Biochemical Pharmacology, Vol. 24, pp. 1937–1942. Pergamon Press, 1975. Printed in Great Britain.

EFFECTS OF LONG-TERM ETHANOL TREATMENT ON ALDEHYDE AND ALCOHOL DEHYDROGENASE ACTIVITIES IN RAT LIVER

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(*Received* 23 *January* 1975; *accepted* 30 *April* 1975)

Abstract—Administration of intoxicating doses of ethanol by gavage for 3 weeks caused weight loss and reduced hepatic aldehyde dehydrogenase activity in the soluble, mitochondrial and microsomal fractions. Rats receiving equivalent amounts of ethanol as a constituent of a liquid diet for 5 weeks gained weight and showed no changes in aldehyde dehydrogenase activity. Alcohol dehydrogenase activity was decreased in the rats treated by gavage and unchanged in those given ethanol in the diet, but in spite of this the rate of ethanol elimination was accelerated in both groups. In the livers of two strains of rats genetically selected for their difference in voluntary alcohol consumption, the mitochondrial and microsomal aldehyde dehydrogenase activities had previously been shown to be significantly higher in the alcohol-consuming (AA) than in the alcohol-avoiding (ANA) rats. Similar differences were now found after long-term intragastric ethanol administration, although in both strains the absolute levels of aldehyde dehydrogenase were reduced. Profound reduction of mitochondrial low-K_m aldehyde dehydrogenase activity and high blood acetaldehyde were observed, especially in the ANA rats. This suggests a possible connection between the low activity of this enzyme and the increased acetaldehyde level.

Numerous workers have sought to establish whether prolonged alcohol consumption causes an increase in the activity of alcohol dehydrogenase (ADH, EC 1.1.1.1), the enzyme mainly responsible for degradation of ethanol in the liver. Increased activity has been reported by several authors, but most studies indicate that, in spite of enhanced rates of alcohol elimination after chronic alcohol intake, ADH is not induced by ethanol (for review see von Wartburg[1]). This is not surprising, however, since it is now generally accepted that the activity of ADH is not a major factor in the regulation of ethanol elimination[2, 3].

Little is known about the effects of chronic alcohol intake on the activity of the second enzyme involved in alcohol breakdown, aldehyde dehydrogenase (EC 1.2.1.3)[4–7]. In this paper we report the effects of long-term ethanol administration, either by intubation or as a constituent of the diet, on the activity of aldehyde dehydrogenase in various subcellular fractions of the liver. Liver ADH activity has also been determined and compared with the effect of alcohol treatment on the rate of ethanol elimination.

MATERIALS AND METHODS

Chemicals. The origin and the purity of the chemicals has been described earlier[8].

Animal treatment. Unless otherwise stated, the rats were kept in individual cages and given standard laboratory food (Astra Ewos, Ab Astra, Södertälje, Sweden) and tap water *ad lib.*

Rats given ethanol by gavage. In the experiments with long-term alcohol treatment by daily stomach tube intubations, 5-month-old male Sprague–Dawley rats were given a balanced liquid diet [9] for 2 weeks before and during the experimental period. This diet was given because the passage of solid food from the stomach to the intestine tends to be retarded during prolonged intubation. In these experiments, 9 rats served as controls (C), 11 rats received a moderate dose of ethanol (E_1), (5 g ethanol/kg body wt by stomach tube as a 20% (w/v) solution every second day for 3 weeks) and 8 rats were given a higher dose of ethanol (6·6 g ethanol/kg body wt per day in two doses, also for 3 weeks) (E_2). The original plan was to give these rats 2×5 g ethanol/kg body wt per day, but this dosage caused profound weight loss and impairment of general condition.

Rats given ethanol in the food. Male Spraque–Dawley rats were also used in experiments where ethanol was given as a constituent of a liquid diet. For the first 2 weeks preceding the experiment, the drinking water of a group of rats was replaced by a 7% (w/v) ethanol solution. During this period the mean daily intake in the ethanol treated group was 5·1 g ethanol/kg body wt. After this the rats were given an ethanol-supplemented liquid diet in Richter tubes as their only source of food and water. The diet resembled that described by Videla *et al.*[3] except that rice starch was used as a carbohydrate source. The salt and vitamin mixtures were as specified by Eriksson[10], except for further supplementation with 0·01% vitamin E (α-tocopherol). Ethanol constituted 36% of the caloric content of the diet. The control animals received a similar diet, except that ethanol was replaced isocalorically by rice starch. A polysaccharide was chosen as carbohydrate source, as being more 'physiological' than the sucrose ordinarily used.

The rats were kept on the liquid diets for 5 weeks. The ethanol treated group consumed 9·1 g ethanol/kg body wt per day during this period.

Treatment of genetically selected strains of rats. In the experiments with two strains of rats

genetically selected for differences in alcohol consumption[11, 12], female rats of the alcohol-consuming AA strain and of the alcohol-avoiding ANA strain were used. Ethanol was given daily for 2 weeks by gavage, (5 g ethanol/kg body wt, as a 20% (w/v) solution). In addition, a 10% (w/v) ethanol solution was given as the sole drinking fluid for the first 4 days. On this treatment, however, most of the animals lost weight, and some appeared to be in poor condition. Water was therefore given to all animals as drinking fluid instead of the ethanol solution and the intubation doses were temporarily reduced for 2 or 3 days for those animals which appeared to be in poor condition. After this, the normal intubation schedule was resumed. On the 13th day the concentration of acetaldehyde in tail blood was determined 30 min after gavage.

Liver sampling and subcellular fractionation. The rats were killed by decapitation and bled, their livers were excised and divided into parts, one of which was transfered to −20° and stored for later determination of the NAD-dependent acetaldehyde uptake in homogenates as described below. A second part was homogenized and fractionated into mitochondrial, microsomal and soluble fractions as described previously[8]. For histological examinations a third piece of liver was fixed in 10% buffered formaldehyde solution.

Determination of ethanol elimination. Ethanol (2·5 g/kg) was administered intraperitoneally as a 9 or 10% solution (w/v) in saline. At regular intervals 0·1-ml blood samples were withdrawn from the tip of the tail and pipetted into 0·6 N perchloric acid containing 25 mM thiourea[13]. The elimination rate was calculated from the elimination time by extrapolation to zero ethanol concentration from the linear part of the blood alcohol curve.

Analytical methods. Activities of aldehyde and alcohol dehydrogenase and those of the marker enzymes, glucose-6-phosphatase and succinate-INT-reductase were measured as previously described in detail[8, 14, 15]. The average yield for the microsomal fraction was 70% and 45% for the mitochondrial fraction. Aldehyde dehydrogenase activities were liberated from particulate fractions by addition of sodium deoxycholate to a final concentration of 0·2%, and assayed directly from detergent-treated samples. The enzymatic activities in the subcellular fractions were corrected on the basis of the yield of the marker enzymes[8]. The capacity of crude liver homogenates to utilize acetaldehyde in the presence of NAD was determined by a method modified from that originally used by Marjanen[8, 16].

Ethanol and acetaldehyde were determined with a Perkin–Elmer F 40 gas chromatograph by the head-space technique[17]. Protein was determined by the biuret method of Gornall *et al.*[18] and corrections were made for the effects of sucrose and deoxycholate. Total lipids of liver samples were determined by extraction with chloroform–methanol[19].

RESULTS

In the first series of experiments, where alcohol was administered by gavage, the drastic alcohol treatment (mean daily alcohol dose 6·6 g ethanol/kg body wt) caused great loss of body weight accompanied by signs of poor condition in some rats (Table 1).

The rate of ethanol elimination was significantly faster both in the heavily dosed group (+19%) and in the moderately dosed group (+11%) than in the controls (Table 2). This observation is in accord with earlier studies[3]. Nevertheless the hepatic alcohol dehydrogenase activity was significantly reduced in both the heavily dosed (−36%) and the moderately dosed (−18%) group as compared with the controls.

The drastic alcohol treatment also caused significant loss of aldehyde dehydrogenase activity (Table 3). Analysis of the distribution of the activity after alcohol treatment showed that the loss of activity was not localized to any specific fraction, because the activity was significantly reduced in the soluble, mitochondrial and microsomal fractions, with either 4·5 mM propionaldehyde or 0·5 mM acetaldehyde as substrate. In the moderately treated animals these activities were also reduced, although the effect was less pronounced.

When 0·5 mM acetaldehyde was used as substrate the contribution of the activities in the soluble and microsomal fractions were rather low. This implies that the absolute difference in the sum of these activities is mainly due to low-K_m enzymes in the mitochondrial fraction, although the difference in activity in this fraction was not statistically significant.

In addition to spectrophotometric determination of aldehyde dehydrogenase activities, the rate of NAD-dependent acetaldehyde uptake in liver homogenates was determined with a gas chromatograph at physiological pH and temperature. The uptake of 0·25 mM acetaldehyde was 2·52 ± 0·09 μmoles/g liver fresh wt per min (mean ± S.E.M.) in the control group ($n = 9$). Corresponding values for the moderately and heavily dosed groups were 1·75 ± 0·07 ($n = 11$) and 1·90 ± 0·13 ($n = 8$), both significantly ($P < 0·001$) lower than the value for the controls.

Table 1. Changes in body weights of rats during the ethanol treatment period

Animal group	Body wt (g) Initial	Final
I. Rats given ethanol by gavage		
Control group (9)	397 ± 12	428 ± 11
Moderately treated alcohol group (11)	414 ± 12	416 ± 9
Severely treated alcohol group (8)	444 ± 12	393 ± 14
II. Rats given ethanol in the food		
Control group (10)	321 ± 10	355 ± 14
Alcohol group (12)	328 ± 7	341 ± 7
III. Genetically selected strains		
AA-rats (8)	215 ± 5	204 ± 6
ANA-rats (8)	222 ± 10	207 ± 10

Values are means ± S.E.M. with numbers of animals in parentheses.

Table 2. Ethanol elimination and liver alcohol dehydrogenase activity after chronic ethanol intubation

		Alcohol dehydrogenase activity (μmoles/g liver fresh wt per min)	Ethanol elimination (mg/kg body wt per hr)
Controls	(C)	1·62 ± 0·10 (8)	329·2 ± 7·4 (9)
Moderately treated	(E_1)	1·33 ± 0·03 (11)*	366·2 ± 8·9 (11)*
Severely treated	(E_2)	1·03 ± 0·06 (8)†	391·0 ± 5·1 (7)†

* $P < 0·01$, † $P < 0·001$ for difference between control and alcohol-treated groups. Values are means ± S.E.M., with numbers of animals in parentheses.

Table 3. Liver aldehyde dehydrogenase activities and subcellular distribution after chronic ethanol intubation

4·5 mM propionaldehyde substrate	μmoles/g wet wt per min		
	Controls (C) ($n = 8$)	Moderately dosed (E_1) ($n = 11$)	Heavily dosed (E_2) ($n = 8$)
Soluble fraction	0·48 ± 0·02	0·39 ± 0·02†	0·38 ± 0·05*
Mitochondrial fraction	1·82 ± 0·12	1·71 ± 0·08	1·21 ± 0·06‡
Microsomal fraction	1·17 ± 0·03	1·17 ± 0·05	0·70 ± 0·06‡
Sum of fractions	3·74 ± 0·16	3·27 ± 0·12	2·29 ± 0·13‡
0·5 mM acetaldehyde substrate			
Soluble fraction	0·138 ± 0·006	0·112 ± 0·007†	0·097 ± 0·010†
Mitochondrial fraction	0·707 ± 0·049	0·612 ± 0·038	0·558 ± 0·053*
Microsomal fraction	0·224 ± 0·007	0·180 ± 0·007‡	0·115 ± 0·014‡
Sum of fractions	1·070 ± 0·053	0·904 ± 0·044†	0·777 ± 0·067†

* $P < 0·05$, † $P < 0·025$, ‡ $P < 0·001$ for difference between control and alcohol-treated groups. Values are means ± S.E.M., with numbers of animals in parentheses.

The drastic enzyme losses were not accompanied by any major changes in the lipid or protein content of the liver. The mean values for hepatic protein content in controls, moderately dosed and heavily dosed rats were 186 ± 3, 176 ± 3 and 179 ± 6 mg/g liver fresh wt (means ± S.E.M.), respectively. Corresponding values for total lipids were 59 ± 3, 65 ± 5 and 63 ± 2 mg/g liver.

The differences observed in hepatic aldehyde and alcohol dehydrogenase activities were basically similar if expressed per mg protein in the subcellular fractions. The activities of the microsomal marker enzyme glucose-6-phosphatase, expressed as μmoles/g liver fresh wt per min, were 7·1 ± 0·7, 6·8 ± 0·7 and 6·8 ± 0·8 for controls, and moderately and heavily dosed rats, respectively. The distributions of glucose-6-phosphatase and succinate–INT-reductase activities after fractionation were similar in all groups. These results indicate that the changes observed in aldehyde dehydrogenase activities are mainly qualitative and not due to changes in microsomal mass or to variations in subcellular fractionation.

When ethanol was given as a constituent of a liquid diet, the alcohol intake was considerably higher (9·1 g/kg body wt per day) than the amount administered in the intubation experiments. In spite of this, the rats gained weight in both groups and remained in good health during the 5-week test period (Table 1). One reason for the ability of the rats to ingest such a large amount of alcohol was the absence of intoxication when ethanol was given in this way. This absence was confirmed by determination of the blood alcohol level. As most of the liquid diet was consumed during the 12-hr dark cycle, blood samples were taken from the tip of the tail early in the morning before the lights were switched on. Of the 12 rats, 11 had detectable blood alcohol levels ranging from 0·8 to 11·4 mM, the mean value being 5·4 ± 1·3 mM.

The capacity to eliminate ethanol was significantly greater in the alcohol-consuming animals than in the pair-fed controls, whether expressed per kg body wt or per g liver wt (Table 4). This indicates that alcohol treatment increases the elimination rate, although it is present at concentrations which do not produce intoxication. On the other hand, no difference in alcohol dehydrogenase activity could be seen between the groups (Table 4).

In all subcellular fractions the aldehyde dehydrogenase activities were very similar in the two groups when 0·5 mM acetaldehyde was used as substrate (Table 5). The same was found in assays with 4·5 mM propionaldehyde: the sum of the

Table 4. Ethanol elimination rates and alcohol dehydrogenase activities in rats fed in alcohol-supplemented liquid diet

		Ethanol elimination		Alcohol dehydrogenase
		mg/kg body wt per hr	μmoles/g liver wet wt per min	μmoles/g liver wet wt per min
Controls	(10)	278 ± 6	2·91 ± 0·11	0·94 ± 0·05
Alcohol-fed	(12)	359 ± 7†	3·30 ± 0·11*	0·93 ± 0·06

* $P < 0·025$, † $P < 0·001$ for difference between control and alcohol-treated groups. Values are means ± S.E.M. with numbers of animals in parentheses.

Table 5. Liver aldehyde dehydrogenase activities and subcellular distribution in rats fed an alcohol-supplemented liquid diet

	μmoles/g liver wet wt per min	
	Controls (n = 10)	Alcohol-fed (n = 12)
Soluble fraction	0·102 ± 0·008	0·096 ± 0·008
Mitochondrial fraction	0·867 ± 0·011	0·919 ± 0·039
Microsomal fraction	0·180 ± 0·010	0·193 ± 0·014
Sum of fractions	1·100 ± 0·017	1·170 ± 0·046

Values are means ± S.E.M. 0·5 mM acetaldehyde was used as substrate.

activities in the fractions was 3·02 ± 0·15 for the control group and 3·10 ± 0·12 (μmoles/g liver wet wt per min) for the alcohol group, and there was no difference in the distribution pattern. Neither was there any difference in the NAD-dependent acetaldehyde uptake between the groups. The activities, expressed as μmoles/g liver wt per min, were 2·87 ± 0·14 for the control group and 2·78 ± 0·09 for the alcohol group when 0·35 mM acetaldehyde was used. The corresponding values at 0·12 mM acetaldehyde were 2·05 ± 0·10 and 1·98 ± 0·06.

Other parameters measured were also similar in the two groups. Glucose-6-phosphatase activities in the microsomal fractions, expressed as μmoles/g liver wet wt per min, were 6·1 ± 0·8 in the controls and 6·3 ± 0·7 in the alcohol group. The protein content was unchanged by alcohol treatment: 167·4 ± 5·8 mg/g liver fresh wt in the controls and 164·3 ± 4·0 in the alcohol group. The total lipid content was slightly higher in livers from alcohol-consuming rats (50·7 ± 3·4 mg/g) than in those from controls (43·7 ± 0·9 mg/g).

In previous experiments with rats of the ethanol-consuming AA strain and the ethanol-avoiding ANA strain which had had no previous access to alcohol, we found that the aldehyde dehydrogenase activities in the microsomal and mitochondrial fractions of the liver were higher in the AA rats and the alcohol dehydrogenase activities were higher in the ANA rats[8]. The present experiments were undertaken to show whether long-term alcohol administration would affect these previously reported strain differences.

The daily intubations (5 g ethanol/kg body wt for 2 weeks) caused some loss of body weight in rats of both strains especially during the first few days of the experiments, when 10% ethanol was given as the only source of fluid (Table 1). Some of the animals appeared to be in poor condition and in these rats the dose of alcohol was temporarily decreased. Interestingly enough, the liver weights were higher in the ANA rats (8·9 ± 0·8 g) than in the AA rats (6·7 ± 0·2 g) after alcohol treatment. Thus the liver/body weight ratio was significantly ($P < 0·025$) higher in the ANA rats. This strain difference was not present in untreated animals[17]. Fatty infiltration was observed both macroscopically and by histologic examination in all livers. The fatty infiltration was classified as mild in 6 and as moderate in 2 of the livers from AA rats. The infiltration was mild in 3, moderate in 3 and severe in 2 livers from ANA rats, indicating that the livers of these rats were slightly more vulnerable to alcohol.

The known differences between the untreated AA and ANA strains[8] in the aldehyde dehydrogenase activities of the subcellular fractions were in general still observable after alcohol treatment. When the higher aldehyde concentration was used, significantly higher activities were observed in the mitochondrial and microsomal fractions from AA rats (Table 6). Furthermore, the summed activities were significantly higher in the AA rats at both substrate levels used. The capacity of liver homogenates to break down acetaldehyde (0·4 mM) was not significantly higher in the AA group (Table 7).

The values observed in this study are considerably lower than in the previous study with untreated animals[8], especially at the lower aldehyde concentration. The most striking decrease was observed in the mitochondrial fraction. In intact rats the contribution of the mitochondrial activity to the summed activities of all fractions was 63% in AA and 65% in ANA livers. After alcohol treatment these contributions decreased to 34 and 36%, respectively.

A few experiments with male rats of the AA and ANA strains showed that alcohol treatment diminished aldehyde dehydrogenase activity, especially that in the mitochondria. In ANA rats mitochondrial activity was reduced almost to zero, whereas in males of the AA strain the decrease in activity paralleled that observed in the females.

Alcohol dehydrogenase activity was significantly higher in ANA rats, in agreement with the previous observation in intact rats (Table 6). The acetaldehyde content of tail blood 30 min after ethanol gavage (5 g/kg) was rather high and significantly higher in ANA rats (Table 7).

Table 6. Liver aldehyde and alcohol dehydrogenase activities in female rats of AA and ANA strain after chronic ethanol intubation

	μmoles/min per g liver wet wt			
	4·5 mM propionaldehyde substrate		0·5 mM acetaldehyde substrate	
Aldehyde dehydrogenase	AA strain	ANA strain	AA strain	ANA strain
Soluble fraction	0·29 ± 0·01	0·27 ± 0·01	0·055 ± 0·004	0·052 ± 0·005
Mitochondrial fraction	0·60 ± 0·02	0·47 ± 0·03‡	0·079 ± 0·001	0·062 ± 0·010
Microsomal fraction	0·87 ± 0·08	0·61 ± 0·05†	0·091 ± 0·004	0·072 ± 0·009
Sum of fractions	1·73 ± 0·09	1·35 ± 0·09‡	0·230 ± 0·007	0·172 ± 0·001‡
	AA		ANA	
Alcohol dehydrogenase (soluble fraction)	1·41 ± 0·09		1·73 ± 0·10*	

* $P < 0·05$, † $P < 0·025$, ‡ $P < 0·01$ for difference between AA and ANA strains. Values are means ± S.E.M. of 8 animals in each group.

Table 7. Blood acetaldehyde and hepatic NAD-dependent acetaldehyde utilization in female rats of AA and ANA strains after long-term ethanol intubation

	Blood acetaldehyde (nmoles/ml)	Acetaldehyde utilization μmoles/g liver wet wt per min
AA strain (8)	148 ± 6	1·06 ± 0·06
ANA strain (8)	277 ± 17*	1·02 ± 0·05

* $P < 0·001$. 0·4 mM acetaldehyde was used as substrate. Values are means ± S.E.M. of 8 animals in each group.

DISCUSSION

Besides the amount of alcohol and the duration of its consumption, dietary factors play an important role in the etiology of alcohol-induced liver damage (for review see French[20]). Although the two-step oxidation of ethanol via acetaldehyde to acetate is accepted to be the cause of the disturbance in hepatic metabolism during alcohol combustion, the role of acetaldehyde in this process, and especially in the effects of long-continued alcohol abuse, is still obscure. Recent reports show that acetaldehyde has major effects, especially on mitochondrial functions[21], although these effects were mostly seen at acetaldehyde levels higher than those reported to occur during ethanol oxidation in the intact liver[22].

The concentration of acetaldehyde in the liver during ethanol oxidation appears to depend on the activity of aldehyde dehydrogenase (Lindros and Koivula, unpublished data). If, therefore, the effects of acetaldehyde are related to its concentration in the liver, changes in the aldehyde dehydrogenase activities of the various subcellular fractions may be of importance. Previous data on the effects of ethanol consumption on aldehyde dehydrogenase activity are sparse and inconsistent. Dajani *et al.*[5] and Majchrowicz *et al.*[4] did not find any significant change in liver aldehyde dehydrogenase activities or blood acetaldehyde clearance rates after long-term ethanol administration. Horton[6] has described induction by ethanol of aldehyde dehydrogenase in the mitochondrial fraction. However, Tottmar *et al.*[7] did not find any significant changes in mitochondrial or microsomal aldehyde dehydrogenase activities after ethanol consumption.

Our results demonstrate that drastic alcohol treatment may lead to significant loss of aldehyde dehydrogenase activity in all subcellular fractions. The absence of such an effect when ethanol was given as a constituent of the liquid diet shows that factors other than the amount of alcohol *per se* are involved. These factors are probably related to the loss of weight and the accompanying impairment of general health observed in the rats given ethanol by intubation. These effects were not seen when ethanol was given in the food. Decreased food intake in intubation experiments is probably due both to intoxication and to disturbed alimentary function. Restricted availability of vital food constituents may disturb hepatic functions including protein synthesis. The decrease in aldehyde dehydrogenase activity observed after drastic alcohol treatment were clearly related to the weight loss. This was demonstrated by the negative correlation found between the individual enzyme activities and weight losses calculated as percentages of the initial body weight. The correlations were highly significant when calculated from the values of aldehyde dehydrogenase activity obtained either with 4·5 mM propionaldehyde ($r = -0·80$, $P < 0·01$) or with 0·5 mM acetaldehyde as substrate ($r = -0·77$, $P < 0·01$). The correlation was even better when the activities were calculated from the values of NAD-dependent acetaldehyde uptake ($r = -0·90$, $P < 0·01$). A significant correlation between weight loss and mitochondrial activity was observed only with 0·5 mM acetaldehyde as substrate ($r = -0·73$, $P < 0·01$). It is notable that there was no significant correlation between weight loss and alcohol dehydrogenase activity.

In the liquid diet series, in which no differences in enzyme activities were seen, the alcohol-treated and control groups did not differ in intake of proteins or of other essential food components. Protein deficiency is known to cause loss of liver ADH activity[23]. Recent studies (Lindros, Koivula, Eriksson and Arpiainen, unpublished) have shown that aldehyde dehydrogenase activity is also sensitive to deficiency of protein and other dietary factors.

The peripheral blood acetaldehyde levels in the

alcohol-treated AA and ANA rats were several times higher than those observed previously in intact animals of the same strains[17]. The difference seemed greater than that to be expected from the larger ethanol dose given. Possibly, the remarkable loss of mitochondrial aldehyde dehydrogenase activity is connected with the increased acetaldehyde levels. The ANA strain, in which higher acetaldehyde levels were observed, had significantly lower aldehyde dehydrogenase activity than the AA strain. Furthermore, ethanol treatment seemed to induce more severe fatty degeneration in livers of ANA rats.

Recent studies strongly suggest that during ethanol oxidation in intact liver tissue most of the acetaldehyde formed is oxidized by the low-K_m mitochondrial aldehyde dehydrogenase[24]. In the AA and ANA rats this low-K_m activity was the very one that decreased profoundly. The accumulation of acetaldehyde in these rats is possibly a consequence of the diminished aldehyde dehydrogenase activity, but equally well might be one of its causes. Acetaldehyde is shown to inhibit protein synthesis at 1 mM concentration *in vitro* [25]. During ethanol oxidation liver acetaldehyde concentrations are considerably higher than those in peripheral blood[26]. The values measured in peripheral blood of the AA and ANA rats during ethanol oxidation varied from 0·15 to 0·28 mM. It is therefore possible that the concentration of acetaldehyde in liver may be high enough to disturb normal protein synthesis also *in vivo*. This may be one of the reasons for the diminution of enzyme activities resulting from the drastic alcohol treatment. The inference is that primary differences in the aldehyde oxidation rate may even be increased by high alcohol consumption. Smith and Packer[27] have suggested a protective role for mitochondrial aldehyde dehydrogenase, which is known to be capable of oxidizing toxic dialdehydes that arise physiologically. The oxidation of acetaldehyde itself can also be considered as a protective function of the mitochondria.

The loss of ADH activity, found in this study after prolonged alcohol administration by gavage, is probably related to the debilitating effects of the treatment as illustrated by the significant loss of body weight and signs of impaired health in some animals. The experiments with ethanol given in the liquid diet demonstrate that alcohol administration *per se* does not necessarily induce changes in ADH activity. Regardless of the effects on ADH activity, ethanol elimination was accelerated in both experiments, supporting the hypothesis[2, 3] that the ethanol elimination rate is not directly dependent on liver alcohol dehydrogenase activity.

Acknowledgements—This investigation was supported by grants from the Sigfrid Juselius Foundation, the National Research Council for Medical Sciences and the Foundation for Studies on Alcohol, Finland. We are indebted to Mrs. Eija Haasanen and Mrs. Hilkka Salohalla for skilful technical assistance and to Associate Professor Martti Koivusalo, Dr. O. Forsander and Dr. C. J. P. Eriksson for valuable criticism of the manuscript. We thank Miss Aino Malila for expert help in the treatment of animals.

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