Relation of Ethanol Inhibition of Hepatic Fatty Acid Oxidation to Ethanol-Induced Fatty Liver (32641)

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Ethanol administration decreases total CO_2 production by the liver (1). In addition, the oxidation of palmitate-[1-^{14}C] to $^{14}CO_2$ by liver slices is decreased when ethanol is added to the incubation medium (2,3). It has been proposed that decreased hepatic fatty acid oxidation could be an important mechanism in the pathogenesis of ethanol induced fatty liver (4), since inhibition of a pathway for the removal of fatty acids from the liver could provide increased substrate for hepatic triglyceride synthesis. Ethanol decreases the oxidation of acetate-[1-^{14}C] to $^{14}CO_2$ in liver slices (3); but it has not been shown that ethanol decreases the oxidation of fatty acids to acetyl-CoA. This is an important distinction; for if ethanol blocked the oxidation of acetyl-CoA to CO_2 without affecting the oxidation of fatty acids to acetyl-CoA, ethanol inhibition of CO_2 production from fatty acids would not be of importance in the pathogenesis of ethanol induced fatty liver.

Ethanol might directly inhibit β-oxidation of fatty acids; or a block in the oxidation of acetyl-CoA could result in an indirect inhibition of β-oxidation. Because ethanol feeding increases the hepatic concentration of α-glycerophosphate (5,6), which results in an enhanced esterification of fatty acids to form complex lipids (5,7), it is difficult to differentiate between the possibilities that ethanol primarily enhances hepatic fatty acid esterification or that the increased incorporation of fatty acids into liver triglycerides and phospholipids after ethanol results from decreased fatty acid oxidation (8,9). Thus it is difficult to decide whether ethanol inhibition of CO_2 production from fatty acids leads of itself to an accumulation of these acids, in the form of complex lipids, in the liver. We have investigated this problem by taking advantage of the fact that medium chain fatty acids are poorly esterified with α-glycerophosphate in rat liver (10,11). The action of ethanol on the hepatic metabolism of octanoate, a C_8 fatty acid, would be expected to be limited to oxidative pathways. Thus, if hepatic fatty acid oxidation is blocked by ethanol in a manner which leads to an increase in the availability of fatty acids for the synthesis of complex lipids, ethanol administration should lead to an accumulation of free octanoate in livers perfused with this acid.

In the present experiments we have compared the effect of ethanol administration on the metabolism of octanoate-[1-^{14}C] and palmitate-[1-^{14}C] to CO_2 and complex lipids in perfused rat livers. The data demonstrate that ethanol blocks CO_2 production from both fatty acids. However, this ethanol inhibition of fatty acid oxidation does not per se lead to an accumulation of fatty acids in the liver, suggesting that ethanol induced inhibition of hepatic fatty acid oxidation is not quantitatively important in the pathogenesis of ethanol induced fatty liver.

Materials and Methods. Rats of the Holtzman strain were used for all experiments. Male rats weighing 350–450 gm were used as liver donors. Red cells for perfusate were obtained from discarded breeders. Sodium octanoate-[1-^{14}C] and palmitate-1-^{14}C were obtained from Volk Radiochemical Company, and unlabeled octanoate and palmitate from the Hormel Institute. These acids were not purified further. Bovine serum albumin, fatty acid poor, was purchased from Pentex Corp.

Rats were fed *ad libitum* on Purina Rat Chow until the start of an experiment. Liver perfusions were carried out according to the method of Miller (12). Perfusate consisted of twice-washed red cells added to Krebs–Henseleit bicarbonate buffer (13), which contained bovine serum albumin and glucose (1 mg/ml buffer). The final perfusate hematocrit was 18% and total perfusate volume was 80 ml. After 30–45 min of perfusion, ethanol and/or labeled fatty acid was added to the

TABLE I. Effect of Ethanol on Recovery of ^{14}C in $^{14}CO_2$ and Liver Lipids After Perfusion with Octanoate-[1-^{14}C] or Palmitate-[1-^{14}C].

Fatty acid	Ethanol	^{14}C (%) recovered as CO_2 per gm liver per 90 min[a]	^{14}C (%) recovered in liver lipids per gm liver per 90 min	p[c]
Octanoate-[1-^{14}C]	—	1.87 ± 0.170 (7)[b]	0.334 ± 0.039 (5)	
	+	0.610 ± 0.067 (7)	0.385 ± 0.027 (5)	<0.2
Palmitate-[1-^{14}C]	—	0.830 ± 0.037 (5)	3.51 ± 0.32 (5)	<0.02
	+	0.242 ± 0.027 (5)	4.75 ± 0.32 (5)	

[a] Mean weight of liver in each experimental group: Octanoate, 12.1 gm; octanoate + ethanol, 11.6 gm; palmitate, 11.3 gm; palmitate + ethanol, 11.5 gm.

[b] Mean ± SE; number of experiments in parentheses.

[c] p for differences in ^{14}C recovered in total liver lipids.

perfusate reservoir. The amount of ethanol added was calculated to give an initial ethanol concentration of 200 mg/100 ml perfusate. In control experiments an equal volume of saline was added. Octanoate and palmitate were added as the albumin solutions of the fatty acid. In octanoate experiments the initial fatty acid concentration was 1.82 mmoles/liter of perfusate plasma, and in palmitate perfusions 2.57 mmoles/liter. The final bovine serum albumin concentration of perfusate was 5% (w/v). The perfusion rate was maintained in excess of 1 ml per min per gm liver in all experiments.

After addition of ethanol and/or labeled fatty acids, the perfusion was continued for 90 min. The CO_2 was collected in a column of hyamine. At the end of the perfusion an aliquot of perfusate was removed from the reservoir and the liver was perfused with cold saline, the livers blotted, weighed, and portions were taken for lipid analysis. Cell-free perfusate and liver aliquots were extracted in $CHCl_3/CH_3OH$ (14), adjusted to pH 2 (15). After washing with 0.2 volumes of 0.1 *N* HCl, the extracts were dried under a stream of nitrogen. With extracts from octanoate perfused livers this was carried out at 0°C on an ice bath (16). Perfusate lipids were separated into fatty acids and neutral lipid fractions by the method of Borgstrom (17). Liver lipids were separated on thin-layer plates using petroleum ether, ether, and acetic acid (90:10:1). Aliquots of the hyamine and perfusate lipids were counted in toluene scintillation solution. Liver lipids were scraped from thin-layer plates and counted in toluene scintillation solution containing 4% cabosil. All counting was done on a Packard Tricarb liquid scintillation counter utilizing external standards or channels ratio for quench correction. Student's t test was used for determination of statistical significance.

Results. The effect of ethanol on fatty acid oxidation to CO_2 and incorporation into liver and perfusate lipids. The data in Table I show that when ethanol was added to the perfusate the recovery of $^{14}CO_2$ production from octanoate-[1-^{14}C] was decreased by a factor of 3. Similarly, $^{14}CO_2$ production from palmitate-[1-^{14}C] was approximately 3-fold lower in ethanol perfusions than in control perfusions. The recovery of $^{14}CO_2$ from octanoate was 2.3 times greater than that for palmitate in control experiments and 2.5 times greater when ethanol was added to the perfusate. Since octanoate-[1-^{14}C] has 8 carbons per ^{14}C label whereas palmitate-[1-^{14}C] has 16 carbons per label, these ratios for $^{14}CO_2$ recovery plus the differences in the initial concentrations of each acid indicate that the amounts of octanoate and palmitate oxidized completely to CO_2 were approximately the same. The data thus demonstrate that ethanol did not have a preferential effect on CO_2 production from either of these acids.

Though ethanol had similar effects on CO_2 production from octanoate or palmitate, there were differences in the recovery of label in liver lipids. Total ^{14}C recovery in liver lipids from octanoate-[1-^{14}C] perfused livers was the same in control and ethanol perfusions.

TABLE II. Percentage Recovery of ^{14}C in Perfusate Lipids.[a]

Fatty acid	Ethanol	Total ^{14}C recovered as perfusate lipid	^{14}C recovered as perfusate neutral lipids
Octanoate-[1-^{14}C]	—	3.37 ± 1.05 (5)	0.550 ± 0.124 (4)
	+	4.89 ± 0.39 (5)	0.924 ± 0.122 (5)
Palmitate-[1-^{14}C]	—	10.05 ± 1.81 (5)	0.40 (2)
	+	6.46 ± 0.59 (5)	0.24 ± 0.01 (3)

[a] Mean ± SE; number of experiments in parentheses.

In contrast, the addition of ethanol to palmitate-[1-^{14}C] perfusions was associated with a significantly greater recovery of ^{14}C in liver lipids. As expected, the recovery of ^{14}C in lipids was considerably greater in palmitate than in octanoate perfusions.

In order to be certain that lipid recovery in the liver accurately reflected the effect of ethanol on hepatic fatty acid metabolism, the amount of ^{14}C fatty acid remaining in the perfusing medium was also measured. These data are shown in Table II. Ethanol did not significantly alter the amount of octanoate-[1-^{14}C] remaining in the perfusate. Separation of the neutral lipid and fatty acid fraction indicated that most of the perfusate lipid was present as fatty acid. In the palmitate experiments there was a greater recovery of lipid in the control experiments than in the ethanol experiments, but this difference was not significant ($.05 > p < .1$). Again, most of the lipid remaining in the perfusate was present as free fatty acid. The recovery of ^{14}C as perfusate neutral lipid was quite low in all experiments. A greater amount of ^{14}C was recovered in perfusate neutral lipids after octanoate perfusion than after palmitate perfusion, especially in view of the lower recovery of ^{14}C from octanoate than from palmitate in liver lipids. There is no obvious explanation for this finding.

The effect of ethanol perfusion on the distribution of recovered ^{14}C in hepatic lipid fractions. In the octanoate-[1-^{14}C] experiments there was a small but significantly greater recovery of ^{14}C in hepatic phospholipids in the presence of ethanol (Table III) as compared to the control perfusions. The recovery of ^{14}C in the other lipid fractions was the same in ethanol and control studies. Despite decreased $^{14}CO_2$ recovery from octanoate in the presence of ethanol, there was no enhanced recovery of octanoate in the free fatty acid fraction of the liver lipids. Also, a relatively large amount of ^{14}C was recovered in the cholesterol fraction, representing oxidation of octanoate-[1-^{14}C] to acetyl-CoA-^{14}C with subsequent recycling of the label into cholesterol. Since gas chromatographic analysis of the chain lengths of fatty acids found in the complex lipid fractions from octanoate-[1-^{14}C] perfused livers was not done, we cannot state whether recovery of octanoate label in liver lipids represents direct esterification

TABLE III. Distribution of Recovered ^{14}C in Hepatic Lipids; Percentage of Total ^{14}C Recovered per Fraction.

		Liver lipid fraction[b]					
Fatty acid	Ethanol	PL[a]	DG	C	FA	TG	CE
Octanoate-[1-^{14}C]	—	29.7 ± 1.19	10.1 ± 1.45	18.65 ± 1.52	10.8 ± 2.26	23.9 ± 2.38	6.83 ± 1.42
	+	37.3 ± 0.90	7.94 ± 0.85	14.9 ± 0.90	7.94 ± 0.46	24.7 ± 1.67	7.01 ± 0.78
Palmitate-[1-^{14}C]	—	41.1 ± 5.09	3.40 ± 0.30	1.72 ± 0.28	1.71 ± 0.37	51.6 ± 5.06	1.16 ± 0.32
	+	34.0 ± 8.01	3.33 ± 0.74	2.53 ± 0.97	2.90 ± 0.90	55.7 ± 5.71	1.55 ± 0.56

[a] Abbreviations: PL, phospholipid; DG, diglyceride; FA, fatty acids; TG, triglycerides; C, cholesterol; CE, cholesterol esters.

[b] Values are the means ± SE for 5 rats.

of octanoate, breakdown of octanoate to acetyl-CoA with a *de novo* synthesis of fatty acids, or chain lengthening reactions.

The distribution of recovered palmitate-[1-^{14}C] in the hepatic lipid fractions was essentially the same in control and ethanol perfusions, but there were differences from the pattern of isotope recovery in octanoate-[1-^{14}C] perfusions. The recovery of ^{14}C in cholesterol and fatty acid fractions was lower in palmitate than octanoate experiments, whereas in palmitate perfusions the percentage of isotope recovered was greater in triglycerides.

Discussion. These experiments demonstrate that ethanol decreases the production of CO_2 from octanoate and palmitate to the same extent. On the other hand, an ethanol induced inhibition of CO_2 production from octanoate did not lead to an accumulation of ^{14}C from octanoate in either liver or perfusate lipids. Most significant, ethanol perfusion did not increase the recovery of octanoate in liver free fatty acids. These findings suggest that ethanol blocks CO_2 production from octanoate without interfering with the entry of octanoate into the β-oxidation cycle. Thus, ethanol inhibition of octanoate oxidation did not increase the amount of fatty acid available for complex lipid synthesis.

In contrast to the data for octanoate, the recovery of palmitate in liver lipids was significantly greater after ethanol as compared to control perfusions. Moreover, the total recovery of ^{14}C from palmitate as CO_2 and lipids was greater in ethanol perfusions than in controls. This resulted from the fact that the decrease in recovery of ^{14}C as CO_2 in ethanol perfusions was 0.59%/gm liver whereas the increase in recovery as liver lipids was 1.24%/gm liver. Since ethanol increased the total recovery of palmitate label in lipid soluble fractions, it must have decreased the entry of palmitate into the β-oxidation cycle. On the basis of the data for octanoate perfusions this effect seems to have resulted from an enhanced esterification of palmitate with α-glycerophosphate rather than blockade of β-oxidation of palmitate. It is possible that the different effects of ethanol on the metabolism of medium and long chain fatty acids in these experiments was due to a specific effect of ethanol on the initial reactions of long chain fatty acid oxidation. Medium and long chain fatty acids not only differ with respect to their esterification with α-glycerophosphate (10,11) but also in their requirements for carnitine (18). Further, the enzymes responsible for β-oxidation differ as the chain length of the acyl group is shortened (18). However, a large reduction in the metabolism of palmitate to acetyl-CoA in ethanol perfused livers should have resulted in a decreased recovery of ^{14}C from palmitate in the cholesterol fractions in comparison to the control perfusions. This was not found.

As a result of an increased hepatic α-glycerophosphate concentration (5,6) ethanol probably did decrease the oxidation of palmitate to acetyl-CoA, by enhancing the incorporation of palmitate into complex lipids, thereby shunting palmitate away from oxidate pathways. Although we cannot definitely exclude some inhibition by ethanol of the activity of the β-oxidation cycle for palmitate, the data suggests that for both palmitate and octanoate the decreased production of CO_2 in the presence of ethanol resulted principally from a block in the metabolism of acetyl-CoA to CO_2. Our data, therefore, suggests that ethanol inhibition of CO_2 production from fatty acids is not of quantitative importance in the pathogenesis of ethanol fatty liver because ethanol does not directly affect the oxidation of fatty acid to acetyl-CoA. Irrespective of its effects on CO_2 production, ethanol increased the recovery of ^{14}C in fatty acids of liver lipids only when the perfused fatty acid could be esterified efficiency with α-glycerophosphate.

Summary. The quantitative importance of the inhibition of hepatic fatty acid oxidation for the pathogenesis of ethanol fatty liver has been investigated in perfused rat livers. Advantage has been taken of the fact that octanoate is poorly esterified in rat liver. Thus, by using octanoate the effects of ethanol on hepatic fatty acid esterification can be separated from effects on fatty acid oxidation. Comparison of the effect of ethanol perfusion on the metabolism of octanoate-[1-^{14}C] and palmitate-[1-^{14}C] indicated that ethanol de-

creased fatty acid oxidation to CO_2 for both acids. However, in contrast to the palmitate data, inhibition of the oxidation of octanoate to CO_2 did not lead to an increase in the recovery of ^{14}C from octanoate in the liver. Ethanol inhibition of CO_2 production from fatty acids did not of itself lead to an accumulation of fatty acids in the liver. Thus, the data suggest that ethanol inhibition of CO_2 production from fatty acids does not contribute to the accumulation of fatty acids (in the form of triglycerides) seen in livers from ethanol treated rats.

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Isolation, Stress, Myocardial Electrolytes, and Epinephrine Cardiotoxicity in Rats* (32642)

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It is known that certain emotional and environmental stresses can provoke or aggravate myocardial necrotization (1).

Prolonged isolation has been found in rats to be associated with a greatly increased catecholamine cardiotoxicity, as manifested by a severalfold decrease of the lethal dose of isoproterenol (2), and a marked intensification of isoprenaline-induced structural lesions (3) after 3 months of isolation: In isolated mice, the toxicity of indirectly adrenergic *d*-amphetamine was likewise augmented (4).

Selye (5) and others (6, 7) have shown that the necrotizing cardiotoxicity of catecholamines is remarkably potentiated by adrenal corticoids especially by 17-hydroxycorticosteroids.

An increased production of 17-hydroxycorticosteroids (8) as well as of total unconjugated corticoids (9) and an increased response to ACTH (9) have been observed in isolated rats. This may account for the isolation-induced exaggeration of catecholamine cardiotoxicity in such animals (2, 3). The mechanism of the catecholamine-"sensitizing" effect (5) of corticoids is not yet clearly understood. However, the myocardial potassium-depleting action of both corticoids (10–

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