Biochemical Pharmacology, Vol. 35, No. 20, pp. 3601–3606, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00
Pergamon Journals Ltd.

ASSESSMENT OF THE ROLE OF NON-ADH ETHANOL OXIDATION *IN VIVO* AND IN HEPATOCYTES FROM DEERMICE

TOSHIKAZU TAKAGI, JEFFREY ALDERMAN, JURGEN GELLERT and CHARLES S. LIEBER*

Alcohol Research and Treatment Center, Bronx V.A. Medical Center and Mount Sinai School of Medicine (CUNY), New York, NY 10468, U.S.A.

(*Received* 14 *December* 1985; *accepted* 6 *March* 1986)

Abstract—Deermice genetically lacking alcohol dehydrogenase (ADH^-) were used to quantitate the effect of 4-methylpyrazole (4-MP) on non-ADH pathways in hepatocytes and *in vivo*. Although primarily an inhibitor of ADH, 4-methylpyrazole was also found to inhibit competitively the activity of the microsomal ethanol-oxidizing system (MEOS) in deermouse liver microsomes. The degree of 4-MP inhibition in ADH^- deermice then served to correct for the effect of 4-MP on non-ADH pathways in deermice having ADH (ADH^+). In ADH^+ hepatocytes, the percent contributions of non-ADH pathways were calculated to be 28% at 10 mM and 52% at 50 mM ethanol. When a similar correction was applied to *in vivo* ethanol clearance rates in ADH^+ deermice, non-ADH pathways were found to contribute 42% below 10 mM and 63% at 40–70 mM blood ethanol. The catalase inhibitor 3-amino-1,2,4-triazole, while reducing catalase-mediated peroxidation of ethanol by 83–94%, had only a slight effect on blood ethanol clearance at ethanol concentrations below 10 mM, and no effect at all at 40–70 mM ethanol. These results indicate that non-ADH pathways (primarily MEOS) play a significant role in ethanol oxidation *in vivo* and in hepatocytes *in vitro*.

The alcohol dehydrogenase (ADH) pathway, located in the cytosolic fraction of hepatocytes, has been considered to be of major importance in the oxidation of ethanol, while there is still some debate concerning the contributions of the two non-ADH pathways, i.e. the microsomal ethanol-oxidizing system (MEOS) and catalase. Several lines of evidence, however, are consistent with a role for non-ADH pathways *in vivo*. Pyrazole and its 4-substituted derivatives, especially 4-methylpyrazole (4-MP), are known to be effective inhibitors of ADH [1–3]. These ADH inhibitors by themselves fail to inhibit ethanol oxidation completely *in vivo* [4, 5] or *in vitro* [6, 7]. In addition, the rate of ethanol oxidation increases at ethanol concentrations well above those needed to saturate the ADH pathway [8]. It is also known that chronic ethanol feeding of rats both induces the activity of microsomal enzymes and increases ethanol oxidation in the liver [6, 9]. All of these observations suggest a significant contribution to hepatic ethanol oxidation by non-ADH pathways.

In this paper, we have addressed the issue of the respective roles of ADH and non-ADH pathways for the metabolism of ethanol in the liver. Attempts to clarify this question usually have relied upon inhibitors of the enzymes ADH and catalase [6, 7, 10]. Although most data obtained with inhibitors have suggested significant non-ADH involvement, the agreement has not been universal [11]. This discrepancy may partly result from the lack of complete specificity of inhibitors. The ADH inhibitors, pyrazole and 4-MP, have been shown to inhibit MEOS also [8, 12, 13] as well as catalase [6]. The catalase inhibitor, azide, is a potent metabolic poison and cannot be applied *in vivo*. The other commonly used catalase inhibitor, 3-amino-1,2,4-triazole (AT), can be applied *in vivo*, but it also inhibits various microsomal enzymes [14] and ADH [15].

The discovery of a deermouse strain genetically lacking ADH (ADH^-) [16] which can consume and eliminate ethanol at rates exceeding 50% of those seen in deermice having ADH (ADH^+) [17] has further suggested an important role for non-ADH mediated ethanol oxidation *in vivo*. The ADH^- deermouse strain also offers a unique opportunity to study the effects of ADH inhibitors on the other pathways. In this instance, we have used it to correct for the effect of the widely used ADH inhibitor, 4-MP, on non-ADH pathways.

In the present study, we measured the ethanol oxidation rates of both strains of deermice *in vivo* and in isolated hepatocytes with or without 4-MP treatment. After correction for the effect of 4-MP on non-ADH pathways, we could calculate relative contributions of the ADH and non-ADH pathways to ethanol oxidation in ADH^+ deermice. AT was also used *in vivo* to estimate whether catalase was significantly involved in non-ADH ethanol metabolism.

MATERIALS AND METHODS

Animals. Animals used in the present studies had the following genotypes for liver alcohol dehydrogenase (ADH): ADH^F/ADH^F (ADH positive,

* Address reprint requests to: C. S. Lieber, M.D., Alcohol Research and Treatment Center, Bronx V.A. Medical Center, 130 West Kingsbridge Road, New York, NY 10468, U.S.A.

ADH^+) and ADH^N/ADH^N (ADH negative, ADH^-). These animals were bred in our laboratory from an original stock provided by Dr. M. Felder.

Male ADH^+ and ADH^- deermice were fed rat Lab-Blox (Wayne food division, Continental Grain Co., Chicago, IL), and given tap water *at lib.* until the experiments were begun.

Materials. NAD (Grade III), NADPH (tetrasodium salt, type I), collagenase (type I), semicarbazide hydrochloride, glucose oxidase (from *Aspergillus niger*, Type II) and sodium azide were purchased from the Sigma Chemical Co. (St. Louis, MO). 4-MP and AT were obtained from the Aldrich Chemical Co. (Milwaukee, WI), acetaldehyde was from the Eastman Kodak Co. (Rochester, NY), and [1-^{14}C]ethanol and [1, 2-^{14}C]acetaldehyde were from New England Nuclear (Boston, MA). To remove trace amounts of acetaldehyde and non-volatile impurities, the [1-^{14}C]ethanol was treated by fractional distillation before use. RPMI 1640 (semicomplete cell culture medium) was bought from GIBCO, Grand Island, NY.

In vitro *studies.* Animals were killed by decapitation. Their livers were excised and pooled (groups of four to six animals). Ten percent homogenates were prepared with 0.25 M sucrose, 10 mM Tris and 1 mM EDTA, pH 7.4. All procedures were carried out at 0–4° unless otherwise specified. 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 the enzyme source for the ADH assays. The pellet was resuspended and washed in 1.15% KCl solution. The microsomal pellet was obtained by spinning down this suspension at 105,000 *g* for another 30 min. Microsomes were suspended in 0.25 M sucrose and stored at −80°.

Proteins in cytosolic and microsomal fractions were determined by the method of Lowry *et al.* [18]. ADH activity in the cytosol was determined at pH 9.6 by the method of Bonnichsen and Brink [19] modified as follows: final ethanol concentration was 25 mM, and 3 mg NAD was used per 3-ml cuvette. The apparent K_m of ADH was determined from double reciprocal plots of velocity versus substrate concentration. Lines were fitted to the data by the least squares method. Kinetic characteristics of 4-MP inhibition were determined using the ADH^+ deermice cytosolic fraction as a source of the ADH activity and using microsomes from both strains to study inhibition on MEOS. The microsomal cytochrome P-450 content was determined according to Omura and Sato [20] with 20% glycerol added to the buffer. MEOS activity was measured by the method of Ohnishi and Lieber [21] using head-space gas–liquid chromatography but with 2 mM NADPH, and omitting EDTA and $MgCl_2$. To eliminate possible contaminating catalase activity, all MEOS assays had 1 mM azide added.

Isolation of hepatocytes. Hepatocytes were isolated by a modification of the collagenase perfusion technique originally described by Berry and Friend [22] adapted for animals of small size according to Deschenes *et al.* [23].

The animals were anesthetized with ketamine hydrochloride (100 mg/kg body wt, i.p.) followed by intraperitoneal injection of sodium heparin (1000 USP units/kg body wt). The thorax was opened, and a 22-gauge intravenous catheter was inserted into the inferior vena cava retrograde through the atrium. The inferior vena cava was ligated above the renal veins. A cut was made in the portal vein to permit free outflow. The initial perfusion solution was O_2 saturated Ca^{2+}-free Krebs bicarbonate buffer with 0.5 mM ethyleneglycolbis(amino-ethylether)tetraacetate (EGTA) (pH 7.4). After 10 min of perfusion of the EGTA buffer (0.5 ml/min), 0.05% collagenase in Krebs bicarbonate buffer with 4 mM $CaCl_2$ was perfused without recirculation at a rate of 1.2 ml/min for 15–20 min depending on the extent of collagenase digestion. Then the liver was transferred to a Petri dish, the capsule was cut, and cells were dispersed in Krebs bicarbonate buffer with 0.05% collagenase and 2.5 mM $CaCl_2$. The cell suspension was incubated for 15 min at 37° and was then filtered through four layers of surgical gauze. The filtered cell suspension was centrifuged at 50 *g* for 90 sec. The cells were washed twice with RPMI 1640 solution and resuspended in RPMI. RPMI 1640 contains all the L-amino acids (including L-glutamine), vitamins, glucose and glutathione in a balanced salt solution.

The average yield of cells was 22.1×10^6 cells/animal and 39.8×10^6 cells/g liver. The average initial viability assessed by the Trypan Blue exclusion test was 87%. Viability declined, on the average, by less than 6% over the 60-min incubation period. Ethanol oxidation rates were corrected for the small changes in viability seen.

Ethanol oxidation in isolated hepatocytes. To determine the rates of ethanol oxidation by the cells, the metabolism of [1-^{14}C]ethanol was assessed according to Berry *et al.* [24]. This method depends upon the fact that acetate is the principal end product of hepatic ethanol metabolism: most of the acetate produced in the liver is further metabolized extrahepatically. Radioactivity in dry matter from deproteinized hepatocyte suspensions after incubations represents acetate and thus directly reflects ethanol metabolism. This method had been tested and found comparable with more conventional measures of ethanol oxidation [25].

Cells (0.5 to 1.0×10^6 in 0.5 ml total volume) were placed in small air-tight vials with or without 1 mM 4-MP added to the medium. After a 5-min preincubation at 37°, the reaction was initiated by adding [1-^{14}C]ethanol containing 50–100 dpm/nmole at a 10 mM or 50 mM final concentration. The glass vials were capped and oxygenated, and the reactions were stopped at 0, 15, 30 and 60 min by adding 0.5 ml of 1 N perchloric acid. The deproteinized solution was centrifuged, and the supernatant fraction was transferred to a scintillation vial; 0.5 ml of 2 N KOH was added, and the contents of the vials were evaporated to dryness and washed three times with absolute ethanol. The residue was redissolved in 0.2 ml of water, neutralized with 2 M H_2SO_4, and 5 ml of Aquasol was added. The radioactivity was determined by liquid scintillation counting in a Beckman LS 7800 with automatic quench correction. The ethanol oxidation rates were then calculated and expressed as nmoles/min/10^6 cells. Corrections for non-

specific inhibition by 4-MP both *in vivo* and in hepatocytes of ADH$^+$ deermice were made by dividing the inhibition seen in the ADH$^-$ systems in the presence of 4-MP as a fraction of control, into the percent inhibition seen in the corresponding ADH$^+$ system. A fixed 4-MP concentration (1 mM) was used throughout the hepatocyte studies, sufficient to inhibit most ADH activity at both 10 and 50 mM ethanol but low enough not to be severely toxic.

Determination of in vivo *ethanol clearance in the presence and the absence of enzyme inhibitors.* Ethanol was injected intraperitoneally at a dose of 1.0 g/kg body wt or 2.5 g/kg body wt. When 4-MP (0.5 mmole/kg body wt) was used to inhibit ADH, it was administered i.p. 15 min prior to the injection of ethanol. Assay of hepatic ADH 6 hr after 4-MP administration (representing the duration of our *in vivo* ethanol elimination experiments) showed no detectable activities with 25 mM ethanol. When AT (1.0 g/kg body wt) was used to inhibit catalase, it was administered i.p. 3 hr prior to the injection of ethanol. Heim *et al.* [26] demonstrated that catalase activity drops to 10% 3 hr after 1 g AT/kg. In our laboratory, it has been confirmed that, 12 hr after AT injection, catalase activity at high H_2O_2 concentrations remained less than 20% of control levels in ADH$^+$ deermice. In addition, actual inhibition of catalase-mediated ethanol peroxidation was measured in this study in liver homogenates from ADH$^-$ deermice treated 3 hr before being killed with 1 g AT/kg body wt. H_2O_2 was provided at low rates (10 or 100 μmoles $H_2O_2 \cdot liter^{-1} \cdot min^{-1}$) using an H_2O_2 generating system consisting of glucose (10 mM) plus glucose oxidase in 50 mM potassium phosphate (pH 7.4) buffer. The H_2O_2 production rates were varied by using different amounts of glucose oxidase based on reported glucose oxidase activity. Peroxidation of 50 mM [1-^{14}C]ethanol (50–100 dpm/nmole) was quantitated from radioactivity trapped by 0.5 ml of 100 mM semicarbazide dissolved in 200 mM potassium phosphate buffer (pH 6.3). The semicarbazide occupied the center well of a two-compartment, stoppered 25-ml Erlenmyer flask, while the outer well contained the ethanol, H_2O_2 generator, and 1 mg of homogenized liver. After 30–60 min at 37° the reaction was quenched with 0.5 ml of 0.3 N $Ba(OH)_2$ followed by 0.5 ml of 0.3 N $ZnSO_4$ and the flasks were left for 24 hr at 25°. The center well contents were dried, then washed three times with absolute ethanol, and counted. Recoveries of acetaldehyde were measured in blank reaction flasks with [1, 2-^{14}C]acetaldehyde and were always higher than 60%.

RESULTS

Effect of 4-*MP on ADH and MEOS activities* in vitro. The average ADH activity in the cytosolic fraction of ADH$^+$ animals was 1.31 ± 0.11 μmoles NAD reduced/min/g liver and 11.55 ± 0.98 nmoles NAD reduced/min/mg cytosolic protein with 25 mM ethanol as a substrate. There was up to 50% substrate inhibition with high ethanol levels (50–100 mM) when the activity was compared to that at 5 mM (Fig. 1).

The apparent K_m of ADH for ethanol, using the ADH$^+$ deermouse cytosolic fraction as the enzyme source, was 0.22 mM. ADH inhibition by 4-MP was competitive with an apparent K_I of 1.22 μM.

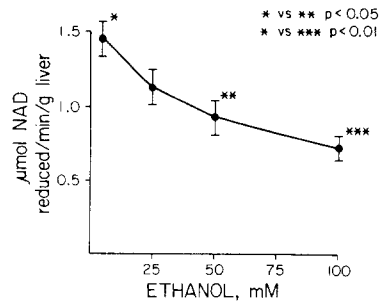

Fig. 1. ADH activity in the cytosol of ADH$^+$ deermice: Substrate inhibition by ethanol. Values are means ± S.E.M., N = 3.

When MEOS assays were carried out at various ethanol concentrations with microsomal fractions from both strains, the K_m (15.6 mM in ADH$^-$ and 15.7 mM in ADH$^+$) and V_{max} (10.5 and 9.8 nmoles/min/nmole P-450 respectively) were found to be similar. When 4-MP was used in the MEOS assays, the inhibition was competitive with ethanol, and apparent K_I values for MEOS of ADH$^-$ and ADH$^+$ microsomes were 135 and 84 μM respectively (Fig. 2, left and right panels).

Peroxidation of ethanol in vitro *after AT administration* in vivo. Ethanol peroxidation by liver homogenates from AT-treated or control ADH$^-$ deermice at rate-limiting H_2O_2 production rates on the order of those reported *in vivo* is shown in Fig. 3. At the lower rate (10 μmoles $H_2O_2 \cdot liter^{-1} \cdot min^{-1}$), AT treatment reduced production of [1-^{14}C]acetaldehyde from [1-^{14}C]ethanol by 83% [from 1271 ± 124 nmoles $\cdot min^{-1} \cdot (g\ liver)^{-1}$ to 234 ± 71; $P < 0.001$]. At the higher rate studied (100 μmoles $H_2O_2 \cdot liter^{-1} \cdot min^{-1}$), AT pretreatment decreased acetaldehyde production by 94% [from 5502 ± 746 nmoles $\cdot min^{-1} \cdot (g\ liver)^{-1}$ to 348 ± 78; $P < 0.001$].

Ethanol oxidation rates of isolated hepatocytes in the presence or absence of 4-*MP.* Isolated hepatocytes of ADH$^-$ deermice metabolized ethanol at a rate 40–60% of that of cells from ADH$^+$ deermice,

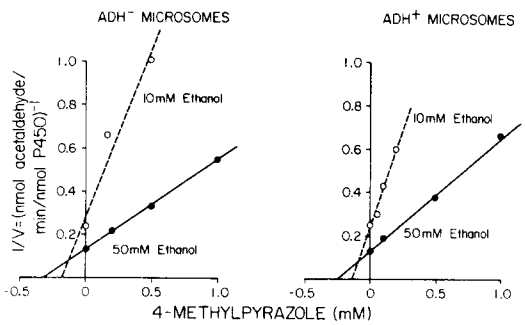

Fig. 2. Inhibition of MEOS activity by 4-MP; Dixon plots of MEOS activities at 10 and 50 mM ethanol concentrations with various concentrations of 4-MP in microsomes from ADH$^-$ (left panel) and ADH$^+$ (right panel) deermice.

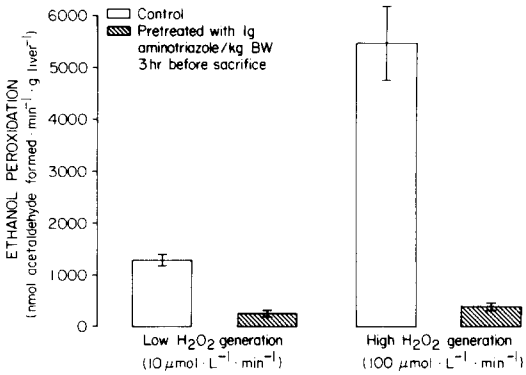

Fig. 3. Peroxidation of ethanol by liver homogenates of AT-treated and control ADH⁻ deermice (N = at least six each). Values after AT treatment (hatched areas) were significantly reduced ($P < 0.001$) versus controls. Values are means ± S.E.M. Details are given in Materials and Methods.

depending upon the ethanol concentration. A 1 mM concentration of 4-MP in the medium decreased the ethanol oxidation rates of ADH⁻ deermouse hepatocytes by 45% of control values ($P < 0.05$) at 10 mM and by 37% ($P < 0.01$) at 50 mM ethanol concentrations. The ethanol oxidation rates of ADH⁺ deermouse hepatocytes were reduced by 4-MP to 15% of control values ($P < 0.001$) at 10 mM and 32% ($P < 0.001$) at 50 mM ethanol (Fig. 4). When the inhibition seen in ADH⁻ animals was used to correct values of ethanol oxidation in ADH⁺ animals, the non-ADH pathways were found to contribute 28% at 10 mM and 52% at 50 mM ethanol concentrations in ADH⁺ isolated hepatocytes. When calculated by subtraction from 100%, the ADH pathway was found to contribute 73 and 49% at 10 and 50 mM respectively.

Ethanol clearance rates in vivo *with or without inhibitors*. In the absence of enzyme inhibitors, the ADH⁺ deermice eliminated ethanol at rates of 142.1 ± 4.0 μmoles/min/kg body wt (below 10 mM ethanol) and 185.6 ± 5.4 (at 40–70 mM ethanol). The ADH⁻ animals eliminated ethanol at rates of 66.1 ± 1.6 (47% of ADH⁺) below 10 mM and 101.8 ± 5.4 (55% of ADH⁺) at 40–70 mM ethanol (Fig. 5).

4-MP at 0.5 mmole/kg body wt (injected 15 min prior to ethanol administration) reduced the ethanol clearance rates of ADH⁻ deermice to 74% ($P < 0.01$) of control at ethanol concentrations below 10 mM and 72% ($P < 0.01$) at ethanol concentrations of 40–70 mM. The ethanol clearance rates of ADH⁺ deermice were also decreased by 4-MP to 31% ($P < 0.01$) of control at ethanol concentrations below 10 mM and 45% ($P < 0.01$) at ethanol concentrations of 40–70 mM. The corrected percent contributions of non-ADH pathways in the elimination of ethanol by ADH⁺ deermice were 42% (below 10 mM ethanol) and 62% (at 40–70 mM ethanol), and the percent contributions of ADH (by subtraction from 100%) were 58 and 38% respectively. Under conditions which resulted in a greater than 83% inhibition of catalase mediated ethanol peroxidation (*vide supra*), the pretreatment by AT reduced the ethanol clearance rates of both strains of deermice only slightly, but significantly ($P < 0.01$) below 10 mM, and not at all at 40–70 mM ethanol (Fig. 5).

DISCUSSION

In this study, we observed that 4-MP significantly reduced both *in vivo* ethanol clearance rates and ethanol oxidation by hepatocytes derived from the ADH⁻ mutant deermouse strain, suggesting a depression of non-ADH pathways by 4-MP. We also found that 4-MP has an inhibitory effect *in vitro* on ethanol oxidation not only by alcohol dehydrogenase but also by MEOS.

Kinetic parameters for ADH obtained from ADH⁺ deermice agree well with some studies using rats [5, 15], but differ from others [11, 27]. When 4-MP was applied *in vitro*, it competitively inhibited MEOS in microsomes of both strains. The type of inhibition agrees with that reported for pyrazole and its derivatives in rats [25, 28]. Apparent K_I values were lower in the present study, perhaps due in part to differences in assay conditions and/or in the species used. It is also possible that MEOS is more sensitive to 4-MP inhibition than had been recognized previously.

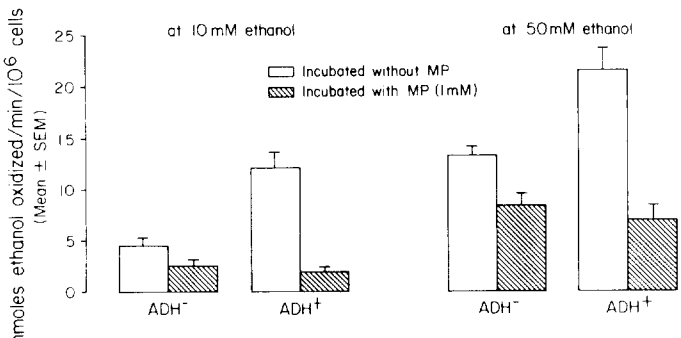

Fig. 4. Ethanol oxidation rates with or without 4-MP in hepatocytes isolated from ADH⁻ and ADH⁺ deermice. Values after 4-MP (hatched areas) were significantly reduced versus controls (ADH⁻, $P < 0.05$ at 10 mM ethanol and $P < 0.01$ at 50 mM; ADH⁺, $P < 0.001$ at both 10 and 50 mM).

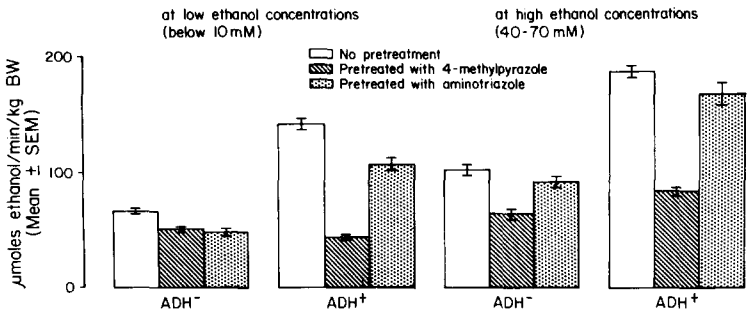

Fig. 5. *In vivo* ethanol clearance (with or without inhibitors) in ADH$^-$ and ADH$^+$ deermice. Animals were given 0.5 mmole 4-MP/kg body wt 15 min prior to ethanol, or 1 g AT/kg body wt 3 hr prior to ethanol. Values after 4-MP (hatched areas) were significantly less than controls ($P < 0.01$) at all ethanol concentrations; values after AT (stippled areas) were significantly less ($P < 0.01$) only at low ethanol concentrations.

Consistent with the sensitivity shown by MEOS *in vitro* to 4-MP, living systems lacking ADH exhibit a diminished clearance of ethanol after 4-MP treatment. This was clearly seen in ADH$^-$ hepatocytes (Fig. 4) and *in vivo* (Fig. 5). To avoid limitation of ADH activity in ADH$^+$ hepatocytes because of factors other than 4-MP, we have employed a semicomplete cell culture medium (RPMI 1640) containing shuttle components or intermediate precursors and various gluconeogenic amino acids. This should permit maximal NADH reoxidation in the system. It is known that the ethanol oxidation by rat hepatocytes can be accelerated by addition of pyruvate or gluconeogenic amino acids, such as lysine, which serve to enhance reoxidation of cytosolic NADH [3, 29].

The phenomenon of non-specific inhibition has been recognized previously but never adequately quantitated (except for *in vitro* systems [13]), because of lack of a proper model. The ADH$^-$ strain provides that model for ADH inhibitors such as 4-MP. Failure to take this inhibition of non-ADH pathways into full account has resulted in an overestimation of the role of ADH in much of the previous literature [2, 11, 30]. Additionally, at high ethanol levels, substrate inhibition (Fig. 1) may further diminish the relative contribution by ADH to total ethanol oxidation. Deermouse ADH activity measured at physiological pH as previously described [11] showed similar decreases with increasing substrate concentration.

Of the two non-ADH pathways, catalase is primarily located in the peroxisomes and oxidizes ethanol *in vitro* in the presence of an H_2O_2-generating system. However, the catalase pathway is unlikely to make a major contribution *in vivo* because catalase-mediated ethanol peroxidation appears to be limited by the very low rate of H_2O_2 generation in living systems rather than by the amount of catalase activity itself [31]. A recent report by Glassman *et al.* [32] has speculated that, when H_2O_2 generation limits ethanol oxidation by catalase, the small amount of catalase activity remaining after AT could metabolize ethanol at near normal rates. However, we have documented that AT effectively inhibits peroxidation of ethanol by catalase (Fig. 3) under the treatment conditions used here *in vivo* (Fig. 5) with H_2O_2 production rates comparable to those estimated *in vivo* [31]. Furthermore, all MEOS assays contained 1 mM azide, effectively precluding catalase participation. At this concentration, azide inhibits more than 99.9% of catalase activity in rat liver homogenates [13]. It was also suggested in the above mentioned report [32] that a significant fraction of *in vivo* ethanol elimination by ADH$^-$ deermice could be due to excretion and expiration rather than oxidation. However, rates of ethanol metabolism measured *in vitro* (in the absence of any losses) (Fig. 4) could account for a large fraction of the metabolism observed *in vivo* (Fig. 5). Furthermore, an earlier study [17] from this laboratory which directly measured loss of ethanol in urine and breath of deermice given ethanol indicated that non-oxidative elimination amounted to only 15% of the total.

Our results obtained with the catalase inhibitor AT indicate that, in deermice, catalase does not participate in non-ADH ethanol metabolism or plays only a minor role. Since we currently lack an appropriate model for estimation of the non-specific effects of AT, we cannot accurately differentiate between these two possibilities. AT depressed ethanol oxidation slightly but significantly *in vivo* at low ethanol levels in both deermouse strains, but did not do so at high ethanol levels, although 83–94% of ethanol peroxidation was inhibited *in vitro* (Fig. 3). Whether this indicates a small contribution by catalase or a non-specific effect of AT remains uncertain. In light of the fact that AT poisons hemeprotein synthesis and reduces many microsomal enzyme activities [14] as well as that of ADH [15], it is probable that the small effect of AT observed in this study is non-specific. Supporting this view is our recent finding [33], using an isotope effect approach not requiring inhibitors, that MEOS can account for ethanol oxidation in ADH$^-$ deermice. The values obtained in the latter study agree remarkably well with the present estimates obtained using an entirely different approach. Using another independent approach, Vind and Grunnet [34] assessed the metabolism of 1R[1-^{3}H]ethanol in rat hepatocytes and found that non-ADH pathways were responsible for 10–50% of ethanol metabolism. These findings tend to support the present results and indicate important par-

ticipation by non-ADH pathways in living systems. Finally, it is noteworthy that, after 4-MP treatment, the absolute values of ethanol elimination by ADH^- deermice *in vivo* (Fig. 5) or by ADH^- hepatocytes (Fig. 4) were the same as the values seen in the corresponding 4-MP-treated ADH^+ models within the limits of experimental error. This suggests that the same pathway underlies the remaining oxidation in each case.

In summary, use of 4-MP to inhibit ADH cannot, by itself, accurately assess the respective roles of pathways involved in ethanol oxidation in hepatocytes and *in vivo*. ADH^- deermice were used in this study to evaluate the effects of 4-MP on the non-ADH pathways and thus permitted more accurate estimation of the relative contributions by ADH and non-ADH pathways in ADH^+ deermice. With this approach, we have demonstrated the significance of non-ADH pathways (primarily MEOS) for ethanol oxidation *in vivo* and in hepatocytes of this species.

Acknowledgements—This study was supported by DHHS grants AA03508 and AA05934 and the Veterans Administration. We thank Ms. N. Bryant for skillful typing of the manuscript, and Ms. T. Wojtowicz for expert technical assistance.

REFERENCES

1. H. Theorell and T. Yonetani, *Biochem. Z.* **338**, 537 (1963).
2. D. Lester, W. Z. Keokosky and F. Felzenberg, *Q. Jl Stud. Alcohol* **29**, 449 (1968).
3. N. W. Cornell, C. Hansch, K. H. Kim and K. Henegar, *Archs. Biochem. Biophys.* **227**, 81 (1983).
4. C. S. Lieber and L. M. DeCarli, *J. Pharmac. exp. Ther.* **181**, 279 (1972).
5. U. Rydberg and A. Neri, *Acta pharmac. tox.* **31**, 421 (1972).
6. C. S. Lieber, E. Rubin, L. M. DeCarli, P. Misra and H. Gang, *Lab. Invest.* **22**, 615 (1970).
7. N. Grunnet, B. Quistorff and H. I. D. Thieden, *Eur. J. Biochem.* **40**, 275 (1973).
8. S. Matsuzaki, E. Gordon and C. S. Lieber, *J. Pharmac. exp. Ther.* **217**, 133 (1981).
9. C. S. Lieber and L. M. DeCarli, *J. biol. Chem.* **245**, 2505 (1970).
10. J. Papenberg, J. P. von Wartburg and H. Aebi, *Enzym. biol. clin.* **11**, 237 (1970).
11. B. V. Plapp, K. G. Leidal, R. K. Smith and B. P. Murch, *Archs Biochem. Biophys.* **230**, 30 (1984).
12. R. Teschke, Y. Hasumura and C. S. Lieber, *Archs. Biochem. Biophys.* **175**, 635 (1976).
13. R. Teschke, S. Matsuzaki, K. Ohnishi, L. M. DeCarli and C. S. Lieber, *Alcoholism: Clin. expl. Res.* **1**, 7 (1977).
14. R. Kato, *Jap. J. Pharmac.* **17**, 56 (1967).
15. E. Feytmans and F. Leighton, *Biochem. Pharmac.* **22**, 349 (1973).
16. K. G. Burnett and M. R. Felder, *Biochem. Pharmac.* **29**, 125 (1980).
17. Y. Shigeta, F. Nomura, S. Iida, M. A. Leo, M. R. Felder and C. S. Lieber, *Biochem. Pharmac.* **33**, 807 (1984).
18. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
19. R. K. Bonnichsen and N. G. Brink, in *Methods in Enzymology* (Eds. C. P. Colowick and N. O. Kaplan), Vol. 1, p. 495. Academic Press, New York (1955).
20. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
21. K. Ohnishi and C. S. Lieber, *Archs Biochem. Biophys.* **191**, 798 (1978).
22. M. N. Berry and D. S. Friend, *J. Cell Biol.* **43**, 406 (1969).
23. J. Deschenes, J-P. Valet and N. Marceau, *In Vitro* **16**, 722 (1980).
24. M. N. Berry, H. V. Werner and E. Kun, *Biochem. J.* **140**, 355 (1974).
25. S. E. Damgaard, *Eur. J. Biochem.* **125**, 593 (1982).
26. W. G. Heim, D. Appleman and H. T. Pyriom, *Am. J. Physiol.* **186**, 19 (1956).
27. C. Delmas, G. de S. Blanquat, C. Freudenreich and J-F. Biellmann, *Alcoholism: Clin. expl. Res.* **7**, 264 (1983).
28. A. I. Cederbaum and L. Berl, *Archs Biochem. Biophys.* **216**, 530 (1982).
29. K. E. Crow, N. W. Cornell and R. L. Veech, *Alcoholism: Clin. expl Res.* **1**, 43 (1977).
30. L. Goldberg and U. Rydberg, *Biochem. Pharmac.* **18**, 1749 (1969).
31. A. Boveris, N. Oshino and B. Chance, *Biochem. J.* **128**, 617 (1972).
32. E. Glassman, G. McLaughlin, D. Forman, M. Felder and R. Thurman, *Biochem. Pharmac.* **34**, 3523 (1985).
33. T. Takagi, J. Alderman and C. S. Lieber, *Alcohol* **2**, 9 (1985).
34. C. Vind and N. Grunnet, *Biochem. Pharmac.* **34**, 655 (1985).