

Ethanol Elimination in Regenerating Rat Liver: The Roles of Alcohol Dehydrogenase and Acetaldehyde

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The rate of ethanol elimination *in vivo* was studied with rats in which the energy consumption of the liver was increased by partial hepatectomy. Immediately after partial hepatectomy the activity of alcohol dehydrogenase in the liver remnant was not changed from that of the livers of sham-operated controls, but the rate of ethanol removal was significantly faster. Twenty-four h after the partial hepatectomy the activity of alcohol dehydrogenase was only 48 % of the activity measured in unoperated control rats. Therefore it is concluded that in normal liver the activity of ADH is in excess. In partially hepatectomized rats the rate of ethanol elimination was linearly correlated with the activity of alcohol dehydrogenase, which suggests that when the rate of NADH reoxidation is markedly increased, as in regenerating rat liver, the rate of ethanol elimination may be limited by the activity of alcohol dehydrogenase. The activity of aldehyde dehydrogenase and the concentration of acetaldehyde in the tail blood were not significantly changed from the level of unoperated rats during oxidation of ethanol.

In low concentrations ethanol is metabolized in the liver mostly by alcohol dehydrogenase, ADH (alcohol:NAD⁺ oxidoreductase, EC 1.1.1.1).¹ Many attempts have been made to correlate the total amount of alcohol dehydrogenase with the rate of ethanol removal, and both positive²⁻⁴ and negative^{3,5-7} results have been obtained.

The amount of alcohol dehydrogenase has been found experimentally to limit ethanol elimination either when the activity is low, as

in developing rat liver² and in livers of thyroid hormone-treated rats,³ or when the rate of ethanol elimination is accelerated as with fructose or pyruvate.^{8,9} The role of alcohol dehydrogenase in the regulation of ethanol elimination in untreated livers has been emphasized as well,^{4,9,10} thus leaving open the question of the role of alcohol dehydrogenase in the regulation of ethanol oxidation.

The rate limiting factor in ethanol elimination in cases where no correlation is found has been suggested to be the increased NADH/NAD⁺ ratio,⁷ the concentration of free NADH,⁸ or the reoxidation of NADH.⁶ Thus, administration of NADH acceptors like pyruvate, fructose or glyceraldehyde,^{9,11,12} or increasing the activity of the respiratory chain either with metabolites like fructose,^{8,11,12} or with uncouplers,⁶ is found to cause an increased rate of ethanol removal. This experiment was designed to determine (i) if the respiratory chain activity could be increased enough, without the addition of substrates or uncouplers, to increase the rate of ethanol elimination *in vivo*, and (ii) if alcohol dehydrogenase activity becomes the rate limiting factor in ethanol oxidation when the respiratory chain activity is sufficiently increased. Increased energy consumption and the activity of the respiratory chain were achieved by partial hepatectomy.¹³⁻¹⁶ Activity of alcohol dehydrogenase, concentration of acetaldehyde, and activity of aldehyde dehydrogenase were studied as the possible regulators of the rate of ethanol elimination in this experimental system.

MATERIALS AND METHODS

Male Sprague-Dawley rats, about 400 g in weight, were used. The rats had free access to pelleted laboratory diet (Orion, Espoo, Finland) and tap water until the start of the experiments. Twenty-six rats were partially hepatectomised under light ether anaesthesia.¹⁷ The mean weight of the liver removed in the operation was 6.8 ± 0.4 g ($n=20$). Control rats were either untreated or sham-operated.

Groups of 4 rats were given an intraperitoneal injection of ethanol (1.5 g kg⁻¹ body weight as 15 % (w/v) solution in water) 24, 48, 72, 96, or 168 h after the operation. Tail blood was taken into tubes containing ice-cold perchloric acid (0.6 N) every 30 min for 3.5 h to measure the rate of ethanol elimination. In a separate experiment 6 rats were given ethanol (2 g kg⁻¹ body weight as 15 % (w/v) solution in water) *via* a stomach tube immediately after the partial hepatectomy and the rate of ethanol elimination was determined. Control rats were sham-operated and received the same dose of ethanol immediately after the sham-operation.

After the last blood sample had been taken the rats were decapitated, and the livers were quickly removed, weighed, and frozen for enzyme activity analysis. Samples of the liver were homogenized in ice-cold sucrose solution (0.25 M) containing 1 % Triton X-100. The pH of the solution was 7.4. The homogenate was passed through a double layer of cheese cloth to remove the cell debris and the filtrate was used as the total homogenate from which protein concentration and activities of aldehyde dehydrogenase, aspartate aminotransferase, and malate dehydrogenase were measured. Part of the filtrate was centrifuged at 30 000 g for 30 min at +4 °C and from the supernatant, which represents the cytoplasmic compartment, were determined the concentration of protein and activities of alcohol dehydrogenase, and α -glycerophosphate dehydrogenase. The mitochondrial fraction was isolated by centrifugation¹⁸ for measurement of the activity of the mitochondrial glycerophosphate oxidase.

Concentrations of ethanol and acetaldehyde were determined gas chromatographically (Perkin-Elmer F 40) by head-space technique.¹⁹ Activities of the enzymes were determined at

37 °C by spectrophotometric methods which have been described previously.²⁰⁻²³ Alcohol dehydrogenase activity was measured at pH 7.4. In the assay the volume fraction of 1:10 liver homogenate was 1/17. This dilution ensured proportionality between rate and homogenate concentration for the accurate measurement of the low activities (24 and 48 h after the operation), and normal *in vivo* activity and the activity in livers 168 h after the operation. The reaction was initiated with ethanol and was followed for 2-3 min, during which time no deviation from linearity was found in the plot of activity against time. When the activity of aldehyde dehydrogenase was determined the initial concentration of acetaldehyde was 0.33 mM. Thus the activity mainly reflects the mitochondrial low K_m enzyme,²⁴ though the high K_m enzymes may also have affected the results. Activities of the enzymes were calculated both per g of liver weight and per mg liver protein. Protein concentration was determined with bovine plasma albumin as standard.²⁵

Student's *t*-test was used to compare the partially hepatectomised rats with sham-operated or unoperated control rats. Paired *t*-test was used when activities from the same liver at different time points were compared (Table 2). Unoperated rats were not taken into account in the linear correlation analysis.

RESULTS

Liver regeneration. About 63 % of the liver mass was resected in the operation (Table 1) and the greatest increase in the liver weight was recorded between 24 and 48 h after the operation. The initial liver weight was not fully recovered after 7 days. A slight loss in the body weight of the rats was also observed, but the liver to body weight ratio remained significantly lower than in unoperated rats.

The protein level both in the total homogenate and in the cytoplasmic fraction was also slightly (statistically insignificantly) lowered after partial hepatectomy (Table 1). During the course of regeneration a slightly higher level of liver protein content was recorded 96 h after the operation. The changes in liver mass, body weight and protein content are similar to those reported previously.²⁶

Table 1. Changes in liver weight, body weight, liver to body weight ratio, and protein content of the liver after partial hepatectomy.

Time after resection/h	n	Body weight/g	Liver weight/g	Liver to body weight ratio/%	Total protein/mg [g liver] ⁻¹
Unoperated	3	409 ± 26	10.8 ± 1.1	2.63 ± 0.20	212 ± 31
24	4	389 ± 23	4.7 ± 0.5 ^b	1.22 ± 0.11 ^b	184 ± 24
48	4	380 ± 19	6.4 ± 0.2 ^b	1.73 ± 0.14 ^b	182 ± 16
72	4	372 ± 10	7.1 ± 0.3 ^a	1.93 ± 0.13 ^a	187 ± 11
96	4	396 ± 13	7.2 ± 1.1 ^a	1.82 ± 0.24 ^a	221 ± 23
168	3	379 ± 26	6.9 ± 0.7 ^a	1.82 ± 0.09 ^a	214 ± 21

The values are the mean ± S.D. ^a=*p*<0.01 and ^b*p*<0.001 for the statistical differences from unoperated control rats.

Table 2. Changes in enzyme activities after partial hepatectomy.

Enzyme activity/ nmol [mg protein] ⁻¹ min ⁻¹	Control n = 4	48h regeneration n = 4	Control n = 3	168h regeneration n = 3
Alcohol dehydrogenase	12.73 ± 3.62	8.44 ± 1.51 ^a	15.72 ± 5.93	13.97 ± 0.83
Aldehyde dehydrogenase	11.32 ± 1.73	10.29 ± 1.26	13.14 ± 0.68	13.45 ± 0.86
Aspartate aminotransferase	1320 ± 117	1324 ± 158	1157 ± 118	1237 ± 103
Malate dehydrogenase	9230 ± 1230	10610 ± 1740	7710 ± 990	7240 ± 300
α-Glycerophosphate dehydrogenase	145.1 ± 18.5	70.3 ± 13.0 ^b	87.9 ± 7.0	55.7 ± 5.4 ^a
Glycerophosphate oxidase	13.4 ± 0.8	15.4 ± 0.7 ^a	13.8 ± 2.4	15.3 ± 0.1

^a and ^b are used to indicate the statistical significance of the differences from the corresponding controls at the level of *p*<0.05 and *p*<0.001, respectively. The results are the mean ± S.D.

Ethanol elimination in partially hepatectomised rats. The rate of ethanol elimination was calculated in two different ways: As per 100 g of body weight (Fig. 1 A), and as per g of liver weight (Fig. 1 B). As could be expected, the rate of ethanol elimination was significantly lower in partially hepatectomised than in control rats when expressed per 100 g of body weight (Fig. 1 A). In regenerating livers the rate of ethanol elimination correlated highly significantly with the liver weight of the rats (*df* = 17, *r* = 0.741, *p* < 0.001). A similar correlation has been found with rabbits after partial hepatectomy.²⁷

The second calculation of the elimination rate of ethanol was based on g of liver weight. This type of calculation should be independent of the total weight of the liver and could thus give information of the regulation of ethanol elimination at cellular level. A great variation in the rate of ethanol elimination was found

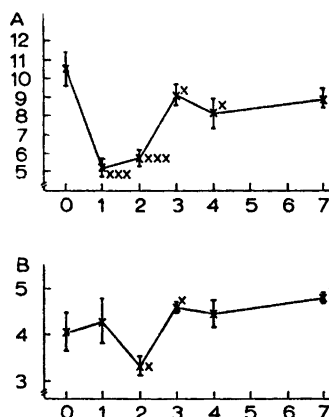


Fig. 1. The rate of ethanol elimination (ordinata; μmol/min) in regenerating rat liver per 100 g of body weight (A), and per g of liver tissue (B), versus time (abscissa; days) after hepatectomy. Each point represents the mean ± S. D. of 3–4 animals. x and xxx are used to indicate the statistical differences *p* < 0.05, and *p* < 0.001 from the unoperated control rats.

during regeneration (Fig. 1 B), so that 48 h after the partial hepatectomy the rate was significantly decreased when compared with unoperated rats ($p < 0.05$) and the 24-hour-regeneration group ($p < 0.05$), and significantly elevated at 72 h ($p < 0.05$ to controls and ns to 24-hour group) and 168 h (statistical difference to controls and 24-hour group $p < 0.05$ and ns, respectively). The results thus suggest that at cellular level the rate of ethanol elimination is regulated by a factor that changes in parallel to the changes observed in the elimination rate.

Activity of alcohol dehydrogenase in regenerating rat liver. After partial hepatectomy there was a remarkable decrease in the activity of alcohol dehydrogenase in the liver remnant (Fig. 2). The percentage decrease in the liver level of alcohol dehydrogenase (52 % from the control to 24-h group) was much higher than the loss of protein (16 %, respectively) (Fig. 2, Table 1). The activity of ADH did not correlate with the protein concentration or the liver weight.

In order to test whether there were changes in other enzyme activities too, the activities of alcohol dehydrogenase, aldehyde dehydrogenase, aspartate aminotransferase, malate dehydrogenase, α -glycerophosphate dehydrogenase, and glycerophosphate oxidase were measured from the pieces of liver removed during the partial hepatectomy and from the livers of the very same rats 48 and 168 h after the operation. It was found (Table 2) that only

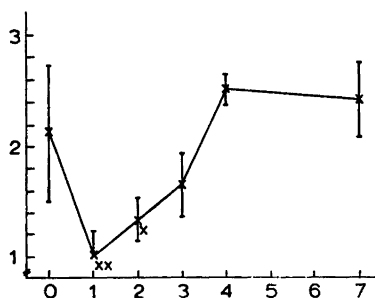


Fig. 2. The activity of alcohol dehydrogenase in rat liver after partial hepatectomy [ordinata; U/(g liver tissue)] versus time (abscissa; days) after partial hepatectomy. Each point represents the mean \pm S.D. of 3–4 rats. x and xx indicate the significance of the statistical difference from unoperated rats at the level of $p < 0.05$ and $p < 0.01$, respectively.

the cytoplasmic enzymes, alcohol dehydrogenase and α -glycerophosphate dehydrogenase, were significantly lower at 48 h. Enzymes located both in the cytoplasm and mitochondria, namely aldehyde dehydrogenase, aspartate aminotransferase and malate dehydrogenase, were not changed, and a significant increase in the activity of the mitochondrial glycerophosphate oxidase was found. One week after the partial hepatectomy only the activity of α -glycerophosphate dehydrogenase was still lower than in the corresponding control livers. According to the present results it seems that the great decrease in the activity of alcohol dehydrogenase is associated with the cytoplasmic location of this enzyme.²⁸

Another reason for the decrease observed in the activity of alcohol dehydrogenase and of α -glycerophosphate dehydrogenase could be the heterogenous distribution of these enzymes in the liver, and thus the following control experiment was performed. Unoperated control rats were decapitated and their livers were dissected into two samples, one representing the part of the liver which is removed in partial hepatectomy and the other the liver remnant. When the activity of alcohol dehydrogenase was measured in the homogenates of these two liver samples, no difference in their level of enzyme activity was found. It therefore appeared improbable that the uneven distribution of alcohol dehydrogenase in the liver would cause an apparent decrease in the activity of this enzyme in the regenerating rat liver.

Correlation between the rate of ethanol elimination and the activity of alcohol dehydrogenase. When calculated per 100 g of body weight, the activity of alcohol dehydrogenase in the liver remnant correlated with the rate of ethanol elimination 24, 48, 72, 96 and 168 h after the partial hepatectomy ($df = 17$, $r = 0.738$, $p < 0.001$). Likewise, a linear correlation ($df = 17$, $r = 0.511$, $p < 0.05$) was found when the activity of alcohol dehydrogenase and the rate of ethanol elimination were expressed as per g of liver weight. In these calculations the unoperated control rats were not taken into account. These results suggest that in regenerating rat liver the total activity of alcohol dehydrogenase regulates the rate of ethanol elimination.

To compare the effect of the activity of alcohol dehydrogenase on the rate of ethanol

Table 3. The rate of ethanol elimination and the activity of alcohol dehydrogenase immediately after partial hepatectomy and sham-operation. The results are the mean \pm S.D.

	Sham-operated	Hepatectomised	<i>p</i>
<i>n</i>	5	6	
Ethanol elimination $\mu\text{mol [g liver]}^{-1} \text{min}^{-1}$	5.2 ± 0.5	9.1 ± 2.3	< 0.01
ADH $\mu\text{mol [g liver]}^{-1} \text{min}^{-1}$	2.44 ± 0.17	2.54 ± 0.27	ns

elimination in sham-operated and partially hepatectomised rats a single dose of ethanol was given to one group of rats immediately after the partial hepatectomy and to another group after sham-operation and the rate of ethanol elimination as well as the activity of alcohol dehydrogenase in the liver were determined. The activity of ADH was similar in both the sham-operated controls and in partially hepatectomised rats (Table 3), but the rate of ethanol elimination was significantly faster in the hepatectomised group (Table 3). Therefore it is concluded that other factors than the activity of ADH mainly regulate the rate of ethanol elimination in intact fed rat liver.

Concentration of acetaldehyde and the activity of aldehyde dehydrogenase. The concentration of acetaldehyde which is regulated by the activity of aldehyde dehydrogenase, sometimes may affect the rate of ethanol elimination.^{29,30} In the present study acetaldehyde concentration

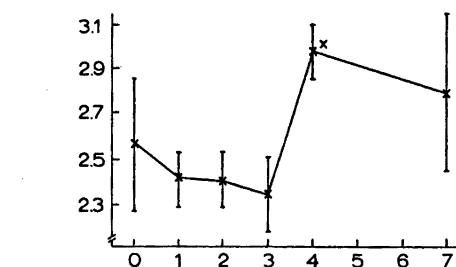


Fig. 3. The activity of aldehyde dehydrogenase [ordinata; U/(g liver tissue)] in rat liver versus time (abscissa; days) after partial hepatectomy. Each point in the figure represents the mean \pm S.D. of 3–4 rats. \times is used to indicate the statistical difference at the level of $p < 0.05$ from unoperated control rats.

was measured in the tail blood, which reflects the concentration of acetaldehyde in the liver.³¹ Although ethanol concentration in the tail blood of partially hepatectomised rats stayed at significantly higher level than in unoperated rats (Table 4), because of the smaller liver mass in relation to body weight, acetaldehyde concentration stayed at the same level as in unoperated rats.

Activity of aldehyde dehydrogenase in the liver was only slightly and insignificantly lower during 3 days after the partial hepatectomy, but rose later on to a significantly higher level than in unoperated rats (Fig. 3). The activity correlated well with the liver protein content ($df = 17$, $r = 0.751$, $p < 0.001$). Accordingly, it seems that acetaldehyde concentration does not rise to a high enough level to affect the rate of ethanol elimination in regenerating rat liver

Table 4. Ethanol and acetaldehyde concentrations in the tail blood of partially hepatectomised rats 2 and 3 h after ethanol administration.

Time after resection/h	<i>n</i>	Ethanol concentration/mM		Acetaldehyde concentration/ μM	
		120 min	180 min	120 min	180 min
Unoperated	3	21.8 ± 1.6	15.6 ± 3.0	26.4 ± 5.0	17.9 ± 2.6
24	4	31.2 ± 0.4^b	27.5 ± 0.8^b	26.7 ± 5.7	20.0 ± 5.3
48	4	22.8 ± 1.5	21.3 ± 1.4^a	31.1 ± 5.7	20.8 ± 6.0
72	4	24.2 ± 0.9	18.6 ± 0.7	29.5 ± 5.6	20.6 ± 5.2
96	4	26.2 ± 1.3^a	20.5 ± 1.3^a	25.4 ± 5.6	22.9 ± 3.5
168	3	25.6 ± 2.0	19.8 ± 1.9	30.5 ± 4.4	22.9 ± 3.0

^a = $p < 0.05$ and ^b = $p < 0.001$ for the statistical difference from the unoperated control rats.

DISCUSSION

In regenerating rat liver the total amount of alcohol dehydrogenase, calculated per 100 g of body weight, was found to be highly significantly correlated with the rate of ethanol elimination, calculated on the same basis. A similar observation has been made for liver mass increased with hypertrophic drug, clofibrate, where the increased liver mass was associated with increased rate of ethanol elimination (calculated per 100 g of body weight).^{32,33} Thus mainly the liver size seems to be reflected in this type of calculation of the rate of ethanol elimination, and even highly significant decreases in the activity of alcohol dehydrogenase are not discovered. Also no information of the regulation of ethanol elimination in the liver is obtained.

At cellular level other factors in addition to the amount of alcohol dehydrogenase appear to regulate the elimination of ethanol. Right after partial hepatectomy the rate of ethanol elimination was significantly increased with no change in the activity of alcohol dehydrogenase and 24 h after the partial hepatectomy the activity of alcohol dehydrogenase was only 48 % of that in unoperated rats, but the rate of ethanol elimination (per g of liver weight) had not decreased correspondingly. Extrahepatic elimination of ethanol could partially explain this discrepancy since extrahepatic elimination increases proportionately to a decrease in liver size. Furthermore, the non-ADH pathways for ethanol elimination that were not measured in this study may have influenced the results. In regenerating rat liver, where the concentration of ethanol is higher than that in untreated control rats, the non-ADH pathways could be more significant. However, such a discrepancy would also occur if the activity of alcohol dehydrogenase were in excess in normal liver, a conclusion that has been reached in many previous studies,^{5-7,34,35} but recently questioned by others.^{4,9,10} Therefore the increased rate of ethanol elimination per unit ADH at 4 and 24 h after partial hepatectomy is probably due to changes in the activity of the respiratory chain¹³⁻¹⁶ and the results of the present study are thus in agreement with previous studies^{5-7,32,34} that have reported that the rate-limiting step in ethanol oxidation

in fed rat liver is the activity of the respiratory chain.

A linear correlation between the rate of ethanol elimination and the activity of alcohol dehydrogenase which was found in regenerating rat liver suggests the rate-limiting role of alcohol dehydrogenase in these livers. These results of the present study can be compared with the studies in which fructose, thyroid hormone, or uncouplers of the respiratory chain have been used to accelerate ethanol elimination. The fructose effect seems mainly to be due to the increased oxidation of NADH in the respiratory chain as a consequence of ATP consumption in the phosphorylation of fructose^{8,12} although the function of fructose and its metabolite glyceraldehyde as cytoplasmic acceptors of NADH may participate in the effect as well.^{8,11,36} In any event, in fructose-treated liver cells the rate of ethanol elimination is stated to be limited by the activity of alcohol dehydrogenase.⁸ After thyroid hormone treatment the rate of NADH reoxidation in the respiratory chain is increased,^{32,37} but simultaneously the activity of alcohol dehydrogenase is decreased³⁸ enough to limit ethanol elimination.³ Similarly, in regenerating rat liver the activity of the respiratory chain¹³⁻¹⁶ is increased enough to change the rate limiting step from NADH reoxidation to the activity of alcohol dehydrogenase, and therefore a linear correlation between the rate of ethanol elimination and the activity of alcohol dehydrogenase is found. The present results strongly suggest that, even though the activity of alcohol dehydrogenase is normally in excess in rat liver, the rate of metabolism can be increased *in vivo*, even without any additional substrates, enough that the activity of alcohol dehydrogenase becomes the rate-limiting step in ethanol elimination.

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