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THE ROLES OF THE HEPATOCELLULAR REDOX STATE AND THE HEPATIC ACETALDEHYDE CONCENTRATION IN DETERMINING THE ETHANOL ELIMINATION RATE IN FASTED RATS

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Abstract—Ethanol administration (2 g/kg i.p.) to fasted male Wistar rats caused, on average, a 64% decrease in the cytosolic free NAD^+:NADH ratio and a 41% decrease in the mitochondrial free NAD^+:NADH ratio measured 90 min after ethanol was injected. Treatment of animals with either Naloxone (2 mg/kg i.p.) 1 hr after ethanol or 3-palmitoyl-(+)-catechin (100 mg/kg p.o. 1 hr before ethanol) prevented these ethanol induced redox state changes, without affecting the ethanol elimination rate or the hepatic acetaldehyde concentration measured at 90 min after ethanol administration. The thiol compounds cysteine and malotilate (diisopropyl-1,3-dithiol-2-ylidene malonic acid) significantly lowered the hepatic acetaldehyde concentrations measured at 0.75, 1.5 and 6.0 hr after ethanol, and caused a 29% and 12% increase respectively in the ethanol elimination rate, without affecting the ethanol induced alterations in the NAD^+:NADH ratio. Pretreatment of animals with the aldehyde dehydrogenase inhibitor, cyanamide (1 mg/kg or 15 mg/kg p.o. one hour before ethanol), caused increases of up to 23-fold in the hepatic acetaldehyde level, without influencing the cytosolic NAD^+:NADH ratio in ethanol dosed rats, while significantly reducing the ethanol elimination rate by up to 44%, compared with controls.

These results suggest that ethanol oxidation by cytosolic alcohol dehydrogenase may be regulated in part by the hepatic acetaldehyde concentration achieved during ethanol metabolism rather than NADH reoxidation, either to supply NAD for the dehydrogenase, or to reduce inhibition of the enzyme by NADH, being a rate-limiting factor in ethanol metabolism in fasted rats.

Ethanol is metabolized mainly in the liver, the major pathway involving oxidation by alcohol dehydrogenase (ADH; EC.1.1.1.1) to acetaldehyde in the cytosol of the hepatocyte. Acetaldehyde is then oxidized by aldehyde dehydrogenase (AlDH) to acetate. In the rat, the latter stage is thought to occur mainly in the mitochondria, although, in humans, the cytosolic AlDH isozyme may be of more importance [1, 2]. A microsomal ethanol oxidizing system (MEOS) has been described, which may be important after chronic ethanol intake, when the activity of this MEOS pathway is enhanced [3].

In naive rats, a number of experimental conditions can influence the rate at which ethanol is metabolized. Fasting decreases the ethanol elimination rate by 40–50% [4], while castration in fed male rats increases the ethanol elimination rate [5, 6]. A number of studies have shown that these changes in ethanol elimination rate may be directly related to changes in the total hepatic ADH activity that these experimental manipulations produce, i.e. fasting decreases, and castration of fed male rats increases the hepatic ADH activity. Thus, a number of workers have concluded that it is the total liver ADH content or ADH activity that is the major rate-determining factor in ethanol elimination [5, 7]. However, other studies have shown a poor correlation between the *in vivo* ethanol elimination rate and the hepatic ADH activity expressed on a "per gram liver" basis [8–10]. Fasting does not in fact change the specific liver ADH activity expressed as units per gram weight or per gram liver protein [11]. This suggests that factors other than simply the hepatic ADH activity may be responsible for regulating the rate of ethanol metabolism *in vivo*, although the mechanisms that influence ethanol metabolism in the presence of a given amount of hepatic ADH activity are still far from clear. Both ADH and AlDH are NAD^+-dependent dehydrogenases, so that while ethanol oxidation is proceeding, there is a marked decrease in the hepatic NAD^+:NADH ratio that is reflected in the cytosolic and mitochondrial compartments of the hepatocyte as increased lactate:pyruvate and 3-hydroxybutyrate:acetoacetate ratios respectively. Kinetic studies using purified rat liver ADH have shown that the inhibitor constant for NADH is of the order of 0.9 μM [4] for this enzyme, so that this redox state alteration, and the associated increase in the cytosolic free NADH concentration, may be a limiting factor for ethanol metabolism. Studies on isolated hepatocytes suggest that this factor could inhibit the oxidation of ethanol *in vivo* by as much as 20%, so that correction of the redox state during ethanol metabolism may enhance the elimination rate [12]. Thus, it has been suggested that the rate of NADH reoxidation is the rate limiting step in

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ethanol metabolism [13], a theory which is partly supported by the finding that methylene blue, which carries out non-enzymatic oxidation of NADH, causes a 40% increase in ethanol oxidation by liver slices [14]. However, convincing evidence for this being an important factor in determining the ethanol elimination rate *in vivo* is still lacking.

An alternative hypothesis is that the ethanol metabolite, acetaldehyde, may act as an inhibitor of ADH, so that the hepatic acetaldehyde concentration during ethanol oxidation may be of importance in determining the elimination rate. Acetaldehyde has an inhibitor constant of 12 μM for rat liver ADH [4], a value that is fairly close to the steady-state hepatic concentration that has been reported after acute ethanol administration in fasted rats [15, 16]. Experiments *in vitro* have shown that ethanol oxidation by the soluble fraction of rat liver is stimulated by the addition of mitochondria to the system, this stimulation being prevented by the presence of the AlDH inhibitor, cyanamide, suggesting a regulatory role for acetaldehyde [17]. This factor may be of particular importance in human alcoholic subjects, who have been shown to have a selective reduction in cytosolic aldehyde dehydrogenase activity, resulting in impaired acetaldehyde oxidation [18–20].

The current study has been carried out to differentiate between the two factors that could regulate ethanol oxidation in fasted rats, that in turn could influence ethanol metabolism independently of changes in the total hepatic ADH activity. The possible role of the hepatic redox state in regulating ethanol metabolism has been assessed by observing the influence that the drugs 3-palmitoyl-(+)-catechin and Naloxone have on the ethanol elimination rate *in vivo*. Both these substances have been reported to reverse the ethanol-induced alterations in the hepatic NAD^+:NADH ratio following ethanol administration, apparently without inhibiting ethanol metabolism [21–25]. The possible regulatory role of acetaldehyde has been assessed by observing the effects that cysteine, Malotilate and the AlDH inhibitor cyanamide have on the ethanol elimination rate. Cysteine is thought to form an adduct with acetaldehyde to yield thiazolidine, thus lowering the hepatic level of ethanol-derived acetaldehyde during ethanol metabolism *in vivo* [26]. Malotilate is a sulphur-containing hepatoprotective drug (diisopropyl-1,3-dithiol-2-ylidenemalonic acid) that has been found to have *in vivo* an effect similar to that of cysteine, although the exact mechanism by which this compound apparently lowers the hepatic acetaldehyde concentration is not understood.

MATERIALS AND METHODS

Chemicals and drugs. All chemicals used were of the highest purity commercially available. 3-Palmitoyl-(+)-catechin was supplied by the Research Department (Zyma SA, Nyon, Switzerland), and malotilate was prepared by Nihon Nohyaku Co. (Tokyo, Japan). Pure naloxone hydrochloride powder was a gift from Du Pont Ltd. Cyanamide and cysteine hydrochloride were obtained from Sigma Chemical Co. (Poole, Dorset, U.K.).

Animals and diets. Male Wister albino rats (University of Surrey strain, 180–230 g) were used. They were housed in pairs in wire-bottomed cages in a room maintained at 22°. Lighting was provided between 0700 hr and 2100 hr daily. They were maintained on Heygates 41B cube diet and water *ad lib.* All animals were fasted for 24 hr prior to ethanol administration.

Ethanol and drug administration. Ethanol (2 g/kg) was administered by the intraperitoneal injection of a 20% (w/v) solution in saline. In experiments where the hepatic acetaldehyde and metabolite concentrations were determined, animals were sacrificed at the appropriate time points after ethanol administration and a piece of liver (about 2 g in weight) rapidly isolated by freeze-clamping using aluminium tongs which had been pre-cooled in liquid nitrogen. In experiments in which ethanol elimination rate was measured, samples of tail blood were obtained at 0.75, 1.5, 3.0, 4.5 and 6.0 hr after ethanol administration and assayed for ethanol by head space gas chromatography using *n*-propanol as the internal standard, under the same conditions as described below for hepatic acetaldehyde. The rate of ethanol disappearance from blood was assumed to obey zero-order kinetics, and the best straight line fit for the blood ethanol disappearance curve for each experimental group was determined using a least-squares regression analysis programme (VisiCalc©) on an Apple computer. The Widmark equation was then applied to determine the ethanol elimination rate in mg/100 ml blood/hr [23].

Malotilate, cysteine and 3-palmitoyl-(+)-catechin were given at doses of 100 mg/kg, 250 mg/kg and 100 mg/kg respectively as suspensions or solutions in 5% (w/v) arabic gum by oral intubation 1 hr before ethanol administration. Naloxone (2 mg/kg) was given as a solution of naloxone hydrochloride in saline by intraperitoneal injection at 1.0, 2.5 and 4.0 hr after ethanol administration. This repeated dosing regime was used in order to maintain fairly constant levels of the drug during the intoxication period, as naloxone has a plasma half-life in the rat of less than one hour [27]. Cyanamide was given as a solution in 0.75% (w/v) methyl cellulose at doses of either 1 mg/kg or 15 mg/kg by oral intubation one hour before ethanol administration.

Hepatic metabolite determinations. Freeze clamped liver samples were obtained 1.5 hr after ethanol administration, a time point at which the steady-state levels of acetaldehyde and metabolites of the various redox pairs should be achieved [28]. In the first series of experiments, liver samples for determination of acetaldehyde were also obtained at 0.75 hr, 3.0 hr and 6.0 hr after ethanol administration. Frozen liver samples were ground to a powder under liquid nitrogen using a pestle and mortar. For acetaldehyde analysis, approximately 300 mg powdered tissue was extracted with 3.0 ml ice-cold 0.6 N perchloric acid (PCA) containing 25 mM thiourea to prevent the non-enzymic production of acetaldehyde from ethanol present in the sample during this procedure [29]. The sample was immediately centrifuged in the cold at 2000 *g* for 5 min, and 2.0 ml supernatant removed into a glass vial containing 1.0 ml propanol internal standard

solution (0.5 mg/ml) which was then sealed with a Teflon lined rubber septum cap. The vial was then heated in a water bath at 60° for exactly 30 min, 2.0 ml head space then being removed using a Pressure-Lok A2 gas syringe and injected into a Hewlett Packard 5710A Gas Chromatograph fitted with a 1.8 m × 4 mm i.d. glass column packed with Porapak Q (50–80 mesh). Running conditions for the gas chromatograph were: column temp. 150°, flame-ionization detector temp. 250°, injection port temp. 200°, nitrogen carrier gas flow 30 ml/min, hydrogen flow 30 ml/min, and air flow 200 ml/min. The retention times for acetaldehyde, ethanol and *n*-propanol were 58, 98 and 260 sec respectively. The gas chromatograph was calibrated daily using freshly prepared aqueous solutions of ethanol and acetaldehyde, the peak height ratios method being used to calculate the concentrations present in the samples. The recovery of acetaldehyde using this method was 94.1%, and providing that the protein precipitate was centrifuged away from the samples as soon as the powdered liver had been mixed with the PCA-thiourea solution, no problems of artefactual formation of acetaldehyde were encountered [30].

For the determination of hepatic redox pair metabolites, approximately 800–1000 mg powdered liver was extracted with 5.0 ml ice-cold 0.6 N PCA, centrifuged in the cold, the residue re-extracted with 2.0 ml 0.3 N PCA, and recentrifuged. The combined supernatants were neutralized to pH 5–6 using 5 M K_2CO_3 with a pH meter. The clear extract, obtained after centrifuging away the potassium perchlorate precipitate, was treated with Florisil (0.1 g/ml extract) as described by Williamson *et al.* [31]. Lactate, pyruvate, 3-hydroxybutyrate and acetoacetate were determined by the methods of Gutmann and Wahlefeld [32], Czok and Lamphrect [33], Williamson and Mellanby [34] and Mellanby and Williamson [35] respectively. Cytosolic and mitochondrial free NAD^+:NADH ratios were calculated from the lactate:pyruvate and 3-hydroxybutyrate:acetoacetate ratios respectively assuming equilibrium constants for rat liver lactate dehydrogenase and 3-hydroxybutyrate dehydrogenase of 1.11×10^{-4} mM and 4.93×10^{-2} mM respectively [31].

Statistical analysis. Unpaired Student's *t*-test was used to analyse the significance of difference between two means. Results are expressed as mean values ± standard deviations.

RESULTS

3-Palmitoyl-(+)-catechin pretreatment did not affect the hepatic acetaldehyde concentrations following ethanol administration, whereas administration of malotilate or cysteine significantly lowered the acetaldehyde level at most of the time points studied (Table 1; Fig. 1). Naloxone did not affect the hepatic acetaldehyde concentration measured 1.5 hr after ethanol administration, whereas cyanamide, at doses of 1 mg/kg and 15 mg/kg orally, caused 10-fold and 23-fold increases respectively in the hepatic level of this ethanol metabolite at the same time point (Table 1).

Ethanol administration caused an increase of between 130 and 230% in the hepatic lactate:pyruvate ratio, and a simultaneous increase of approximately 70% in the 3-hydroxybutyrate:acetoacetate ratio at 1.5 hr after dosing, when values were compared with those from saline dosed controls (Table 2). Thus, ethanol caused a calculated 58–68% decrease in the cytosolic free NAD^+:NADH ratio, and a 41% decrease in the mitochondrial free NAD^+:NADH (Table 3). Treatment of animals with either naloxone or 3-palmitoyl-(+)-catechin prevented these ethanol induced redox state changes, the hepatic lactate:pyruvate, 3-hydroxybutyrate:acetoacetate and associated cytosolic and mitochondrial NAD^+:NADH ratios being restored virtually to control values by the administration of these drugs to ethanol treated animals. These effects of naloxone and 3-palmitoyl-(+)-catechin were statistically significant. Malotilate and cysteine were found to have no influence on the alteration of the hepatocellular redox state induced by ethanol

Table 1. Effects of various drug treatments on hepatic acetaldehyde concentrations following ethanol administration

Treatment	Hepatic acetaldehyde 90 min after ethanol (nmoles/g)
Experiment 1	
Control	6.52 ± 1.21 (5)
Ethanol + arabic gum	24.02 ± 6.02 (8)
Ethanol + 3-palmitoyl-(+)-catechin	22.91 ± 5.13 (5)
Experiment 2	
Ethanol + saline	22.53 ± 4.12 (4)
Ethanol + naloxone	27.82 ± 6.17 (4)
Experiment 3	
Ethanol + methyl cellulose	22.04 ± 4.83 (8)
Ethanol + cyanamide (1 mg/kg)	221.7 ± 27.5* (6)
Ethanol + cyanamide (15 mg/kg)	509.4 ± 81.2* (6)

3-Palmitoyl-(+)-catechin was given at a dose of 100 mg/kg, as a suspension in 5% (w/v) arabic gum by the oral route 1 hr before ethanol administration (2 g/kg). Naloxone hydrochloride (2 mg/kg i.p.) was given in saline 1 hr after the same ethanol dose. Cyanamide was given orally as a solution in 0.75% (w/v) methyl cellulose 1 hr before ethanol. Each value given is the mean ± S.D., the number of determinations being given in parentheses.

* $P < 0.001$ vs the ethanol-methyl cellulose group.

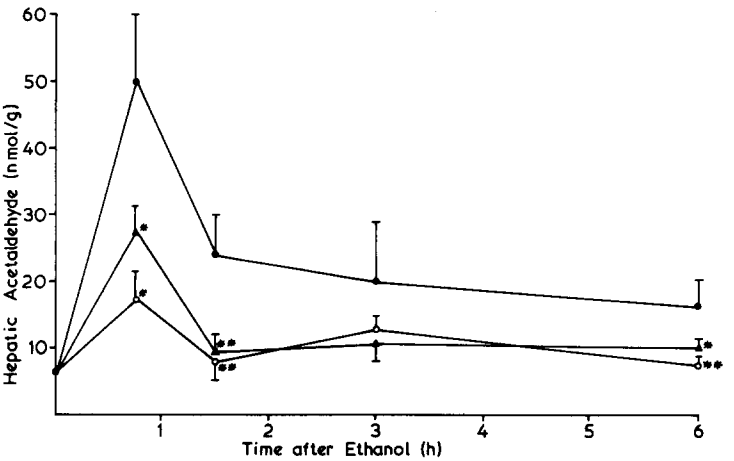

Fig. 1. The effect of malotilate or cysteine on hepatic acetaldehyde after acute ethanol administration. Malotilate and cysteine were given at doses of 100 mg/kg and 250 mg/kg respectively by the oral route one hour after intraperitoneal injection of ethanol (2 g/kg). Each point represents the mean + S.D. of at least four animals: ●, ethanol only; ▲, ethanol and cysteine; ○, ethanol and malotilate. *P < 0.05 vs ethanol only group; **P < 0.02 vs ethanol only group.

administration (Tables 2 and 3). The effect of cyanamide on the hepatocellular redox state was studied only in ethanol dosed animals at the higher dose of this AlDH inhibitor (15 mg/kg orally). Cyanamide was found to have little effect on the lactate: pyruvate ratio or the cytosolic NAD^+: NADH ratio in ethanol treated animals (Tables 2 and 3). However, the drug did decrease the 3-hydroxybutyrate: acetoacetate ratio, presumably as a result of effective inhibition of the mitochondrial NAD^+ dependent AlDH isoenzyme (Table 2).

Administration of 3-palmitoyl-(+)-catechin or Naloxone to ethanol dosed animals, made no obvious difference to the blood ethanol concentrations at any of the time points studied or to the calculated ethanol elimination rates (Table 4). Malotilate slightly lowered the blood ethanol level at all of the time points studied, an effect which became statistically significant 4.5 hr after ethanol administration ($P < 0.05$; Fig. 2). This compound did increase the ethanol elimination rate, although this effect was not statistically significant (Table 4). Cysteine actually increased the initial blood ethanol concentration measured 0.75 hr after ethanol administration, but as a result of the significant stimulating effect the compound had on the ethanol elimination rate ($P < 0.05$), at the 6.0 hr time point, cysteine had significantly lowered the blood ethanol concentration

Table 2. Effect of the various drug treatments on the hepatic lactate/pyruvate and 3-hydroxybutyrate/acetoacetate ratios after acute ethanol administration

Drug	Treatment regime			
	Saline control	Saline and drug	Ethanol only	Ethanol and drug
	(i) Lactate/pyruvate			
Naloxone	16.5 ± 3.0	15.7 ± 5.1	38.5 ± 8.0*	18.2 ± 3.2*
3-Palmitoyl-(+)-catechin	13.0 ± 2.4	15.2 ± 3.4	41.6 ± 3.2*	12.0 ± 2.6†
Malotilate	13.2 ± 4.0	10.3 ± 1.4	32.1 ± 5.5*	28.0 ± 2.3
Cysteine	13.2 ± 4.0	14.6 ± 2.1	32.1 ± 5.5*	35.8 ± 2.2
Cyanamide (15 mg/kg)	N.D.	N.D.	41.5 ± 3.9	39.4 ± 4.5
	(ii) 3-Hydroxybutyrate/acetoacetate			
Naloxone	2.58 ± 0.30	2.75 ± 0.49	4.35 ± 0.10*	2.54 ± 0.66*
3-Palmitoyl-(+)-catechin	N.D.	N.D.	N.D.	N.D.
Malotilate	2.60 ± 0.30	1.89 ± 0.41	4.43 ± 0.18*	5.15 ± 0.57
Cysteine	2.60 ± 0.30	1.71 ± 0.22†	4.43 ± 0.18*	3.78 ± 0.17
Cyanamide (15 mg/kg)	N.D.	N.D.	7.60 ± 1.02	5.00 ± 0.80§

Determinations were made on pieces of liver freeze clamped 90 min after administration of ethanol (2 g/kg i.p.) or an equivalent volume of saline.

Details of drug dosages and regimes are given in Table 1. Each value represents the mean ± S.D. of five experimental animals.

*$P < 0.001$ vs saline only group.
† $P < 0.001$ vs ethanol only group.
‡ $P < 0.05$ vs saline only group.
§ $P < 0.01$ vs ethanol only group.
N.D. = not determined.

Table 3. The effect of drug treatments on cytosolic and mitochondrial free NAD^+:NADH ratios following ethanol administration

Drug	Treatment regime: Saline control	Saline + drug	Ethanol only	Ethanol + drug
	(i) Cytosolic NAD^+:NADH			
Naloxone	546 ± 99	574 ± 190	234 ± 49*	495 ± 87†
3-Palmitoyl-(+)-catechin	693 ± 128	593 ± 133	217 ± 17*	751 ± 163†
Malotilate	683 ± 207	874 ± 119	281 ± 48*	322 ± 26
Cysteine	683 ± 207	617 ± 89	281 ± 48*	252 ± 15
Cyanamide (15 mg/kg)	N.D.	N.D.	217 ± 20	229 ± 26
	(ii) Mitochondrial NAD^+:NADH			
Naloxone	7.9 ± 0.9	7.4 ± 1.3	4.7 ± 0.1**	8.0 ± 2.1*
3-Palmitoyl-(+)-catechin	N.D.	N.D.	N.D.	N.D.
Malotilate	7.8 ± 0.9	10.7 ± 2.3	4.6 ± 0.2**	3.9 ± 0.4
Cysteine	7.8 ± 0.9	11.9 ± 1.5†	4.6 ± 0.2**	5.4 ± 0.2
Cyanamide (15 mg/kg)	N.D.	N.D.	2.7 ± 0.4	4.1 ± 0.7‡

Ethanol was given at a dose of 2 g/kg (i.p.) and redox state measurements were made on frozen liver samples taken 90 min after ethanol dosing.

Values were determined from the lactate/pyruvate and 3-hydroxybutyrate/acetoacetate ratios shown in Table 2. Other experimental details are given in Table 2.

* $P < 0.001$ vs saline control group.
† $P < 0.001$ vs ethanol only group.
‡ $P < 0.05$ vs saline control group.
§ $P < 0.01$ vs ethanol only group.

($P < 0.05$). Malotilate and cysteine caused 12% and 29% increases in the ethanol elimination rate respectively (Table 4).

Both the low and high doses of cyanamide significantly increased the blood ethanol concentration at nearly all the time points studied. The low dose (1 mg/kg) caused a 31% decrease in the ethanol elimination rate ($P < 0.001$) and the high dose (15 mg/kg) inhibited ethanol disappearance by 44% compared with controls ($P < 0.001$; Table 4).

DISCUSSION

This study has shown that correction of the ethanol induced alterations in the hepatic redox state (NAD^+:NADH ratio) after acute ethanol administration does not affect the rate of ethanol elimination in fasted male Wistar rats. However, administration of compounds that lower the hepatic acetaldehyde concentration under identical conditions, and which do not reverse the ethanol induced alteration in the redox state, increased the rate of ethanol disappearance from blood. In addition, elevation of the hepatic acetaldehyde concentration by administration of the AlDH inhibitor, cyanamide, causes marked inhibition of ethanol metabolism, in an approximately dose-dependent manner. Apart from a slight effect on the mitochondrial NAD^+:NADH

Table 4. Effect of drug treatments on ethanol elimination from blood following acute ethanol administration

Treatment group	Ethanol elimination rate (mg/100 ml/hr)
Experiment 1	
Ethanol + arabic gum	26.83 ± 2.14 (8)
Ethanol + malotilate	30.00 ± 2.33 (5)
Ethanol + cysteine	34.57 ± 3.80* (5)
Ethanol + 3-palmitoyl-(+)-catechin	26.20 ± 2.71 (5)
Experiment 2	
Ethanol + saline	31.00 ± 1.21 (5)
Ethanol + naloxone	32.33 ± 1.36 (5)
Experiment 3	
Ethanol + methyl cellulose	34.44 ± 2.69 (8)
Ethanol + cyanamide (1 mg/kg)	23.66 ± 3.39† (6)
Ethanol + cyanamide (15 mg/kg)	19.30 ± 1.87† (6)

Ethanol was given at a dose of 2 g/kg (i.p.) and the elimination rate calculated from blood ethanol determinations carried out in the first six hours after ethanol dosing. Drug dosages and other experimental details are as given in the legends to the other Figures and Tables. Each value represents the mean ± S.D., the number of determinations being given in parentheses, and was calculated as described in the methods section.

* $P < 0.05$ vs ethanol only group.
† $P < 0.001$ vs ethanol only group.

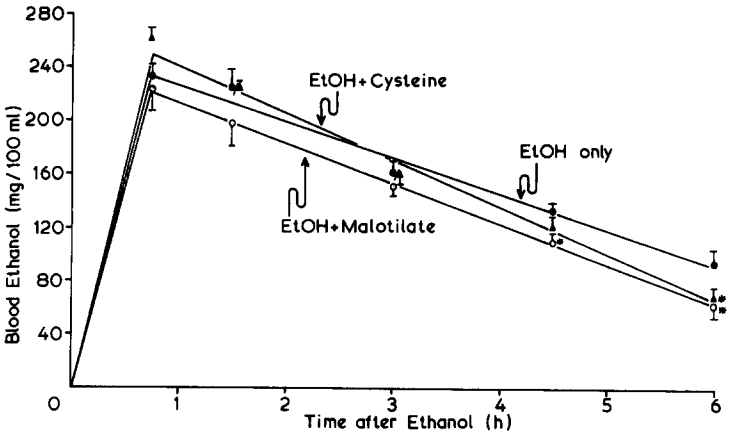

Fig. 2. The effect of malotilate or cysteine on blood ethanol concentrations following acute ethanol administration (2 g/kg i.p.). Experimental details as in legend to Fig. 1. ●, ethanol only; ▲, ethanol and cysteine; ○, ethanol and malotilate. *$P < 0.05$ vs ethanol only group.

ratio, cyanamide did not influence the hepatic redox state in ethanol treated animals. It therefore seems that it is the hepatic acetaldehyde concentration and not the hepatic NAD^+:NADH ratio (or similarly, the rate of NADH oxidation) that influences ethanol metabolism in fasted rats. This finding is perhaps not surprising when one considers that the equilibrium for rat liver ADH lies in favour of the reverse reaction. Under physiological conditions, rat liver supernatants metabolise ethanol to acetaldehyde at a rate of 5.16 μmol/min/g liver, whereas identical preparations will reduce acetaldehyde to ethanol at a rate of 26.9 μmol/min/g liver [36]. Thus, *in vivo*, effective removal of acetaldehyde by AlDH is probably very important in maintaining the ADH reaction in the forward direction (i.e. ethanol to acetaldehyde).

Recent studies have shown that rat liver ADH obeys a Theorell–Chance mechanism, and that at low ethanol concentrations (<10 mM), ethanol does in fact exert some substrate inhibition consistent with the formation of a dead end ADH–NADH–ethanol complex. This theory was supported by data indicating that the coenzyme bound to ADH is not in equilibrium with the free NADH pool [37]. The alternative controlling factor related to the redox state, namely that NAD^+ may become limiting, is not supported by a recent study in which the authors suggested that the free NAD^+ concentration does not change significantly after ethanol administration, nearly all of the decrease in the hepatic NAD^+:NADH ratio being accounted for by an increase in the hepatic free NADH concentration [12]. Thus, in the present study, if it is assumed that the hepatic free NAD^+ concentration remains constant at 500 μM [38, 39], administration of 2.0 g/kg ethanol would cause an increase in the free cytosolic NADH concentration from 0.78 μM to 2.05 μM in the present study. On the basis of the quoted inhibitor constant of 0.9 μM with respect to NADH for ADH [10], this change could certainly exert considerable inhibition on the rate of ethanol oxidation by ADH. Thus, our results may not be inconsistent with the theory that NADH at the active site of ADH is not equilibrated with the free NADH pool. Correction of the free NAD^+:NADH ratio by Naloxone and 3-palmitoyl-(+)-catechin and consequently correction of the free NADH concentration at the same time, should release ADH from inhibition by NADH and thus increase the rate of ethanol metabolism, whereas this was not found to be the case. However, these drugs may not be affecting the rate of dissociation of NADH from ADH so that if there was some way this could be enhanced, the ethanol elimination rate may be stimulated.

The effects of cyanamide presented here, namely increased hepatic acetaldehyde concentrations and a decreased ethanol elimination rate, agree with previous reports [40]. Such a central role of acetaldehyde in regulating ethanol metabolism is further supported by our findings with cysteine and malotilate. While these two compounds tend to enhance the ethanol elimination rate, they exerted a clear ability to reduce hepatic acetaldehyde concentrations, by an average of about 11 nmoles/g during ethanol oxidation, a value close to the quoted inhibitor constant of acetaldehyde for rat liver ADH [10]. One alternative explanation for the stimulating effect that these substances had on ethanol metabolism is that they increased the total liver ADH content. However, although ADH activities were not determined in the present study, this explanation seems unlikely in view of the short time period following drug administration during which ethanol elimination was studied, and the fact that rat liver ADH has a half life in fasted rats of 2.8 days [11].

Thus, in conclusion, this study has shown that the hepatic acetaldehyde concentration may be of importance in governing the ethanol elimination rate in fasted rats, while the free NAD^+:NADH ratio does not seem to be of obvious importance. This study is not, however, inconsistent with theories which suggest that the rate of dissociation of NADH from ADH could also influence the rate of oxidation of ethanol by this enzyme. Whether these considerations also apply to rats in the fed state is

difficult to assess, as the total liver ADH activity is considerably higher under these conditions, so that this, and other dietary factors may be of more importance in determining the ethanol elimination rate under these conditions. These findings may however, be of importance to human alcoholics who have been shown to have impaired acetaldehyde oxidation [20].

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REFERENCES

1. T. J. Peters, *Br. med. Bull.* **38**, 17 (1982).
2. W. J. Jenkins and T. J. Peters, *Cell. Biochem. Function.* **1**, 37 (1983).
3. C. S. Lieber, *Pharmac. Biochem. Behav.* **18**, Suppl. 1, 181 (1983).
4. D. W. Crabb, W. F. Bosron and T.-K. Li, *Archs Biochem. Biophys.* **224**, 299 (1983).
5. E. Mezey, J. J. Potter, S. M. Harmon and P. D. Tsitouras, *Biochem. Pharmac.* **29**, 3175 (1980).
6. T. J. Cicero, J. D. Bernard and K. Newman, *J. Pharmac. exp. Ther.* **215**, 317 (1980).
7. G. Rachamin, J. A. Macdonald, S. Wahid, J. J. Clapp, J. M. Khanna and Y. Israel, *Biochem. J.* **186**, 483 (1980).
8. T. J. Braggins and K. E. Crow, *Eur. J. Biochem.* **119**, 633 (1981).
9. H. Wallgren and H. Barry, *Actions of Alcohol*, Vol. 1, pp. 77–153. Elsevier, Amsterdam (1970).
10. L. Lumeng, W. F. Bosron and T.-K. Li, *Biochem. Pharmac.* **28**, 1547 (1979).
11. W. F. Bosron, D. W. Crabb, T. A. Howinger and T-K. Li, *Alchsm. clin. exp. Res.* **8**, 196 (1984).
12. K. E. Crow, K. M. Newland and R. D. Batt, *Pharmac. Biochem. Behav.* **18**, Suppl. 1, 237 (1983).
13. A. A-B. Badawy, *Clin. Endocrin. Metab.* **7**, 247 (1978).
14. D. E. Smith and H. W. Newman, *J. biol. Chem.* **234**, 1599 (1959).
15. L. Lumeng and D. W. Crabb, *Biochem. Pharmac.* **33**, 2623 (1984).
16. C. J. P. Eriksson, T. V. Widenius, R. H. Ylikahri, M. Harkonen and P. Leinonen, *Biochem. J.* **210**, 29 (1983).
17. A. G. Dawson, *Biochem. Pharmac.* **32**, 2157 (1983).
18. W. J. Jenkins and T. J. Peters, *Lancet* **ii**, 628 (1980).
19. M. Thomas, S. Halsall and T. J. Peters, *Lancet* **ii**, 1057, 16 (1982).
20. K. R. Palmer and W. J. Jenkins, *Gut* **23**, 729 (1982).
21. A. A-B. Badawy and M. Evans, *Br. J. Pharmac.* **74**, 514 (1981).
22. A. A-B. Badawy and S. U. Aliyu, *Alcohol. Alchsm* **19**, 199 (1984).
23. P. R. Ryle, J. Chakraborty and A. D. Thomson, *Alcohol. Alchsm* **20**, in press (1985).
24. A. Gajdos, M. Gajdos-Torok and R. Horn, *Biochem. Pharmac.* **21**, 595 (1972).
25. P. R. Ryle, J. Chakraborty and A. D. Thomson, *Pharmac. Biochem. Behav* **18**, Suppl. 1, 473 (1983).
26. C. Hirayama, Y. Kishimoto, T. Wakushima and Y. Murawaki, *Biochem. Pharmac.* **32**, 321 (1983).
27. S. H. Ngai, B. A. Berkowitz, J. C. Young, J. Hempstead and S. Spector, *Anaesthesiology* **44**, 398 (1976).
28. L. Lumeng, M. B. Waller, W. J. McBride and T.-K. Li, *Pharmac. Biochem. Behav* **16**, 125 (1982).
29. C. J. P. Eriksson, H. W. Sippel and O. A. Forsander, *Analyt. Biochem.* **80**, 116 (1977).
30. C. J. P. Eriksson, *Alcoholism* **4**, 22 (1980).
31. D. H. Williamson, P. Lund and H. A. Krebs, *Biochem. J.* **103**, 514 (1967).
32. I. Gutmann and A. W. Wahlefeld, in *Methods of Enzymatic Analysis* (Ed. H. U. Bergmeyer), p. 1415. Academic Press, London (1974).
33. R. Czok and W. Lamprecht, in *Methods of Enzymatic Analysis* (Ed. H. U. Bergmeyer), p. 1446. Academic Press, London (1974).
34. D. H. Williamson and J. Mellanby, in *Methods of Enzymatic Analysis* (Ed. H. U. Bergmeyer), p. 1836. Academic Press, London (1974).
35. J. Mellanby and D. H. Williamson, in *Methods of Enzymatic Analysis* (Ed. H. U. Bergmeyer), p. 1840. Academic Press, London (1974).
36. N. W. Cornell, *Pharmac. Biochem. Behav.* **18**, Suppl. 1, 215 (1983).
37. W. F. Bosron, D. W. Crabb and T.-K. Li, *Pharmac. Biochem. Behav.* **18**, Suppl. 1, 223 (1983).
38. T. Cronholm, *Pharmac. Biochem. Behav.* **18**, Suppl. 1, 229 (1983).
39. L. Lumeng, W. F. Bosron and T-K. Li, in *Alcohol and Aldehyde Metabolising Systems* (Ed. R. G. Thurman), p. 489. Plenum Press, New York (1980).
40. O. Tottmar and H. Marchner, *Acta Pharmac. Toxic.* **38**, 366 (1976).