The Acute Effect of Alcohol on Hepatic Coenzyme A and Acetyl CoA Concentrations (36058)

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Several investigations have shown convincingly that ethanol[1] inhibits the citric acid cycle in the liver (1, 2), but the exact nature of this effect is still uncertain (3). One prior study employing low alcohol levels in a perfused liver system has suggested that the impairment of the citric acid cycle is due to a coordinated inhibition of citrate synthase and isocitrate dehydrogenase mediated by an increased NADH/NAD ratio (2). It is not established, however, whether these observations apply also to the *in vivo* situation and to higher blood alcohol levels. Another aspect of this problem previously studied has dealt with the effect of ethanol on hepatic coenzyme A (CoA) and acetyl-CoA concentrations. The results of these investigations, however, have been contradictory. As regards CoA, Ammon *et al.* (4, 5) have shown that alcohol given intravenously to mice induces a striking depression of hepatic CoA, and this was felt to be due to complexing of the CoA with acetaldehyde generated by alcohol metabolism. On the other hand, no decrease of hepatic CoA has been demonstrated by Williamson *et al.* (2), using a rat liver perfusion system and employing a relatively low alcohol concentration in the perfusate; or by Bode *et al.* (6), 2 hr after the oral administration of a single large alcohol load to rats kept on a standard diet. The available data regarding the effect of ethanol on hepatic acetyl-CoA are equally confusing. Decreased (7), increased (8), or unaltered (2) liver acetyl-CoA concentrations have been reported after alcohol administration to rats. These various inconsistencies have been so far either left unanswered or have been attributed to differences in species or manner of alcohol administration. Since the concentration of CoA and acetyl-CoA in liver may influence importantly the citric acid cycle, it was the purpose of this study to resolve the present controversy by assessing in detail the acute effect of alcohol on hepatic CoA and acetyl-CoA levels.

Materials and Methods. Two sets of experiments were carried out using nonfasted female Sprague-Dawley rats (80–100 g) and female and male Swiss Albino mice (20 g). In the first studies, groups of rats and mice were given alcohol in various doses orally, intraperitoneally, or intravenously; and at specified times thereafter, liver CoA and acetyl-CoA concentrations and pyruvate decarboxylase activity were measured and correlated with the blood alcohol level. Pyruvate decarboxylase activity was investigated, since this enzyme participates in the reaction wherein pyruvate is converted to acetyl-CoA. In the second group of studies, we assessed the concept of Ammon *et al.* (5) that acetaldehyde derived from alcohol binds and depresses hepatic CoA. Accordingly, some animals were given disulfiram (which inhibits acetaldehyde metabolism) prior to being injected with alcohol, while other rats were given acetaldehyde directly. Controls consisted of uninjected animals and those given isocaloric glucose or saline. The details of the experimental protocol for each study are given in Tables I, II, and III.

The alcohol was given as a 20–40% solution in water (depending on the dose) with care to administer only about 1 ml to rats and 0.4 ml to the mice. Disulfiram was finely pulverized in water and acetaldehyde was freshly dissolved in 0.89% saline.

For tissue analysis, the animals were decapitated; and the liver samples were removed immediately and frozen in liquid ni-

[1] Alcohol and ethanol are used interchangeably.

TABLE I. Effect of Alcohol on Hepatic Acetyl-CoA and CoA Concentration and on Hepatic Pyruvate Decarboxylase Activity in Rats.

Alcohol dose	Sampling time after alcohol (min)	Blood alcohol (mg/100 ml)[a]	Alcohol group			Glucose-injected control group[d]		
			Acetyl-CoA[b]	CoA[b]	Pyruvate decarboxylase activity	Acetyl-CoA[b]	CoA[b]	Pyruvate decarboxylase activity
3.0 g/kg, po	45	111.2 ± 30.1	43.67 ± 5.54[e]	132.5 ± 3.99		36.94 ± 2.08	140.2 ± 8.74	
1.5 g/kg, ip	15	101.5 ± 6.7	35.28 ± 1.69[e]			28.99 ± 3.52		
	45	79.5 ± 17.8	27.59 ± 0.98			22.12 ± 2.60		
3.0 g/kg, ip	15	231.3 ± 31.7	32.12 ± 1.80[e]			32.58 ± 1.66		
	45	133.3 ± 35.8	27.56 ± 2.52			32.49 ± 3.01		
4.0 g/kg, ip	15	295.3 ± 59.2	32.84 ± 2.14[e]		111.4 ± 12.46	28.52 ± 2.61		143.8 ± 24.64
	45	168.5 ± 47.3	29.05 ± 2.04[e]	121.6 ±12.75	82.4 ± 12.70	36.34 ± 1.69	119.3 ± 9.36	106.1 ± 10.95
4.0 g/kg, iv	30	306.6 ± 46.1	42.05 ± 2.79[e,f]			25.00 ± 1.76		

[a] Each value is the mean ± SE of 4 animals.

[b] Nanomoles per gram of liver wet weight. Each value is the mean ± SE of 6–10 animals.

[c] Micromoles of pyruvate decarboxylated per gram of tissue per 30 min. Each value is the mean ± SE of 6–12 determinations.

[d] Uninjected control values were: acetyl-CoA, 24.11 ± 2.04; and CoA, 113.1 ± 6.51 nmoles/g of wet wt, while pyruvate decarboxylase activity was 137.9 ± 23.5 μmoles of pyruvate decarboxylated/g of tissue/30 min. Each value is the mean ± SE of 8–12 determinations.

[e] Significantly higher ($p < .05$ or less) than uninjected controls.

[f] Significantly higher ($p < .005$) than corresponding glucose control. The other alcohol treated animals are not significantly different from the corresponding glucose controls.

trogen for acetyl-CoA and on Dry Ice for CoA determinations. Use of the *in situ* freeze-stop technique in some animals gave more erratic and no higher values for acetyl-CoA. Unfrozen fresh liver was used for pyruvate decarboxylase measurements since tissue freezing lowers the activity of this enzyme (9). Acetyl-CoA was measured by the procedure of Wieland and Weiss (10) using Pearson's correction for the calculations (11). Each assay was carried out in triplicate and the results were averaged. Recovery of weighed amounts of acetyl-CoA, added to liver homogenates, averaged 90%. For CoA analysis, the boiling extraction method was employed (12), since this procedure has been shown previously to give the highest CoA yield (13). CoA was assayed by the method of Srere using highly purified citrate cleavage enzyme (14, 15). This assay was highly reproducible and gave recoveries of 95% with known quantities of CoA. *In vitro* studies indicated that alcohol in concentrations comparable to those present in blood of the experimental animals did not interfere with the acetyl-CoA or CoA assays. Pyruvate decarboxylase activity was measured by the procedure of Dreyfus and Hauser (9). Alcohol in blood was analyzed with alcohol dehydrogenase (16). Statistical analysis was carried out by the nonparametric Mann-Whitney U test (17).

Results and Discussion. Table I shows the acute effect of alcohol on hepatic acetyl-CoA concentration. The control acetyl-CoA values are comparable to data obtained by other investigators (2, 6, 8). It is evident that in general alcohol, variously administered, failed to alter significantly hepatic acetyl-CoA as compared to values in glucose-injected controls. Animals given intravenous alcohol, however, did manifest an increase in hepatic acetyl-CoA concentration ($p<.005$). Furthermore, the alcohol-injected rats had somewhat higher hepatic acetyl-CoA levels than the uninjected controls, and in most instances this difference was significant (Table I). These data agree with and amplify some prior studies (2, 6, 8) but do not corroborate the report that alcohol acutely lowers the hepatic acetyl-CoA concentration (7). In addition it is apparent that hepatic pyruvate decarboxylase activity, wherein pyruvate is converted to actyl-CoA, is not significantly altered by alcohol ($p>.05$) (Table I). There are several possible explanations for the sometimes observed increase in hepatic acetyl-CoA after alcohol. First, anesthesia may increase the hepatic acetyl-CoA (18, 19) and this is why our studies were carried out in unanesthetized animals. This possibility, however, is unlikely since some of the increased acetyl-CoA levels were obtained in alcohol-injected rat showing no depression of consciousness, as is corroborated by the modest blood alcohol levels in these animals. Second, alcohol may generate acetyl-CoA in the course of its metabolism. This mechanism is possible but alcohol is now felt to be converted primarily to acetate which is metabolized extrahepatically (3); and, in our studies, the level of hepatic acetyl-CoA does not seem to correlate well with the administered alcohol load. The last possibility is that alcohol impairs the metabolism of acetyl-CoA in the citric acid cycle, resulting in acetyl-CoA accumulation. This mechanism deserves experimental verification.

As shown in Table I, alcohol given acutely to rats did not alter the hepatic CoA concentration. Since Ammon *et al.* (5) reported a striking depression of hepatic CoA in mice at similar blood alcohol levels, comparable studies were also carried out in mice to rule out specie differences. These data, presented in Table II, clearly show that alcohol as compared to isocaloric glucose failed to depress the hepatic CoA level. Unaccountedly, female mice given glucose ip showed more variation in hepatic CoA and the mean value was modestly reduced ($p<.05$) compared to other groups. However, the corresponding alcohol-injected animals had somewhat higher, not lower, hepatic CoA levels. In addition, uninjected mice had a similar hepatic CoA concentration to that observed in animals given alcohol. Our studies, therefore, do not support the concept that alcohol lowers the hepatic CoA concentration. Diverse methodology probably explains our inability to corroborate the prior findings of Ammon *et al.* (4, 5). The CoA assay used by these investiga-

TABLE II. Effect of Alcohol on Hepatic CoA Concentration in Mice.

Alcohol dose	Sex	Blood alcohol (mg/100ml)	CoA conc (nmoles/g of liver wet wt)		
			Alcohol group	Control group	
				Glucose-injected	Uninjected
4.0 g/kg, ip	Female	295.3 ± 7.9[a] (9)	154.6 ± 5.89 (24)	119.2 ± 10.13[b] (20)	155.5 ± 5.20 (26)
4.0 g/kg, iv	Female	304.3 ± 61.6 (12)	178.9 ± 8.64 (11)	157.7 ± 7.08 (9)	
4.0 g/kg, ip	Male	285.3 ± 24.3 (12)	162.6 ± 11.21 (10)	171.9 ± 8.82 (12)	163.1 ± 11.48 (10)

[a] Mean ± SE of number of animals given in parentheses. For plasma alcohol measurement, groups of 3 animals were pooled for each assay. All assays were performed at 60 min after alcohol (or glucose) administration.

[b] Significantly below ($p < .05$) the comparable uninjected and alcohol groups. All other corresponding alcohol and control groups of mice had comparable CoA levels ($p > .05$).

tors appears to be nonspecific and is believed to measure substantial amounts of other substances in addition to CoA (13). Indeed, the specific enzymatic procedure employed in this study gives CoA values for control mouse liver about half of those reported by Ammon *et al.* (4). The CoA concentrations we obtained in normal rat liver, however, agree well with data reported by others for this species using alternate but specific assays (2, 6, 13).

TABLE III. Effect of Acetaldehyde on Hepatic CoA Concentration in Rats.

Experimental group	CoA (nmoles/g of liver wet wt)
Uninjected	113.1 ± 6.51[a] (6)
Disulfiram, 0.5 mg/kg, po[b]	85.5 ± 5.97 (6)
Disulfiram, 0.5 mg/kg, po;[c] alcohol, 1.5 g/kg, ip	81.5 ± 3.76 (6)
Disulfiram, 0.5 mg/kg, po;[c] glucose, 2.6 g/kg, ip	87.5 ± 5.60 (6)
Acetaldehyde, 5 μmoles/g, ip[d]	137.7 ±16.81 (6)
Normal saline, ip[d]	81.4 ± 6.41 (6)

[a] Mean ± SE of number of animals shown in parentheses.

[b] Animals sacrificed 90 min after disulfiram administration.

[c] Alcohol or glucose given 30 min after disulfiram. Animals sacrificed 90 min after disulfiram administration.

[d] Acetaldehyde was dissolved in normal saline. Animals were sacrificed 5 min after acetaldehyde or saline injection. The acetaldehyde-injected rats exhibited hyperventilation and drowsiness at 3–5 min.

Finally, we tested the hypothesis that acetaldehyde, generated from alcohol metabolism, depresses hepatic CoA. In these studies we paralleled the protocol of Ammon *et al.* (4, 5) but applied it to rats. Table III shows that rats given disulfiram and alcohol exhibited the same hepatic CoA concentration as controls given disulfiram alone or together with isocaloric glucose. In addition, rats given acetaldehyde and sacrificed immediately after the onset of neurologic signs also failed to show a depression of hepatic CoA.

Our composite data therefore clearly indicate that acutely administered ethanol does not alter significantly hepatic pyruvate decarboxylase activity or its CoA content and results in a normal or somewhat increased acetyl-CoA concentration in the liver. It appears that the acute depressive effect of alcohol on the hepatic citric acid cycle is mediated at another biochemical site which remains to be precisely elucidated.

Summary. Since prior studies of the effect of alcohol on hepatic coenzyme A (CoA) and acetyl-CoA concentrations have yielded conflicting data, this investigation aimed to assess in detail the acute effect of alcohol on the concentration of these substances in liver. Rats and mice were given various loads of alcohol and, at selected time intervals, hepatic CoA and Acetyl CoA concentrations were

determined and correlated with blood alcohol levels.

The present study clearly shows that alcohol given acutely does not alter significantly hepatic CoA content and results in a normal or somewhat increased acetyl-CoA concentration in the liver. Acetaldehyde likewise failed to influence significantly the hepatic CoA concentration. We conclude that the acute depressive effect of alcohol on the hepatic citric acid cycle is not exerted at the CoA–acetyl-CoA level.

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