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

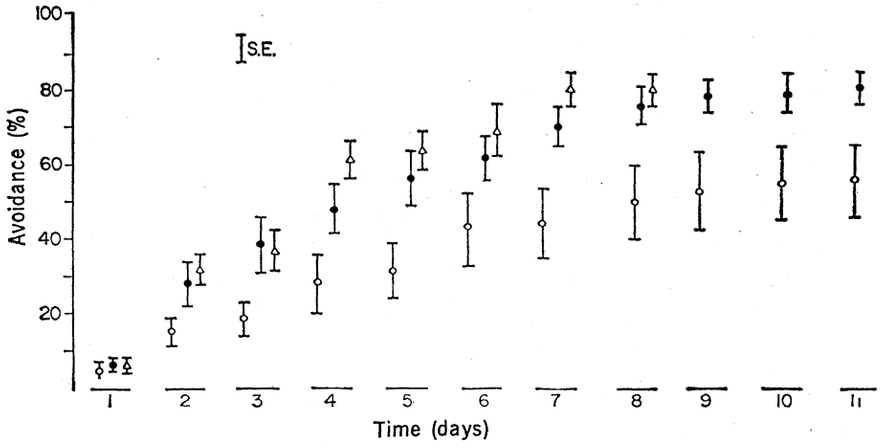

Fig. 1. Performance of mice in a shock-avoidance task in a shuttle box, expressed as mean, and standard error (S.E.) of 30 trials per session. Open circles, performance of ethanol-consuming mice; filled circles, performance of sucrose-consuming mice; triangles, performance of mice on a diet of laboratory chow.

had to remain in the original compartment upon presentation of the conditioned stimuli in order to avoid shock (*6*). The criterion for passive avoidance was five correct responses in five trials. Autopsies were obtained at the end of the last testing session, and sections of kidney, liver, lung, and heart stained with hematoxylin and eosin were examined.

The mean body weights (± the standard deviation) of the mice at the beginning of behavioral testing were 22.4 ± 1.3 g for the group consuming water, 23.6 ± 1.1 g for the group consuming sucrose, and 23.3 ± 1.3 g for the alcohol group. The maximum weight loss of the ethanol-consuming mice was 1.2 g on day 8, but the majority of this group of mice lost no weight. The mean daily alcohol consumption calculated as absolute ethanol was 0.58 ml per mouse. Loss of ethanol from the liquid diet as determined by gas chromatographic analysis of samples obtained from the end of the drinking tubes (*7*) at the end of a 24-hour period was less than 2 percent of the amount of ethanol contained in the diet. Upon substitution of laboratory chow for the liquid diet a withdrawal reaction was observed in all mice that formerly received alcohol in the diet: nine mice had nonlethal convulsions, and five mice developed tremors and forward arching of the tail described as Stage II severity of withdrawal (*4*).

Blood analyses of mice killed during the last day of ethanol or sucrose consumption showed the following results. Mean microhematocrit: ethanol, 45.7 percent and sucrose, 46.2 percent. The mean concentration of folic acid in the serum (normal for man in this laboratory is 7 to 16 ng/ml) was 30.5 ng/ml for the ethanol group and 30.0 ng/ml for the sucrose group. The mean concentration of vitamin B_{12} in the serum (normal for man in this laboratory: 200 to 900 pg/ml) was 527 pg/ml for the ethanol-consuming group and 476 pg/ml for the sucrose-consuming group. The mean weight of the adrenal glands (paired) was 8.3 mg for the ethanol-consuming group and 7.0 mg for the sucrose group. The mean weights of adrenal glands for animals consuming laboratory chow ranged from 6.5 to 9.0 mg. Histological sections (*8*) of the adrenal glands failed to reveal cortical hyperplasia. Sections of lung, heart, and kidneys were interpreted as being normal. The sections of the liver revealed 1+ to 2+ small fat droplets in liver cells graded according to Lieber and Rubin (*9*). The degree of fatty infiltration was equally distributed between sucrose- and ethanol-consuming groups. There was no evidence of hepatocellular degeneration or portal cirrhosis. Sections of kidney, heart, lung, and liver of the mice killed after testing in the shuttle box revealed no abnormalities; specifically, the liver sections of the sucrose- and ethanol-consuming groups showed no histologically demonstrable fat, liver cell necrosis, or portal cirrhosis.

The rate of learning of ethanol-consuming mice after discontinuation of alcohol diet was slower than that of mice on sucrose and laboratory chow diets (Figs. 1 and 2). Analysis of variance of groups versus days (*10*) resulted in a statistically significant group effect ($P < .005$), a days effect ($P < .001$), and interaction ($P < .001$). By Newman-Keuls comparisons the ethanol-consuming group performed significantly worse than either the sucrose or laboratory chow groups ($P < .001$). The performance of the laboratory chow and sucrose groups was statistically indistinguishable. The rate of learning became asymptotic at a mean of 56 percent correct responses on day 11 for the ethanol-consuming mice after discontinuation of alcohol diet as compared with an asymptotic rate of 81 percent for the controls ($P < .005$). The performance of the sucrose- and ethanol-consuming groups (after discontinuation of alcohol diet) continued to be asymptotic to day 16 (not shown in figures). There were no "freezing responses" observed under the conditions of this experiment. Passive avoidance to criterion (five correct responses in five

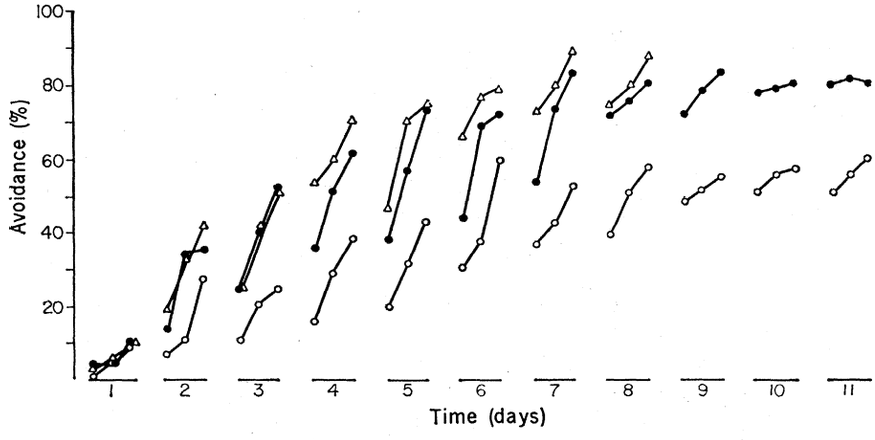

Fig. 2. Progression of performance during each daily session recorded in blocks of ten trials. Open circles, performance of ethanol-consuming mice; filled circles, performance of sucrose-consuming mice; triangles, performance of mice on a diet of laboratory chow.

trials) was achieved after a mean of eight trials with no significant differences between the three groups, which probably indicates no deficit in inhibiting a previously learned response.

The behavioral effects of acutely elevated concentrations of alcohol in the blood are well documented (*11*). However, there are no reports of the effects of chronic ingestion of ethanol and appropriate control diets upon animal learning after the cessation of the acute effect of ethanol. Whereas it appears well established that thiamine deficiency may cause a memory defect and neurological symptoms in man, whether associated with chronic alcoholism or not (*1*), the possibility must be considered that chronic ethanol consumption itself may also be deleterious to the brain. The demonstration of such a direct effect of ethanol or its metabolites upon brain function may imply that not all toxic effects of chronic ethanol consumption upon the brain can be prevented by the administration of vitamins.

The diets selected for this investigation were nutritionally adequate as documented by composition (*2*, *4*), body weight comparison, and absence of clinical signs recognized as associated with vitamin deficiencies of mice (*12*). There was no significant weight loss due to consumption of the ethanol-containing diet in the mouse strain (C-57) used in this study, which has a high preference for alcohol. The previously described temporary weight loss (*2*, *4*) in a mouse strain with a low preference for ethanol (ICR-DUB) consuming a comparable diet was not observed under the conditions of this experiment. The possibility that ethanol in the diet resulted in decreased absorption of vitamins under the conditions of this experiment is highly unlikely in view of the nearly identical serum folate and vitamin B_{12} concentrations in both ethanol- and sucrose-consuming groups. There was no evidence of anemia in any of the groups. The calculated intake of all vitamins consumed under the conditions of this experiment (*2*, *4*) ranged from 1½ to 2 times that required for growing mice (*13*), whereas decreased absorption of vitamins reported in some chronic alcoholic patients is on the order of magnitude from 0 to 40 percent of the administered dose (*14*).

The possibility exists that the differences in performance between the ethanol- and sucrose-consuming groups is secondary to a stress reaction and chronically increased release of adrenal corticotropic hormone (ACTH) and adrenal corticoid caused by the effects of ethanol. It is highly unlikely that under the conditions of this experiment ethanol represented a significant stressor in view of normal adrenal weights and histology. Chronic release of ACTH and stress are known in rodents to increase the adrenal weight two- to threefold by the end of the second week (*15*). Furthermore, almost all available evidence suggests an improved performance of avoidance conditioning under various conditions of stress or increased concentrations of adrenal steroid (*16*). If ethanol had induced stress under the conditions of this experiment, then this should have served to diminish the observed differences in performance between the ethanol- and sucrose-consuming groups.

The mice were tested in the shuttle box 10 to 14 days after drinking the last dose of alcohol. This excluded the possibility that acutely elevated concentrations of alcohol in the blood could have interfered with performance. There was no significant difference between the performance of animals receiving laboratory chow and the liquid diet containing sucrose, so that any difference in performance of mice which had previously ingested ethanol must be attributed to an effect of ethanol per se and not to a dietary deficiency. The significance of learning the shuttle box avoidance tasks has been discussed (*5*). The possibility that impaired performance of ethanol-treated mice is due to decreased activity is unlikely because the intertrial interval responses of all three groups were not significantly different. It is possible that mice drinking ethanol have become less sensitive to the shock stimulus. An impairment in processing painful stimuli by the peripheral or central nervous system in the animals treated with ethanol may possibly explain the observed results (*17*). It is more likely that ethanol or its metabolites impair associative processes of learning in the central nervous system.

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Forgetting: Trace Erosion or Retrieval Failure?

Abstract. *A series of lists of random words was presented. Following each list, the subject attempted to recall the words of the list prior to the list just presented. Recall probability for a given word depended on the length of the list in which it was embedded, not on the length of the list intervening between presentation and test. These results indicate that forgetting is a failure in the memory search during retrieval rather than a degradation of the memory trace occurring between presentation and test.*

Models of forgetting may be divided into two classes. The first holds that the memory trace formed during presentation is degraded between that moment and the later time of test. In a decay theory, time alone is sufficient to cause degradation of the trace (*1*). An interference theory, however, as-