein was electrophoresed at 15°. The gel was fixed, stained with Coomassie Brilliant Blue R, destained, and dried on thick filter paper.

Electron microscopy. For electron microscopy a piece of liver was immersed in 2.5% glutaraldehyde in cacodylate buffer (pH 7.4), followed by fixation in 2% osmium tetroxide. After dehydration the specimen was embedded in Epon 812 sectioned with an LKB IV ultramicrotome and examined under a Zeiss 10C electronmicroscope.

Statistical analysis. Results are expressed as mean ± S.E.M., and the significance of the difference between experimental and control values was assessed by paired or group Student's *t* tests.

RESULTS

Ethanol consumption. Both the ADH-positive and the ADH-negative strains of deermice consumed relatively large amounts of ethanol when the latter was given in a liquid diet. As shown in Table 1, the intake of ethanol increased considerably after chronic ethanol feeding as we increased the concentration of ethanol from 1% (w/v) to 3–5% gradually. Surprisingly, the ethanol intake exceeded 20 g per kg body weight per day after 2–4 weeks of chronic ethanol feeding even in ADH-negative animals. We did not find appreciable leakage of diets or loss of ethanol by evaporation. Urinary and respiratory losses of ethanol amounted to about 5 and 10% of total intake, respectively. The blood ethanol levels of ethanol-fed animals (measured randomly between 9:00 a.m. and 2:00 a.m.) varied from 10 to 70 mM in ADH-negative and from 10 to 35 mM in ADH-positive deermice.

Table 1. Ethanol intake by ADH-positive and ADH-negative deermice*

	Ethanol intake [g·(kg body wt)$^{-1}$·day^{-1}]	
	After 3–5 days	After 2–4 weeks
ADH$^-$	15.5 ± 1.8	27.7 ± 1.4
ADH$^-$	13.3 ± 1.4	22.7 ± 1.2

* Data are means ± S.E.M.; N = 11. Absolute ethanol amounts were calculated from the daily dietary intake.

Blood ethanol elimination study. The maximal blood ethanol levels were reached within 30 min after injection of 3 g/kg body wt of ethanol. From the elimination curve of each animal, rates of ethanol disappearance from the blood were calculated both at high concentrations (between 40 and 70 mM) and at low concentations (between 5 and 10 mM). Figure 1 shows the mean values (±S.E.M.) in ethanol-fed and pair-fed control ADH-positive and ADH-negative deermice. In ADH-positive deermice, the blood ethanol disappearance rate, when measured at high concentrations, was significantly higher in ethanol-fed animals (262 ± 24 μmoles per kg body wt per min) than in pair-fed controls (198 ± 9; $P < 0.0125$; eight pairs). Similarly, in ADH-negative deermice, the rates at high concentrations of ethanol-fed animals were also significantly higher (168 ± 9) compared to pair-fed controls (132 ± 8; $P < 0.05$; 7 pairs). These high ethanol concentrations (40–70 mM) would be expected to saturate all ethanol-metabolizing pathways including MEOS. The rate of ethanol disappearance increased significantly after chronic ethanol feeding in ADH-positive as well as ADH-negative animals. By contrast, at low ethanol concentration (5–10 mM), there were no significant differences in elimination rates between ethanol-fed and pair-fed animals in both ADH-positive and ADH-negative strains.

To determine how much catalase activity is involved in ethanol oxidation *in vivo* in deermice, 1 g/kg body wt of aminotriazole was injected 6 hr before the ethanol elimination study. A week before this aminotriazole treatment study, the same animals underwent the same ethanol elimination study with injection of physiological saline instead of aminotriazole. In the ADH-positive chow-fed deermice at high ethanol concentrations (between 40 and 70 mM), aminotriazole-treated animals eliminated ethanol at a rate not significantly different than controls (197 ± 14 μmoles per kg body wt per min vs 191 ± 9). Also in ADH-negative chow-fed deermice, there was no significant difference (107 ± 4 in controls vs 94 ± 2). In ADH-positive ethanol-fed deermice, there was no significant difference in ethanol elimination rates after aminotriazole treatment (220 ± 17 in controls vs 262 ± 25). The liver catalase activities were inhibited by 90–95%, when measured at the end of the elimination studies.

Alcohol dehydrogenase (ADH). In ADH-positive animals, hepatic ADH activities decreased significantly after chronic ethanol feeding (1.31 ± 0.09 μmoles NAD reduced per g liver per min to 0.87 ± 0.16; $P < 0.05$) (Fig. 2). Expressed in μmoles per kg body wt per min, the corresponding values were 70.2 ± 25.4 and 41.4 ± 21.1 respectively. The ADH activity was completely absent, as expected, in ADH-negative animals, even after chronic ethanol consumption (Fig. 2).

Catalase. Catalase activity was not changed significantly after chronic ethanol consumption in either ADH-positive or ADH-negative animals. In ADH-negative naive animals, catalase activity was half of

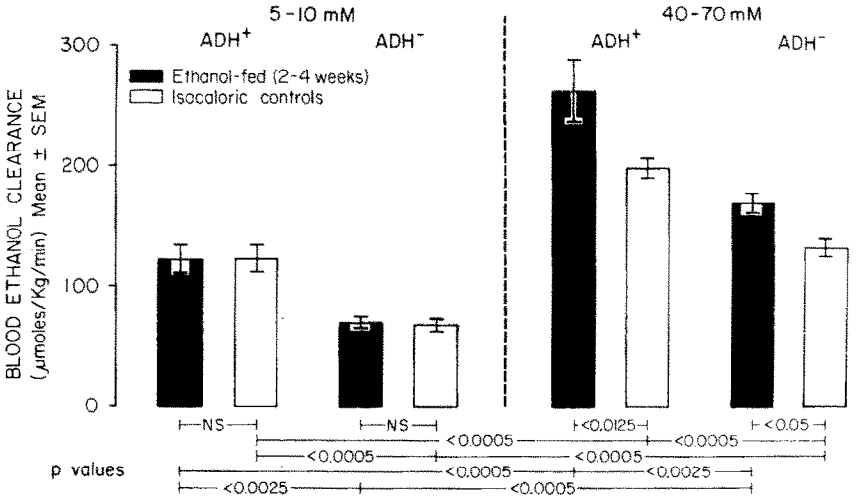

Fig. 1. Effect of chronic ethanol feeding on blood ethanol elimination rate in ADH-positive and ADH-negative deermice given ethanol. Ethanol, 3 g/kg of body weight (10% w/v solution), was injected; each blood ethanol elimination curve was plotted, and elimination rates were calculated at 5–10mM and 40–70 mM in each ethanol-fed and pair-fed control animal of both strains.

that in ADH-positive naive animals but there was a trend towards an increase after chronic ethanol consumption (Fig. 2).

Microsomal ethanol-oxidizing system (MEOS) and cytochrome P-450. MEOS activity was significantly higher in the ethanol-fed animals, especially in ADH-negative deermice (Fig. 2). When expressed per mg microsomal protein, the mean value was 5.93 ± 0.73 nmoles acetaldehyde formed/min in ADH-positive naive controls, 19.45 ± 3.28 in ADH-positive ethanol-fed animals, 10.12 ± 1.86 in ADH-negative naive controls, and 34.56 ± 3.52 in ADH-negative ethanol-fed animals respectively. Expressed as μmoles per kg body wt per min, the corresponding values were 13.6 ± 4.9 and 40.1 ± 12.5 in ADH-positive naive controls and ethanol-fed animals, respectively, and 22.9 ± 7.3 and 107.0 ± 37.1 in ADH-negative naive controls and ethanol-fed animals respectively. Interestingly, the activity of MEOS in ADH-negative naive animals was higher than in the ADH-positive naive animals ($P < 0.01$). Moreover, the increase of MEOS activity in ADH-negative animals after chronic ethanol consumption was almost 3-fold. The increase of MEOS activity was associated with an increase in cytochrome P-450 content. Cytochrome P-450 contents were 0.65 ± 0.05 nmoles/mg protein in ADH-positive pair-fed controls, 1.23 ± 0.19 in ADH-positive ethanol-fed animals, 0.56 ± 0.11 in ADH-negative pair-fed controls, and 1.69 ± 0.16 in ADH-negative ethanol-fed animals. When cytochrome P-450 content was expressed per gram of liver, it also increased significantly after chronic ethanol feeding in both ADH-positive and ADH-negative strains (Fig. 3). The increase

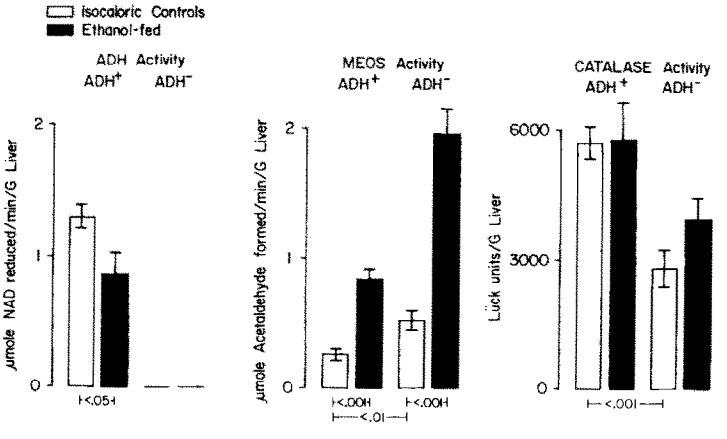

Fig. 2. Effect of chronic ethanol consumption on the activities of hepatic ADH, the microsomal ethanol-oxidizing system (MEOS), and catalase in ADH-positive and ADH-negative deermice. Activities were measured in chronic ethanol-fed and pair-fed control deermice of both strains. The values are expressed as means ± S.E.M. (per g of liver).

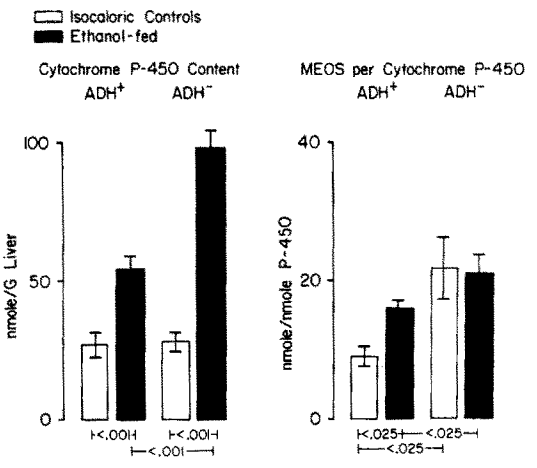

Fig. 3. Effect of chronic ethanol consumption on the microsomal cytochrome P-450 content and the activity of the microsomal ethanol-oxidizing system (MEOS) in ADH-positive and ADH-negative deermice. MEOS activity is expressed per cytochrome P-450 content. The data represent means ± S.E.M.

of cytochrome P-450 content in ADH-negative animals was almost 300% after chronic ethanol consumption.

To assess the capacity of the cytochrome P-450 to sustain ethanol oxidation, MEOS activities were also expressed per cytochrome P-450 content (Fig. 3): this ratio was the highest in the ADH-negative animals and, after chronic ethanol consumption, this ratio increased significantly in ADH-positive animals.

SDS-polyacrylamide gel electrophoresis. Solubilized microsomal proteins of ADH-positive and ADH-negative deermice (both ethanol-fed and pair-fed control animals) were analyzed by electrophoresis (Fig. 4). At the same time, the microsomes of phenobarbital (PB)- or 3-methylcholanthrene (MC)-treated deermice were also subjected to protein electrophoresis. We found no significant difference in patterns between ADH-positive and ADH-negative strains. However, the microsomal fraction from ethanol-fed deermice contained several strongly stained bands, one of which was easily distinguished from control preparations or from the bands seen in microsomal preparations from PB- or MC-treated deermice.

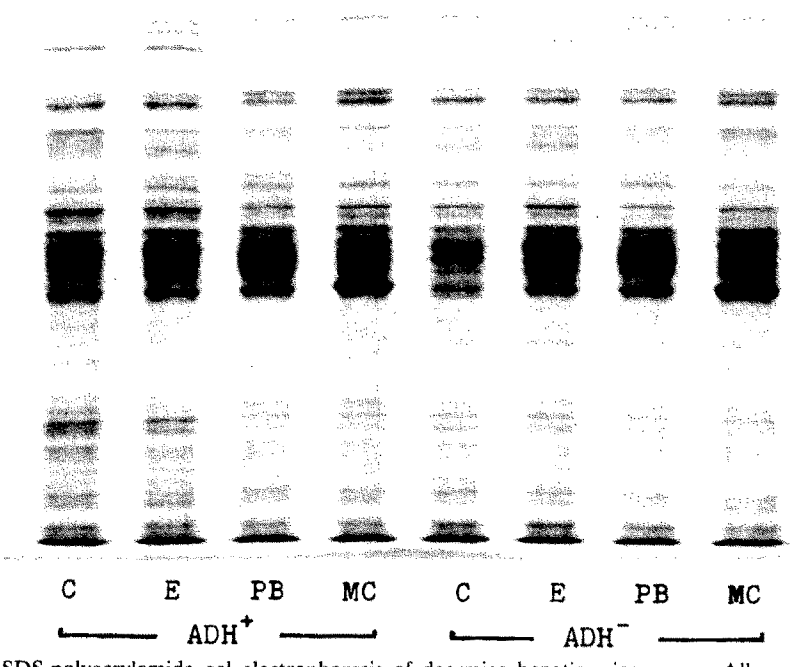

Fig. 4. SDS-polyacrylamide gel electrophoresis of deermice hepatic microsomes. All samples were treated and electrophoresed by the method of Laemmli [25]. Wells marked ADH+ contain microsomes from ADH-positive deermice, whereas wells marked ADH- contain microsomes from ADH-negative deermice. Key: C, pair-fed controls; MC, 3-methylcholanthrene-treated deermice, PB, phenobarbital-treated deermice; and E, ethanol-treated deermice.

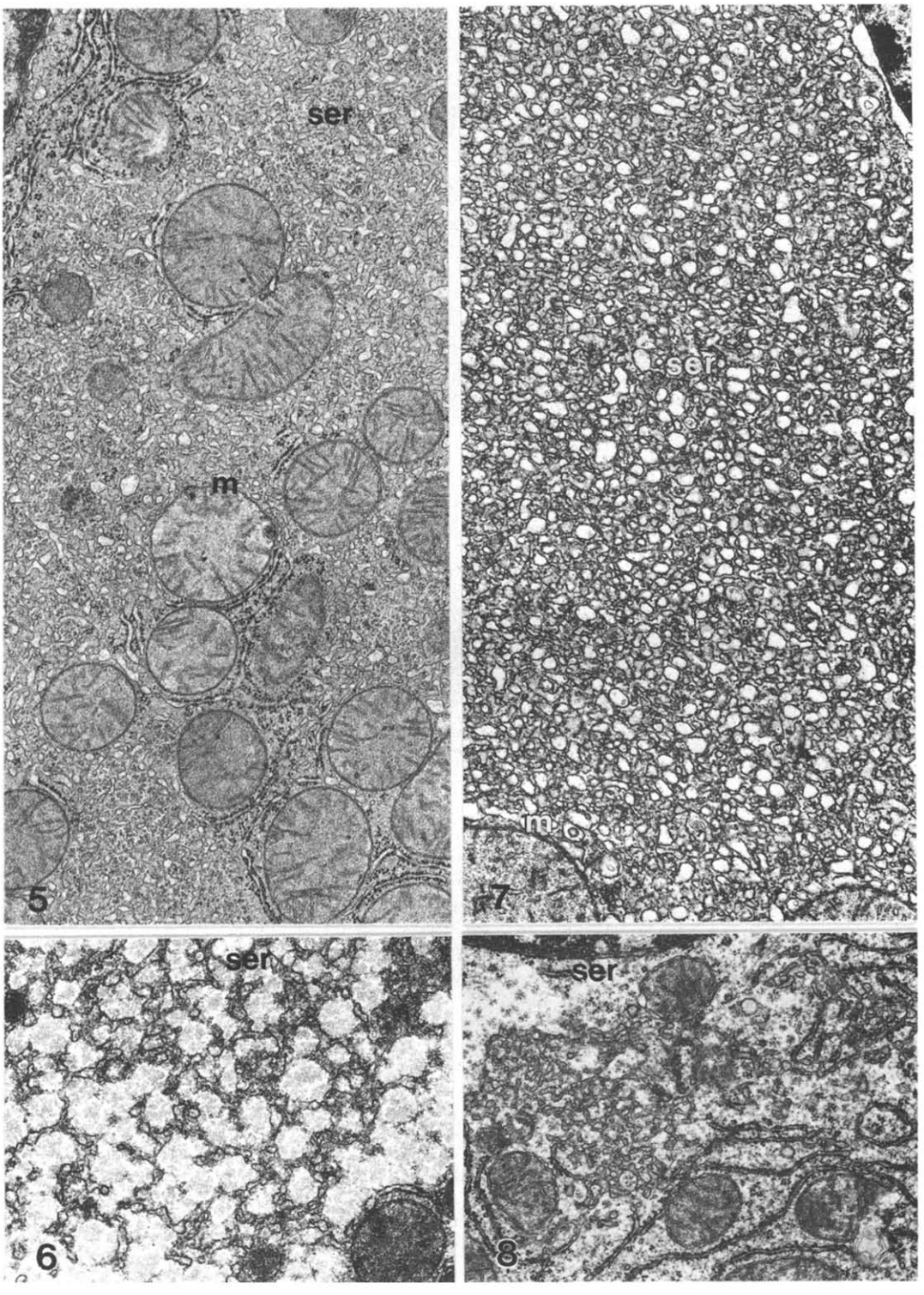

Fig. 5–8. Electronmicrographs of ADH-positive (ethanol-fed), ADH-positive (control), ADH-negative (ethanol-fed) and ADH-negative (control) deermice respectively. After chronic ethanol consumption, the liver became literally packed with the proliferated membranes of the smooth endoplasmic reticulum (SER), particularly in ADH-negative deermice (Fig. 7). Mitochondria (M) are also seen.

K_m *of MEOS.* The apparent K_m of *in vitro* MEOS was measured by constructing a Lineweaver–Burk plot. The mean values were 12.9 mM in the ADH-positive chow-fed deermice and 13.2 mM in the ADH-negative chow-fed deermice. In some aminotriazole-treated animals, the kinetics of blood ethanol disappearance *in vivo* were determined as described by Makar and Mannering [26]. The blood ethanol concentration curve was used to construct a Lineweaver–Burk plot from which the apparent K_m *in vivo* was derived as described previously for the rat [27]. These apparent K_m values *in vivo* were 8.4 mM in ADH-positive and 8.7 mM in ADH-negative aminotriazole-treated deermice respectively.

Ultrastructure. The induction of MEOS activity in the microsomes of deermice by chronic ethanol consumption had its morphologic counterpart (Figs. 5–8). Even in the naive ADH-negative deermice, hepatic smooth endoplasmic reticulum (SER) was somewhat abundant compared to the ADH-positive animals. The most striking change was noted after chronic ethanol consumption, particularly in ADH-negative deermice. Their livers became literally packed with the proliferated membranes of the smooth endoplasmic reticulum (Fig. 7).

DISCUSSION

This report demonstrates the role of a non-ADH ethanol-metabolizing pathway, the microsomal ethanol-metabolizing system (MEOS), in ethanol metabolism *in vivo.* The proposal by Lieber and DeCarli [4, 5, 27] that this system is distinct from ADH and catalase and plays a role in ethanol metabolism has been the subject of a long standing controversy. Because ADH-negative deermice have no liver ADH activity whatsoever, they lend themselves to the study of non-ADH mediated ethanol metabolism. It is noteworthy that, despite the total absence of ADH activity, the ADH-negative deermice consumed ethanol at a rate almost two-thirds that of ADH-positive deermice. At low ethanol concentrations, blood ethanol elimination rates did not change after chronic ethanol consumption both in ADH-positive and ADH-negative animals. On the other hand, at high ethanol concentrations, they increased significantly both in ADH-positive and ADH-negative animals. These results suggest that a high K_m system is present which can be "induced" by chronic ethanol consumption. In ADH-negative animals fed ethanol chronically, blood ethanol disappearance rates reached a level comparable to that of naive ADH-positive animals. Nevertheless, ADH-negative deermice had lower catalase activity than ADH-positive deermice. Moreover, administration of aminotriazole, which blocks the catalase activity *in vivo*, had no signficant effect on blood ethanol elimination in ADH-positive and ADH-negative deermice, although at the dose used it effectively inhibited catalase activity. These results suggest that catalase does not participate significantly in ethanol metabolism *in vivo* in those deermice.

In ADH-positive deermice, liver ADH activities decreased significantly after chronic ethanol consumption as was reported before by us in rats [5] and baboons [28].

Wendell and Thurman [6] described an adaptive increase of ethanol elimination rates after chronic ethanol feeding in rats. They reported that NADH-reoxidation is rate-limiting for ethanol utilization at the low dose whereas the activity of ADH becomes limiting at high dose and after pretreatment with ethanol in the fed state *in vivo*, and they incriminated both ADH and catalase-H_2O_2 dependent components for this enhancement of ethanol elimination. It is obvious, however, that in ADH-negative deermice such a mechanism must be reconsidered, and it is most likely, therefore, that MEOS must play an important role in ethanol metabolism, at least in the ADH-negative strain. It is apparent from many studies that the mechanism of MEOS is similar to that of microsomal drug metabolism and involves cytochrome P-450 [5, 29]. Ohnishi and Lieber [9] reported that a reconstituted system consisting of partially purified cytochrome P-450 from ethanol-fed rats, NADPH–cytochrome *c* reductase, and synthetic phospholipid not only metabolizes benzphetamine, a substrate for microsomal cytochrome P-450, but also oxidizes ethanol, propanol and butanol, and that the ethanol oxidation depends on the concentration of cytochrome P-450 and NADPH–cytochrome *c* reductase.

It is now well recognized that there are many types of cytochrome P-450 in rats and rabbits. We have shown before that chronic ethanol consumption induces a form of cytochrome P-450 in rats which is distinct from those induced by phenobarbital or 3-methylcholanthrene, and which has a higher capacity to sustain oxidation in a reconstituted system [9]. A similar system appears to operate in the deermice. Indeed, MEOS activity increased significantly after chronic ethanol consumption whether expressed per mg of microsomal protein, per g of liver or per 100 g body weight, and its increase paralleled that of cytochrome P-450, especially in ADH-negative animals. When the MEOS activity was expressed per cytochrome P-450 content, it increased after chronic ethanol consumption in ADH-positive animals and reached a level comparable to that in ADH-negative animals; the latter seemed to have the most effective form of cytochrome P-450 in terms of ethanol oxidation. The results of SDS-gel electrophoresis suggest that, in deermice, there are ethanol-inducible forms of cytochrome P-450, as shown before for the rat [9] and rabbit [11]. Efforts to purify this form in the deermice are presently underway.

The observation that the daily average blood ethanol levels in ADH-negative ethanol-fed animals were about 40 mM indicates that ADH-negative ethanol-fed animals maintain ethanol at a level sufficient to effectively saturate MEOS, which was found to have an *in vitro* K_m of about 13 mM. In baboon studies, Pikkarainen and Lieber [30] and Nomura *et al.* [31] reported concentration-dependent ethanol elimination rates with kinetics which suggested that a non-ADH, high K_m ethanol-metabolizing system, such as MEOS, significantly contributes to ethanol oxidation *in vivo*, at high blood ethanol concentrations comparable to those we found in ADH-negative animals. As for other membrane bound systems, it is difficult to extrapolate quantitatively from *in vitro* activity to *in vivo* metabolism.

Furthermore, even in ADH-negative deermice, some ethanol elimination persisted at low ethanol concentrations (Fig. 1). This was apparently unaffected by alcohol feeding; a non-ADH, non-MEOS mechanism may be involved, including, at least in part, urinary and pulmonary losses.

In conclusion, in ADH-negative deermice, substantial ethanol metabolism and therefore substantial ethanol consumption can be sustained by non-ADH pathways, particularly after chronic ethanol consumption, most likely because of a striking adaptive increase in activity of the microsomal ethanol-oxidizing system.

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