Biochemical Pharmacology, Vol. 45, No. 10, pp. 1989–1994, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

DEHYDROGENASE-DEPENDENT METABOLISM OF ALCOHOLS IN GASTRIC MUCOSA OF DEER MICE LACKING HEPATIC ALCOHOL DEHYDROGENASE

GUNILLA EKSTRÖM, TOMAS CRONHOLM, CARINA NORSTEN-HÖÖG and MAGNUS INGELMAN-SUNDBERG*

Department of Physiological Chemistry, Karolinska Institutet, S-104 01 Stockholm, Sweden

(*Received* 28 *August* 1992; *accepted* 23 *February* 1993)

Abstract—Deer mice (*Peromyscus maniculatus*) lacking hepatic alcohol dehydrogenase (ADH) have been used as a model for studies of ethanol elimination catalysed by non-ADH systems like catalase and cytochrome P450. However, in an *in vivo* study on these animals (ADH^- deer mice), we detected reversibility in the oxidation of [^{2}H]ethanol, indicating that a major part of the ethanol elimination was due to a dehydrogenase (Norsten *et al.*, *J Biol Chem* **264**: 5593–5597, 1989). In the present investigation, we found significant ethanol oxidizing activity in the gastric mucosa of the deer mice. Reversibility was demonstrated by the use of [^{2}H]acetaldehyde and gas chromatography–mass spectrometry of the products. The kinetic ^{2}H isotope effect of the gastric system was about 3.0 and the system was comparatively insensitive to inhibition by 4-methylpyrazole. The behavior of the deer mice gastric ADH in isoelectric focusing and its higher activity with longer alcohols as substrates indicated similarity with the previously described human class IV enzymes. Our data are in agreement with results obtained *in vivo* and indicate that ethanol is oxidized extrahepatically in ADH^- deer mice. This has to be taken into account when deer mice are used to study non-ADH-dependent ethanol oxidation *in vivo*.

The contribution of non-alcohol dehydrogenase (ADH†) pathways to ethanol elimination is still under debate. Systems under investigation as to their role in the oxidation of ethanol *in vivo* are cytochrome P450 [1, 2] and catalase [3]. Deer mice (*Peromyscus maniculatus*) of a strain lacking detectable ADH activity in the liver [4] have been used for a long time in studies of the relative contribution of non-ADH enzymes to the *in vivo* metabolism of ethanol. Results presented in favour of P450 as the major catalyst [5–9] include the fact that the rate of microsomal ethanol oxidation, when expressed in relation to the content of cytochrome P450 in the liver microsomal membrane, is higher in the strain of deer mice lacking ADH (ADH^-) than in those containing ADH (ADH^+) [5, 6]. The catalase inhibitor 3-amino-1,2,4-triazole has been described to be without effect on the rate of ethanol elimination in ADH^- deer mice [7]. Experiments with the ADH inhibitor 4-methylpyrazole *in vivo* and in isolated hepatocytes have suggested that P450 plays a major role in ethanol elimination in deer mice [8]. Such a conclusion has also been supported by calculations of isotope effects for ethanol elimination *in vivo* [9].

On the other hand, experiments with fructose and aminotriazole, added to inhibit peroxidation of ethanol via catalase-H_2O_2, have provided support for the importance of this enzyme system in ethanol elimination [10, 11]. Antibodies against ethanol-inducible cytochrome P450 from rabbit liver were used to identify a homologous protein in deer mice [12]. A comparison of the rates of microsomal ethanol oxidation *in vitro* with rates of ethanol elimination *in vivo* indicated that deer mouse P450 could account for only a minor part of total ethanol elimination in both strains. By contrast, peroxisomal β-oxidation capacity was increased by 40% over control values by ethanol treatment, consistent with the hypothesis that the increase in ethanol elimination in the ADH^- deer mice after chronic ethanol treatment is mediated predominantly via catalase-H_2O_2 [12].

In a previous study of the mechanism of ethanol elimination in deer mice, we found reversibility of ethanol oxidation *in vivo* using both ADH^+ and ADH^- animals. Deuterium from [1,1-$^2H_2$]ethanol was transferred to acetaldehyde at an equal rate in animals of both strains [13]. It could be calculated that ethanol elimination in the ADH^- deer mice, having a rate of about 50% of that in ADH^+ deer mice, was mainly catalysed by dehydrogenase systems. In addition, we found deuterium transfer between ethanol and butanol in ADH^- deer mice [14]. The inhibitory role of butanol on ethanol elimination in ADH^- deer mice has previously been taken as evidence for the involvement of P450 in this process [1]. Based upon butanol–butanal exchange in liver perfusion systems, we found almost no ADH activity in the liver of ADH^- deer mice [14].

In the present paper we addressed the question of the origin of the *in vivo* dehydrogenase activity previously found in ADH^- deer mice.

MATERIALS AND METHODS

Materials. [1,1-$^2H_2$]Ethanol (99.6% ^{2}H), [2,2,2-

* Corresponding author: Dr Magnus Ingelman-Sundberg, Department of Physiological Chemistry, Karolinska Institutet, P.O. Box 60 400, S-104 01 Stockholm, Sweden. FAX (46) 8-338453; Tel. (46) 8-7287735.

† Abbreviations: ADH, alcohol dehydrogenase; ADH^+ deer mice, deer mice lacking hepatic ADH; ADH^- deer mice, deer mice having hepatic ADH; GC–MS, gas chromatography–mass spectrometry.

$^2H_3$]ethanol (99.0% ^2H), [1,1,2,2,2-$^2H_5$]ethanol (99.2% ^2H) and [$^2H_4$]acetaldehyde (99.5% ^2H) were from Alfred Hempel GmbH & Co. (Düsseldorf, Germany). [1-^{13}C]Ethanol (90% ^{13}C) was from Merck Sharp and Dohme (Montreal, Canada). NAD^+, NADH and 4-methylpyrazole were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Dinitrophenylhydrazine and 3,5-dinitrobenzoyl chloride were obtained from Merck AG (Darmstadt, Germany) and recrystallized from ethanol and benzene, respectively. Calcium cyanamide was from the American Cyanamide Co (Wayne, NJ, U.S.A.) and semicarbazide from Merck AG.

Animals. Male deer mice (*P. maniculatus*), one strain with reported normal levels of hepatic ADH (*AdhF/AdhF*) (ADH^+) and one strain reported to lack hepatic ADH (*AdhN/AdhN*) (ADH^-), were kindly provided by Dr R. G. Thurman, University of North Carolina at Chapel Hill. These animals were established from breeding pairs supplied by Dr M. R. Felder of the University of South Carolina. The deer mice were fed chow diet and water *ad lib.*

Preparation of cytosol. The organs were quickly removed and rinsed in 1.15% KCl. All tissues were homogenized in four volumes of 1.15% KCl and centrifuged at 500 *g* for 10 min. The resulting supernatants were centrifuged at 105,000 *g* for 60 min. The protein concentration was determined according to the method of Lowry *et al.* [15].

Oxidation of alcohols. Incubations with alcohols were carried out in 25-mL stoppered tubes containing 5-mL tubes of reagent for derivatization of the aldehydes formed [16]. All incubation mixtures had a final volume of 2 mL and contained cytosol corresponding to 0.05–1 mg of protein, 2 mM calcium cyanamide, 2 mM NAD^+ and 0.25–500 mM alcohol in 0.1 M Na-glycine buffer, pH 10.0. The semicarbazone was quantitated spectrophotometrically at 224 nm [16].

Measurements of $^D(V/K)$ in vitro. Incubations with deuteriated ethanols (100 mM of 1:1 mixtures) contained cytosol corresponding to 1 mg of protein and 6 mM NAD^+ in 0.1 M Na-glycine buffer, pH 10.0. The 5-mL tubes contained 10 mM 2,4-dinitrophenylhydrazine in 1 mL of dimethylformamide and 5 μg of butyraldehyde as internal standard [17]. GC–MS analysis and calculations of the $^D(V/K)$ values were performed as described by Ekström *et al.* [17].

Reduction of [$^2H_4$] *acetaldehyde.* Incubations were carried out with cytosol prepared from gastric mucosa from both strains of animals, for 15 min at 37°. The reaction mixtures contained cytosol corresponding to 1 mg of protein, 5 mM calcium cyanamide, 2 mM NADH and 6.5 mM freshly distilled [$^2H_4$]acetaldehyde in 50 mM potassium phosphate buffer, pH 7.4. [1,1-$^2H_2$]Ethanol was added as internal standard after termination of the incubations with perchloric acid and the ethanols were derivatized with 3,5-dinitrobenzoyl chloride and analysed by GC–MS [18].

Reduction of unlabeled acetaldehyde. The acetaldehyde-dependent oxidation of NADH was followed at 340 nm at 25°. The cuvettes contained cytosol corresponding to 0.1 mg of protein, 100 mM acetaldehyde and 0.5 mM NADH in 0.1 M potassium phosphate buffer, pH 7.5.

Isoelectric focusing. Isoelectric focusing was carried out in a Phast system (Kabi-Pharmacia, Sweden) at a pH range of 3–9. Cytosol from gastric mucosa (30 μg of protein) or liver (60 μg of protein) was applied.

ADH activity staining. The gels were stained for activity with 35 mM ethanol or pentanol in 0.1 M Tris–HCl, pH 8.5, containing 0.078 mM phenazine metosulfate, 0.390 mM nitroblue tetrazolium and 1.1 mM NAD^+ at 40° for 60 min in the dark [19].

Table 1. Ethanol oxidation in cytosol prepared from different organs from two strains of deer mice

Cytosol origin	Acetaldehyde production	
	nmol/mg, min	μmol/hr
ADH^+ deer mice		
Gastric mucosa	14.7 ± 4.4 (4)	0.20
Liver	18.3 ± 5.3 (8)	2.7
ADH^- deer mice		
Gastric mucosa	17.0 ± 3.8 (4)	0.22
Liver	1.6 ± 2.0 (5)	0.24

The incubations contained cytosol corresponding to 1 mg of protein, 37.5 mM ethanol and 2 mM NAD^+ in 0.1 M Na-glycine buffer, pH 10.0.

The cytosol preparations were obtained from individual animals (liver) or from four animals (gastric mucosa).

The mean ± SD is given from the number of experiments shown in parentheses.

RESULTS

ADH activity in cytosol from deer mice tissues.

Cytosol preparations from liver and gastric mucosa obtained from ADH^+ and ADH^- deer mice were evaluated for the presence of ADH by measurement of acetaldehyde formation from ethanol (37 mM). Cyanamide was added to prevent further oxidation of acetaldehyde [20]. As seen in Table 1, the ADH activity, on a protein basis, was about the same in cytosol from the gastric mucosa from both strains and in liver cytosol from ADH^+ deer mice. By contrast, liver cytosol from ADH^- deer mice did not exhibit any significant ADH activity using ethanol as substrate (Table 1).

Characteristics of gastric ADH activity

The ADH activity in gastric mucosa from ADH^- deer mice was dependent on NAD^+ and constant for at least 30 min. The apparent K_m for ethanol was about 40 mM and the apparent V_{max} about 70 nmol/mg and min. Butanol was a better substrate having a lower K_m and a higher V_{max} (Table 2).

At an ethanol concentration of 100 mM, 4-methylpyrazole did not inhibit the gastric mucosal ADH activity in ADH^- deer mice at low concentrations (up to 0.1 mM), whereas inhibition was seen of the liver cytosolic activity (ADH^+ deer mice) already at 10 μM (Fig. 1). Inhibition of the gastric ADH activity was only seen at very high

Table 2. Apparent kinetic parameters of gastric mucosal ADH of ADH$^+$ and ADH$^-$ deer mice

Substrate	K_m (mM)	V_{max} (nmol/mg, min)
ADH$^+$		
Ethanol	31	100
Butanol	0.9	147
ADH$^-$		
Ethanol	42	68
Butanol	3.8	194

The data represent mean values of two different experiments performed in duplicate at six diferent substrate concentrations each using 0.1 mg protein/sample.

The SD of the slopes in the Lineweaver–Burk plots was 9–16% except for butanol in ADH$^+$ animals (34%).

concentrations (0.5–1 mM) of 4-methylpyrazole, thus indicating a difference in sensitivity towards the inhibitor between the two types of ADH activity. Aminotriazole, an inhibitor of both catalase and ethanol-inducible cytochrome P450 (CYP2E1) [21], was found to inhibit also gastric ADH. At 10 mM concentration, the gastric mucosal ADH in ADH$^-$ deer mice was inhibited by 35%, and 50% inhibition was seen at 25 mM (N = 2).

The kinetic isotope effects, $^D(V/K)$, of ethanol oxidation catalysed in cytosol from the gastric mucosa of ADH$^+$ and ADH$^-$ deer mice were determined by a competitive method, involving GC–MS analysis of acetaldehyde produced during the oxidation of mixtures of ethanols labeled with stable isotopes. The experiments were carried out with two sets of differently labeled ethanols in order to detect possible errors due to interference during oxidation or analysis. As shown in Table 3, kinetic isotope effects of about 3 were reached for the gastric ADH activity in cytosolic preparations from the ADH$^+$ and ADH$^-$ deer mice.

Isoelectric focusing of gastric and hepatic ADH

Liver cytosolic fractions from both strains of deer mice were subjected to isoelectric focusing and the gel was subsequently stained for ethanol-dependent dehydrogenase activity. As seen in Fig. 2, no band was detectable in the preparation from ADH$^-$ deer mice, whereas an enzyme with apparent pI of 3, indicative of ADH of class I [22], was detected in the cytosol from ADH$^+$ deer mice. Analysis of cytosol, prepared from the gastric mucosa of both strains of deer mice, revealed two bands with pI around 4.8 and 6.2, when incubated with pentanol. These were not found in the liver samples and probably belong to ADH class II and IV, respectively [22]. In neither of the gastric samples could ADH class I be detected, whereas ADH class III was seen in both the liver and gastric mucosal preparations of both strains.

Acetaldehyde reductase activity

In order to ascertain that the ethanol oxidizing activity in gastric mucosa was due to a dehydrogenase, reduction of [$^2H_4$]acetaldehyde with NADH as

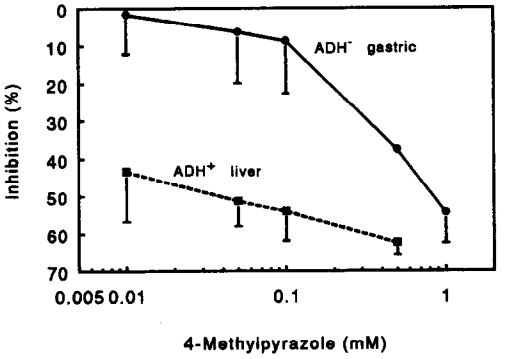

Fig. 1. Effect of 4-methylpyrazole on ethanol oxidation in cytosol from gastric mucosa of ADH$^-$ deer mice (100 mM ethanol, 0.1 mg of protein) (●) and liver cytosol from ADH$^+$ deer mice (50 mM ethanol, 0.5 mg of protein) (■). Acetaldehyde formation in the presence of the indicated concentrations of 4-methylpyrazole was measured as acetaldehyde semicarbazone at 224 nm. Incubations contained cytosol, 2 mM calcium cyanamide and 2 mM NAD$^+$ in 0.1 M Na-glycine buffer, pH 10.0. The data shown are means ± SD of three different experiments performed in duplicate.

Table 3. Kinetic deuterium isotope effects of ethanol oxidation in cytosol preparations from gastric mucosa of ADH$^+$ and ADH$^-$ deer mice and reduction of [$^2H_4$]acetaldehyde measured as formation of [$^2H_4$]ethanol

Type of deer mouse	Isotope effects		Formation of [$^2H_4$]ethanol (nmol/mg min)
	[1,1-$^2H_2$]- and [2,2,2-$^2H_3$]ethanol	[$^2H_5$]- and [1-^{13}C]ethanol	
ADH$^+$	2.98 ± 0.58 (8)	2.92 ± 0.01 (3)	24.9 (2)
ADH$^-$	3.20 ± 0.21 (5)	2.89 ± 0.05 (3)	16.7 (2)

The isotope effects and the formation of ethanol were determined by GC–MS analysis. The product ratio was calculated after oxidation of 1:1 mixture of either [1,1-$^2H_2$]ethanol and [2,2,2-$^2H_3$]ethanol or [1,1,2,2,2-$^2H_5$]ethanol and [1-^{13}C]ethanol.

The values are means ± SD of the analysis, and the number of experiments is in parentheses.

cofactor was measured by GC–MS analysis of derivatized ethanol (Table 3). The rate of formation of [$^2H_4$]ethanol was similar in cytosols from both strains. No reduction of acetaldehyde was seen in the absence of NADH. The optimal rate of reduction of acetaldehyde was followed by measuring the oxidation of NADH at substrate saturation (100 mM) [23]. The rate of NADH oxidation under these conditions was 78 and 55 nmol/mg protein, min (N = 2) in cytosol from ADH$^+$ and ADH$^-$ deer mice, respectively.

DISCUSSION

The results presented are consistent with the presence of ADH activity in the cytosolic fraction of the gastric mucosa isolated from deer mice. Thus, a high rate of ADH$^+$-dependent ethanol oxidation was observed in the cytosol of gastric mucosa, and on a protein basis this activity was about the same as in the liver of the ADH$^+$ strain. The rate of oxidation *in vivo* in the gastric mucosa was calculated to be about 15 μmol/hr, which is 25% or less of the total rate of ethanol oxidation in an ADH$^-$ deer mouse [6, 13]. The remaining oxidation might be explained by extrahepatic ADH in other tissues, e.g. the skin [24].

The efficient NADH-dependent reduction of acetaldehyde in these cytosolic preparations shows that the activity was due to a dehydrogenase. Furthermore, the $^D(V/K)$ value of 3.0 for ethanol oxidation in cytosol is compatible with the isotope effect determined *in vivo* for ethanol elimination in deer mice. We have previously reported *in vivo* $^D(V/K)$ values of 2.2 and 3.2 in ADH$^-$ and ADH$^+$ deer mice, respectively [13]. Previous results from experiments *in vivo* in deer mice utilizing isotope techniques have been interpreted in favour of P450 being the major enzyme responsible for ethanol elimination [9]. However, these investigators

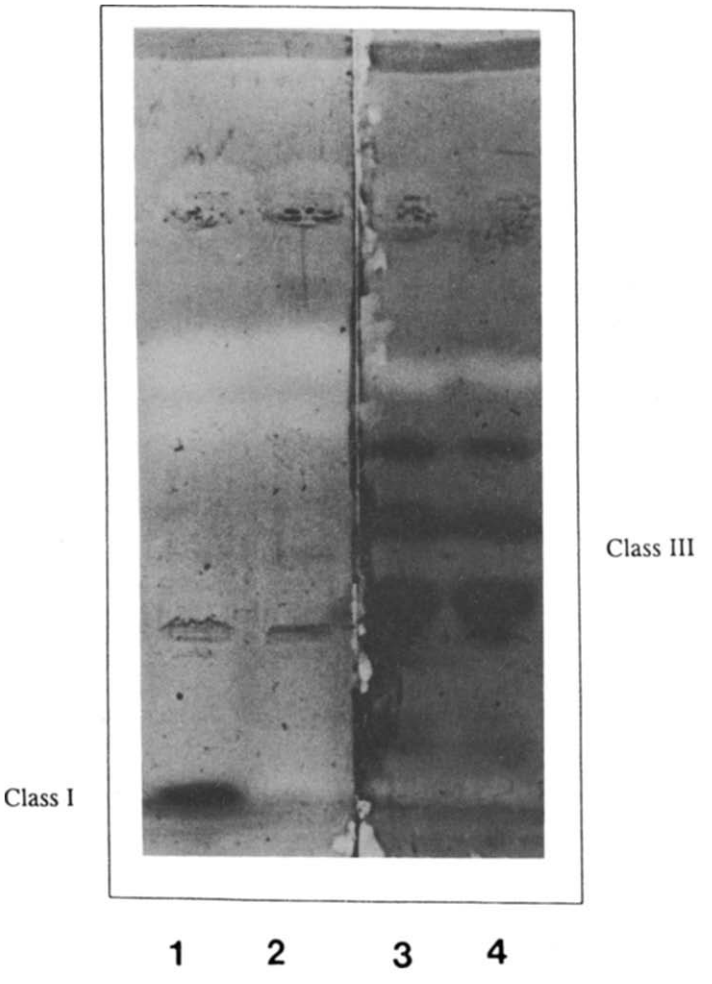

Fig. 2. Isoelectric focusing gel with samples from gastric mucosa and liver from ADH$^+$ and ADH$^-$ deer mouse. The first two lanes were loaded with liver cytosol from ADH$^+$ (lane 1) and ADH$^-$ (lane 2) deer mice and stained with ethanol as a substrate. Cytosol from gastric mucosa of ADH$^+$ (lane 3) and ADH$^-$ (lane 4) deer mice was stained with pentanol. The indicated positions of class I ADH, determined by staining with ethanol as substrate, and of class III ADH, determined using formaldehyde and glutathione as substrates, were obtained in parallel experiments.

assumed absence of reversibility of the oxidation process, an assumption that is not valid [13, 14] (present investigation).

Isoelectric focusing indicates that liver cytosol from ADH$^+$ deer mice contains a dehydrogenase enzyme active with ethanol as a substrate, belonging to ADH class I. The corresponding enzyme in the ADH$^-$ animals could not be detected. Using formaldehyde and glutathione as the substrates, class III ADHs were tentatively identified in both strains of animals. Analysis of the cytosol isolated from gastric mucosa revealed three bands of activity suggested to belong to ADH classes II, III and IV, based on pI values. The more pronounced staining with the longer alcohol (pentanol) is in accordance with the results *in vitro*, where incubations revealed that butanol is a better substrate for the gastric enzyme than ethanol, having a lower K_m value and a higher V_{max}.

The ADH in gastric mucosa from deer mice has many enzymatic and physicochemical properties in common with those described for the corresponding human enzyme [25]. In both species, the K_m for ethanol is about 40 mM, and butanol constitutes a much better substrate, with a K_m of around 2 mM. Furthermore, it appears that the gastric enzyme is less sensitive to inhibition with 4-methylpyrazole [25]. This difference can also be seen when examining deer mice ADH in cytosol from the liver and stomach (Fig. 1). A difference in the 4-methylpyrazole effect on ethanol elimination *in vivo* has been observed between ADH$^+$ and ADH$^-$ deer mice. Thus, 4-methylpyrazole given at a dose of 0.5 mmol/kg prior to ethanol caused 50% inhibition of ethanol elimination in ADH$^+$ deer mice, but only 25% inhibition in ADH$^-$ deer mice [13]. These data further support the importance of extrahepatic ADH for ethanol elimination in ADH$^-$ deer mice. The difference in sensitivity to 4-methylpyrazole of ethanol elimination between the two different deer mice strains has previously been observed by other authors, but interpreted in favour of cytochrome P450 as the major enzyme responsible for ethanol elimination [8]. It seems likely that the incomplete inhibition of ethanol elimination by 4-methylpyrazole in ADH$^+$ deer mice [8] and in rats [26] might be due to extrahepatic oxidation [24].

The ADH$^-$ deer mice have been used extensively in order to evaluate non-ADH-dependent ethanol elimination *in vivo*. We have here presented evidence for the existence of a very active ADH system in the gastric mucosa of both ADH$^+$ and ADH$^-$ strains of deer mice. We therefore conclude that deer mice are not a useful animal model for studying non-ADH-dependent ethanol oxidation. The results further indicate that gastric ADH may be of great importance for ethanol elimination also in other species.

Acknowledgements—We are indebted to Ann-Louise Hagbjörk and Inger Backman for skilful technical assistance, to Dr Mats Estonius for valuable aid with the isoelectric focusing gels and to Dr Jan-Olov Höög for valuable discussions. This work was supported by grants from The Swedish Alcohol Research Fund and from The Swedish Medical Research Council (Grants No. 2189 and 5949).

REFERENCES

1. Kato S, Alderman J and Lieber CS, *In vivo* role of the microsomal ethanol-oxidizing system in ethanol metabolism by deermice lacking alcohol dehydrogenase. *Biochem Pharmacol* **37**: 2706–2708, 1988.
2. Kato S, Alderman J and Lieber CS, Respective roles of the microsomal ethanol oxidizing system and catalase in ethanol metabolism by deermice lacking alcohol dehydrogenase. *Arch Biochem Biophys* **254**: 586–591, 1987.
3. Handler AH, Bradford BU, Glassman E, Ladine JK and Thurman RG, Catalase-dependent ethanol metabolism *in vivo* in deer mice lacking alcohol dehydrogenase. *Biochem Pharmacol* **35**: 4487–4492, 1986.
4. Burnett KG and Felder MR, Genetic regulation of liver alcohol dehydrogenase in *Peromyscus*. *Biochem Genet* **16**: 443–454, 1978.
5. Shigeta Y, Nomura F, Leo MA, Iida S, Felder MR and Lieber CS, Alcohol dehydrogenase (ADH) independent ethanol metabolism in deermice lacking ADH. *Pharmacol Biochem Behav* **18** (Suppl 1): 195–199, 1983.
6. Shigeta Y, Nomura F, Iida S, Leo MA, Felder MR and Lieber CS, Ethanol metabolism *in vivo* by the microsomal ethanol-oxidizing system in deermice lacking alcohol dehydrogenase (ADH). *Biochem Pharmacol* **33**: 807–814, 1984.
7. Gellert J, Alderman J and Lieber CS, Interaction between ethanol metabolism and mixed-function oxidation in alcohol dehydrogenase positive and negative deermice. *Biochem Pharmacol* **35**: 1037–1041, 1986.
8. Takagi T, Alderman J, Gellert J and Lieber CS, Assessment of the role of non-ADH ethanol oxidation *in vivo* and hepatocytes from deermice. *Biochem Pharmacol* **35**: 3601–3606, 1986.
9. Alderman J, Takagi T and Lieber CS, Ethanol-metabolizing pathways in deermice. Estimation of flux calculated from isotope effects. *J Biol Chem* **262**: 7497–7503, 1987.
10. Handler JA, Bradford BU, Glassman EB, Forman DT and Thurman RG, Inhibition of catalase-dependent ethanol metabolism in alcohol dehydrogenase-deficient deermice by fructose. *Biochem J* **248**: 415–421, 1987.
11. Bradford BU, Handler JA, Seed CB, Forman DT and Thurman RG, Inhibition of ethanol metabolism by fructose in alcohol dehydrogenase-deficient deer mice *in vivo*. *Arch Biochem Biophys* **288**: 435–439, 1991.
12. Handler JA, Koop DR, Coon MJ, Takei Y and Thurman RG, Identification of $P\text{-}450_{ALC}$ in microsomes from alcohol dehydrogenase-deficient deermice: contribution to ethanol elimination *in vivo*. *Arch Biochem Biophys* **264**: 114–124, 1988.
13. Norsten C, Cronholm T, Ekström G, Handler JA, Thurman RG and Ingelman-Sundberg M, Dehydrogenase-dependent ethanol metabolism in deer mice (*Peromyscus maniculatus*) lacking cytosolic alcohol dehydrogenase. Reversibility and isotope effects *in vivo* and in subcellular fractions. *J Biol Chem* **264**: 5563–5597, 1989.
14. Cronholm T, Norsten-Höög C, Ekström G, Handler JA, Thurman RG and Ingelman-Sundberg M, Oxidoreduction of butanol in deermice (*Peromyscus maniculatus*) lacking hepatic cytosolic alcohol dehydrogenase. *Eur J Biochem* **204**: 353–357, 1992.
15. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
16. Ingelman-Sundberg M and Johansson I, The mechanism of cytochrome P-450-dependent oxidation of ethanol in reconstituted membrane vesicles. *J Biol Chem* **256**: 6321–6326, 1981.

17. Ekström G, Norsten C, Cronholm T and Ingelman-Sundberg M, Cytochrome P-450 dependent ethanol oxidation. Kinetic isotope effects and absence of stereoselectivity. *Biochemistry* **26**: 7348–7354, 1987.
18. Cronholm T, Hydrogen transfer between ethanol molecules during oxidoreduction *in vivo*. *Biochem J* **229**: 315–322.
19. Parês X, Moreno A, Cederlund E, Höög J-O and Jörnvall H, Class IV mammalian alcohol dehydrogenase. Structural data of the rat stomach enzyme reveal a new class well separated from those already characterized. *FEBS Lett* **277**: 115–118, 1990.
20. Marchner H and Tottmar O, A comparative study on the effects of disulfiram, cyanamide and 1-aminocyclopropanol on the acetaldehyde metabolism in rats. *Acta Pharmacol Toxicol* **43**: 219–232, 1978.
21. Koop DR, Inhibition of ethanol-inducible cytochrome P450IIE1 by 3-amino-1,2,4-triazole. *Chem Res Toxicol* **3**: 377–383, 1990.
22. Vallee BL and Bazzone TJ, Isozymes of human liver alcohol dehydrogenase. *Isozymes Curr Top Biol Med Res Isozymes* **8**: 219–244, 1983.
23. Handler JA and Thurman RG, Catalase-dependent ethanol oxidation in perfused rat liver. Requirement for fatty-acid-stimulated H_2O_2 production by peroxisomes. *Eur J Biochem* **176**: 477–484, 1988.
24. Boleda MD, Julia P, Moreno A and Parês X, Role of extrahepatic alcohol dehydrogenase in rat ethanol metabolism. *Arch Biochem Biophys* **274**: 74–81, 1989.
25. Moreno A and Parês X, Purification and characterization of a new alcohol dehydrogenase from human stomach. *J Biol Chem* **266**: 1128–1133, 1991.
26. Rydberg U and Neri A, 4-Methylpyrazole as an inhibitor of ethanol metabolism: differential metabolic and central nervous effects. *Acta Pharmacol Toxicol* **31**: 421–432, 1972.