Pyrazole and Induction of Fatty Liver by a Single Dose of Ethanol

Abstract. *Pyrazole (4 millimoles per kilogram or 272 milligrams per kilogram of body weight), given to fasted rats 10 minutes before gavage with ethanol (4 grams per kilogram), completely prevented both the disappearance of ethanol from the blood over a 16-hour period and the ethanol-induced reduction in the ratio of oxidized to reduced hepatic nicotinamide-adenine dinucleotide. However, it did not affect the accumulation of triglycerides in the liver after the administration of ethanol. These results indicate that metabolism of ethanol is not required for production of fatty liver by a single, large dose of ethanol.*

The administration of a single, large dose of ethanol, at least 4 g/kg and commonly 6 g/kg or more, to fasted rats results in a significant increase in triglyceride content in the liver, reaching a peak at about 16 hours after administration (*1*). Adrenalectomy or hypophysectomy prevents the induction of fatty liver by a single dose of ethanol (*2*), and it was believed that ethanol must be stimulating peripheral fatty acid mobilization by way of activation of the pituitary-adrenal system. However, it was later pointed out (*3*) that fatty liver could result from impaired oxidation of fatty acids or triglycerides in the liver, even in the presence of a normal rate of mobilization. In this view, the adrenals were required only for normal mobilization. Lower doses of ethanol (less than 0.5 g/kg) in man have been reported to cause an initial reduction in plasma free fatty acids, arguing against an increase in peripheral mobilization (*4*). An alternative hypothesis was therefore advanced, stating that intrahepatic accumulation of reduced nicotinamide-adenine dinucleotide ($NADH_2$) resulting from the oxidation of ethanol inhibits the oxidation of free fatty acids, increases the production of α-glycerophosphate, and thus increases esterification to form triglycerides (*5*). However, since ethanol oxidation is a zero-order process at all but very low ethanol concentrations (*6*), this hypothesis would not explain the dose dependence of the induction of fatty liver by a single dose of ethanol (*1*).

Pyrazole effectively inhibits the oxidation of ethanol, both in vivo and by liver alcohol dehydrogenase in vitro (*7*). It should therefore prevent the change in the ratio of hepatic nicotinamide-adenine dinucleotide (NAD) to $NADH_2$ which results from ethanol oxidation. If metabolism of ethanol is required for production of fatty liver by a single dose of ethanol, then pyrazole should also inhibit the production. In contrast, if the over-mobilization hypothesis is correct, a single dose of ethanol should still induce fatty liver even in the presence of pyrazole. This has been subjected to experimental test.

Female Wistar rats were divided into four groups. After a period of 7 hours without food, one group (E) received ethanol (4 g/kg), given as a 25 percent solution (by volume) in water by stomach tube. Controls (G) received equal volumes of an equicaloric solution of glucose. Two additional groups (EP and GP) received the same doses of ethanol and glucose, respectively, 10 minutes after the intraperitoneal injection of a 1.7 percent solution (weight to volume) of pyrazole (4 mmole/kg; 272 mg/kg) in water. Sixteen hours after administration of ethanol or glucose the animals were decapitated; the livers were rapidly excised, rinsed in saline solution, and homogenized in phosphate buffer 0.066*M*, *p*H 7.0. Triglycerides were extracted (*8*) and measured colorimetrically (*9*). This experiment was carried out on three separate occasions, with six animals per treatment group on each replication. In the second replication, tail vein blood samples of 0.05 ml were taken at 4, 8, and 16 hours after intubation from each rat receiving ethanol, for measurement of ethanol by the internal standard technique of gas-liquid chromatography (*10*). In a separate experiment, the same four treatments were employed, but the animals were decapitated at 4 hours after intubation, and frozen samples of liver were obtained as rapidly as possible by the freeze-clamp technique (*11*) for analysis of NAD and $NADH_2$ (*12*).

This dose of pyrazole almost completely inhibited ethanol metabolism (Table 1). The concentration of ethanol in the blood did not decrease between 4 and 16 hours in the EP animals, while in the E group it declined at a mean rate of about 28 mg/100 ml per hour during the 4- to 8-hour period, and 14 mg/100 ml per hour during the subsequent 8 hours.

In keeping with the observed in-

Table 1. Concentrations of ethanol in the blood of rats in the presence and absence of pyrazole. Values expressed are the number of milligrams per 100 ml of blood (mean ± S.E.). There were six animals in each group. Ethanol was given intragastrically at a dose of 4 g/kg. Pyrazole (272 mg/kg) was given intraperitoneally 10 minutes before ethanol administration. The *P* values shown refer to differences between ethanol and ethanol-pyrazole animals as estimated by Student's *t*-test.

Time after administration of ethanol (hours)	Ethanol concentration		*P* values
	Without pyrazole	With pyrazole	
4	382 ± 48	564 ± 71	$.05 < P < .1$
8	268 ± 38	600 ± 36	$P < .001$
16	160 ± 42	593 ± 34	$P < .001$

Table 2. Ratios of hepatic NAD to $NADH_2$ and the triglyceride concentrations after administration of ethanol in the presence and absence of pyrazole. The numbers in parentheses indicate the number of animals per group. Ethanol and pyrazole doses are as indicated in Table 1. The glucose solution contained the same number of calories as did the ethanol dose, both administered by stomach tube. The NAD/$NADH_2$ ratios and the triglyceride concentrations were determined at 4 and 16 hours, respectively, after administration of ethanol. Values are expressed as the mean ± S.E.

Hepatic NAD (μg/g, wet wt)	Hepatic $NADH_2$ (μg/g, wet wt)	NAD/$NADH_2$	Hepatic triglycerides (mg/g of liver, wet wt)
	Ethanol		
241 ± 27 (7)	188 ± 14 (7)*	1.32 ± 0.15 (7)†	29.17 ± 3.91 (18)‡
	Ethanol + pyrazole		
240 ± 10 (8)	88 ± 10 (8)	2.98 ± 0.37 (8)	32.13 ± 4.40 (18)‡
	Glucose		
239 ± 21 (7)	97 ± 12 (7)	2.66 ± 0.32 (7)	13.80 ± 1.41 (18)
	Glucose + pyrazole		
254 ± 13 (7)	94 ± 7 (7)	2.76 ±0.19 (7)	14.77 ± 1.32 (18)

* Significantly higher than values for the other groups (see text for *P* values). † Significantly lower than ratios for the other groups (see text for *P* values). ‡ Significantly higher than triglyceride concentrations in corresponding glucose groups (see text for *P* values).

hibition of ethanol disappearance, pyrazole also prevented the reduction in the ratio of hepatic NAD to $NADH_2$ produced by ethanol alone (Table 2). There was no significant difference among the EP, G, and GP groups, but all three were significantly higher than the E group ($P < .005$, $P < .005$, and $P < .001$, respectively). Correspondingly, the concentrations of $NADH_2$ in these three groups were all lower than those in the E group ($P < .001$ in all cases).

However, pyrazole did not prevent the accumulation of hepatic triglycerides caused by ethanol (Table 2). The pooled values from the three separate experiments for E and for EP were not significantly different from each other, but were significantly higher than those for G and GP, respectively ($P < .001$ in both cases). The same results were obtained in each of the three separate replications. Analysis of variance confirmed that there were significant differences both between treatments and between days ($P < .001$ for each).

These results are not consistent with the view that the fatty liver produced by a single dose of ethanol results from $NADH_2$ accumulation as a result of the metabolism of ethanol. They do support the hypothesis that large doses of ethanol increase peripheral mobilization of fatty acids (*1, 2*), and it is worth noting that high concentrations of alcohol in the blood of man were found to raise the plasma concentration of free fatty acids (*4*). The present results would also be compatible with a direct effect of ethanol per se on hepatic utilization of fatty acids, as postulated by Lelbach (*13*). It is clear that the mechanism of production of fatty liver by ethanol cannot yet be considered settled.

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Impairment of Shock Avoidance Learning after Long-Term Alcohol Ingestion in Mice

Abstract. *Chronic alcohol consumption impaired the learning of a two-way shuttle box avoidance task in mice 10 to 14 days after the discontinuation of ethanol in the diet. Control groups received laboratory chow ad libitum or were pair-fed with the alcohol-consuming mice by diets containing isocaloric amounts of sucrose. The performance of the two control groups was indistinguishable from each other, and only the ethanol-consuming mice performed poorly. It was therefore concluded that alcohol consumption per se and not a nutritional deficiency was responsible for the impairment of learning.*

Brain damage in patients suffering from chronic alcoholism is generally attributed to malnutrition, particularly thiamine deficiency (*1*). However, the effect of chronic ingestion of alcohol per se with a diet adequate in all essential nutrients upon learning ability in laboratory rodents under controlled conditions has not been reported. It is the purpose of this study to investigate whether chronic ingestion of ethanol with a nutritionally adequate diet may result in impairment of performance in a shock-avoidance task in mice. The learning rate of alcohol-consuming mice was impaired 2 weeks after the acute effects of ethanol consumption had subsided.

Forty-two 8-week-old female mice (C-57 Bl/6j) were divided into three equal groups. One group received laboratory chow and water ad libitum. The remaining two groups were pair-fed with liquid diets containing either ethanol or sucrose as 35 percent of the total calories as described (*2*). Two additional groups of eight mice were pair-fed with the alcohol- and sucrose-containing diets for 3 months and were decapitated on the last day of consuming the liquid diets. Blood was collected from individual animals for microhematocrit determinations and pooled from two mice each for determination of serum folate and vitamin B_{12} concentrations (*3*). Sections from liver, kidney, heart, and lungs were fixed immediately in buffered formaldehyde. Adrenal glands were dissected out, weighed, and then fixed for tissue sections.

After 3 months the diet of all groups was changed to laboratory chow, and the degree of severity of withdrawal reaction was determined (*4*). The mice were then maintained on laboratory chow and water for 10 to 14 days before they were tested in a shuttle box (*5*). The box was modified to keep the cage floor in its original position for the duration of the delivery of shock in order to prevent the mouse from tripping the floor switch by bouncing without actually escaping into the other compartment. A sound and a light (conditioned stimulus) were presented simultaneously for 5 seconds. The unconditioned stimulus was a 0.3-ma scrambled shock delivered to the cage floor for a minimum of 5 seconds or until the mouse escaped to the other side of the cage. The intertrial interval was 30 seconds, and the intersession interval was 24 hours. Each session consisted of 30 trials recorded in blocks of ten. An avoidance response was recorded when the animals moved to the other side of the shuttle box during the 5-second warning period preceding the shock. After training for active avoidance for 16 days, passive avoidance training was begun 1 minute after the last active avoidance session by applying current to the opposite side of the shuttle box floor. The mice previously trained to actively avoid shock by escaping to the other compartment now