Effect of chronic ethanol feeding on hepatic microsomal glycerophosphate acyltransferase activity

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Abstract The activity and submicrosomal distribution of α-glycerophosphate acyltransferase (GPAT) were studied in rats fed ethanol for 6 wk. GPAT activity was also measured in rats after 10 days of alcohol feeding, 22 days of phenobarbital administration, or 24 days on a high fat (71% of total calories) diet. After 6 wk of ethanol feeding, GPAT activity was increased 73% when expressed per milligram of protein and 133% when expressed per 100 g of body weight ($P < 0.005$). GPAT activity was more abundant in the smooth than in the rough microsomes of both control and ethanol-fed rats when expressed per milligram of microsomal protein and when expressed per gram of liver; the smooth microsomes accounted for most of the increased GPAT activity after ethanol. 10 days of ethanol feeding or 22 days of phenobarbital administration did not increase GPAT activity. Feeding a high fat diet for 24 days increased GPAT activity per milligram of protein to an extent similar to that observed after chronic ethanol administration. When expressed per 100 g of body weight, however, the increase was much greater after ethanol. The significance of these findings in vivo has not been elucidated. Increased GPAT activity might contribute to the persistence of alcoholic fatty liver and the development of hyperlipemia.

Supplementary key words fatty liver · fatty acid esterification · phenobarbital · smooth microsomes · rough microsomes · cytochrome P-450 · high fat diet · endoplasmic reticulum

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Abbreviations: GPAT, α-glycerophosphate acyltransferase; RM, rough microsomes; SM, smooth microsomes; P-450, cytochrome P-450; MEOS, microsomal ethanol-oxidizing system.

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Chronic ethanol administration has been shown to produce progressive accumulation of hepatic triglyceride (1), increased lipoprotein production, and hyperlipemia (2). Fatty acid synthesis is increased (3, 4) and fatty acid oxidation is depressed (3–5) in the presence of ethanol; this results in deposition in the liver of endogenously synthesized and dietary fatty acids (6, 7). The metabolism of ethanol is associated with an increase in the NADH/NAD ratio (8, 9) and a rise in the concentration of α-glycerophosphate in the liver (10). The increased production of liver lipids as a result of ethanol feeding suggested that hepatic lipid esterifying activity might also be enhanced. Radioautographic studies have shown a participation of the endoplasmic reticulum in fatty acid esterification (11). The first specific reaction in hepatic glycerolipid synthesis is catalyzed by the enzyme L-α-glycerophosphate acyltransferase (GPAT) (EC 2.3.1.15), which is present in the microsomal fraction obtained from liver homogenate (12). Since chronic ethanol consumption results in the proliferation of the endoplasmic reticulum (13–16), we wondered whether it also gave rise to an increase in GPAT activity which might contribute to the production and persistence of alcoholic fatty liver (17).

MATERIALS AND METHODS

Animal procedures

Sprague-Dawley strain rats were purchased from Charles River Breeding Laboratories (Wilmington, Mass.) in groups of weanling littermates. They were fed Purina laboratory chow and tap water ad lib. until they reached a weight of 100–150 g. They were then housed in individual wire-bottomed cages. A first group

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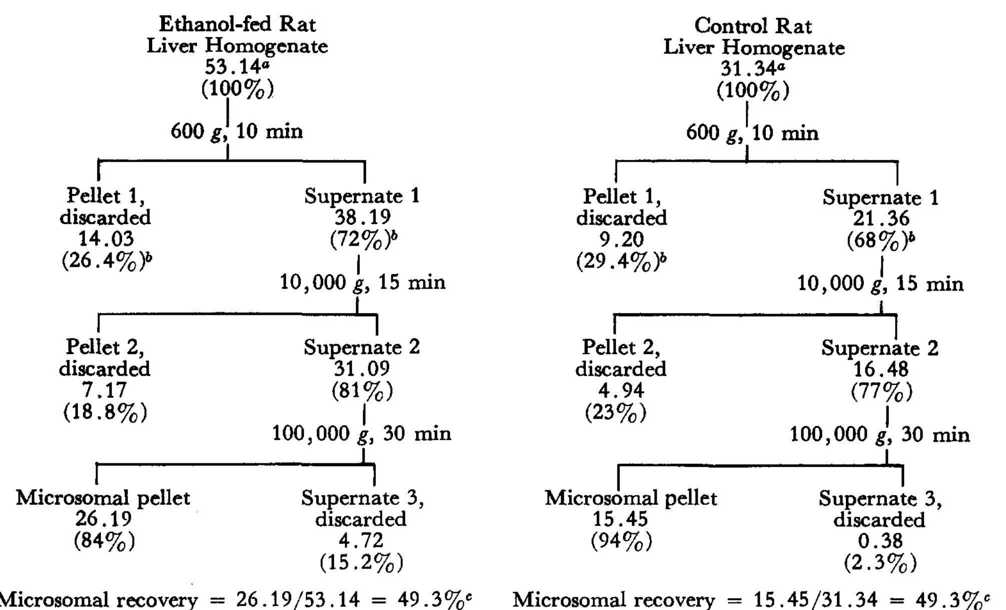

Total nmoles recovered during the centrifugation:
ethanol = 14.03 (pellet 1) + 7.17 (pellet 2) + 4.72 (supernate 3) + 26.19 (microsomal pellet) = 52.11
control = 9.20 (pellet 1) + 4.94 (pellet 2) + 0.38 (supernate 3) + 15.45 (microsomal pellet) = 29.97

Ratio, nmoles recovered during the centrifugation/nmoles measured in homogenate:
ethanol, 52.11/53.14 = 98.1%
control, 29.97/31.34 = 95.6%

Fig. 1. Cytochrome P-450 was measured after every step of differential centrifugation and expressed in nmoles/g of liver. Each fraction further centrifuged was considered 100% of the following two fractions. As shown here, after every centrifugation between 96.4 and 100.0% of the cytochrome P-450 in the starting material was recovered. The total recovery of cytochrome P-450 after the whole procedure was 98.1% for the ethanol-fed rat and 95.6% for the control rat. The microsomal recovery was 49.3% in both animals.

[a] All figures are nmoles of cytochrome P-450 per gram of liver.

[b] The percentage refers to the proportion of cytochrome P-450 recovered from the previous step (100%) during centrifugation.

[c] The recovery is the percentage of cytochrome P-450 of the homogenate remaining in the microsomal pellet.

(10 pairs of females, 8 pairs of males) was isocalorically pair-fed once daily a nutritionally adequate liquid diet as previously described (18) for 4–6 wk until they were killed. These diets supply 18% of total calories as protein, 35% as fat, 11% as carbohydrate (Dextri-Maltose, generously provided by Mead, Johnson & Co., Evansville, Ind.), and 36% of remaining calories either as additional carbohydrate (controls) or as ethanol. A second group (eight pairs of females, three pairs of males) was similarly fed for 6 wk, but these rats were fasted for 18 hr prior to being killed. A third group (eight pairs of females, three pairs of males) was fed the same diets, but for only 10 days. In a fourth group of six pairs of male rats, one littermate of each pair received a 0.1% aqueous solution of sodium phenobarbital (Gane's Chemical Works, Carlstadt, N.J.) in tap water as the only source of water for 22 days. These rats had free access to Purina laboratory chow. In this group, the animals were killed 48 hr after their last dose of phenobarbital. In a fifth experiment, rats were fed in groups of three for 24 days. Two littermates were given, respectively, the control and the alcohol diet as described above, and the third littermate received 36% of total calories as additional fat instead of carbohydrate or ethanol. On the last day, the diet was given by gastric intubation 10 hr and 2 hr before killing. All animals were killed by decapitation; the liver from each rat was quickly perfused with 0.15 M ice-cold KCl (except for the animals in group II, for which 0.25 M sucrose was used), excised, and weighed, and an aliquot was homogenized in ice-cold 0.15 M KCl or 0.25 M sucrose in a Potter-Elvehjem homogenizer with a motor-driven Teflon pestle.

Differential centrifugation

In the first experimental group, microsomes were harvested as described by Fallon and Kemp (19). In the second experimental group, the homogenate was spun at 12,000 g for 20 min. The supernatant fraction was carefully transferred and mixed with 1 M CsCl to a final

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TABLE 1. Hepatic lipids, microsomal components, and GPAT activity in rats fed alcohol for 6 wk and in their pair-fed controls (group I)[a]

	Body Wt	Liver Wt	Total Lipids	Triglycerides	Cytochrome P-450: Per g Liver	Cytochrome P-450: Per mg Microsomal Protein	Microsomal Protein[b]	GPAT Activity: Per mg Microsomal Protein	GPAT Activity: Per g Liver	GPAT Activity: Per 100 g Body Wt
	g	*g/100 g body wt*	*mg/g liver*	*mg/g liver*	*nmoles*	*nmoles*	*mg/g liver*	*nmoles CoA min^{-1}*	*nmoles CoA min^{-1}*	*nmoles CoA min^{-1}*
Alcohol	200.1 ± 09.0[c]	4.3 ± 0.1	102.6 ± 9.6	58.4 ± 9.7	54.2 ± 3.8	1.206 ± 0.083	44.94 ± 1.50	28.3 ± 2.3	1114 ± 68	4721 ± 327
Control	214.4 ± 10.6	3.4 ± 0.1	45.0 ± 2.2	9.7 ± 1.4	29.9 ± 1.9	0.768 ± 0.069	37.74 ± 0.85	16.4 ± 2.2	582 ± 73	2022 ± 287

[a] The results are means ± SEM. For all parameters except body weight, the difference between alcohol-fed and control animals was significant ($P < 0.005$).
[b] Corrected for losses during preparation.
[c] The level of significance for the difference in body weight is $P < 0.05$.

concentration of 0.015 M; rough (RM) and smooth (SM) microsomes were prepared according to Bergstrand and Dallner (20). The intermediate fraction between the fluffy double layer of SM and the pellet of RM was also collected.

In the remaining groups (III–V), the same procedure as for the first group was used except that the initial centrifugation at 600 *g* was omitted. In all experiments, microsomal recovery was estimated from the amount of cytochrome P-450 remaining in the microsomal fraction compared with that in the total homogenate of the same weight of liver, according to Greim (21). A typical recovery experiment is reported in Fig. 1. The method of Omura and Sato (22) was used for cytochrome P-450 measurement. Protein was determined according to Lowry et al. (23). RNA was extracted according to Dallner, Siekevitz, and Palade (24) and determined by the orcinol reaction (25). In experiments where SM and RM were isolated, the values were corrected for their losses into the intermediate phase by assessing the RNA/protein ratio in each fraction. Mitochondrial contamination of microsomes was assessed by measuring succinic dehydrogenase activity according to Eibl, Hill, and Lands (26); it was found to be negligible. The exact determination of the recovery value of rough and smooth microsomes in total microsomal suspensions was not possible because of the lack of specific markers for each of these microsomal subfractions (20). However, since recovery of total microsomes was comparable in the various groups of animals, it was assumed that this also applied to the subfractions.

Enzyme assays

The assay of GPAT was based on the method of Brandes, Olley, and Shapiro (27), according to the procedure described by Fallon and Kemp (19) and Fallon and Lamb (28) with minor modifications as to the pH (7.5 at 35°C) and to palmitoyl CoA concentrations (0.4 mM). This method consists of the measurement of CoA release corrected for deacylase activity (27). Our apparent K_m values for each substrate, D,L-α-glycerophosphate and palmitoyl CoA, were identical with those published by these authors and were the same whether measured in rats fed Purina laboratory chow or in animals given our control or ethanol-containing liquid diets. The amount of soluble CoA liberated was measured on a Gilford spectrophotometer at 260 nm. Results were converted to nmoles of CoA using the extinction coefficient $\epsilon = 16$ mM^{-1} cm^{-1} (19, 28). Under these conditions, the reaction was linear for 20 min for concentrations of microsomal protein of 0.25–0.35 mg/ml. Microsomal protein concentration was 0.3 mg/ml of incubation mixture for all assays. Dithiothreitol and palmitoyl CoA were purchased from Nutritional Biochemicals Corp. (Cleveland, Ohio), D,L-α-glycerophosphate from Calbiochem (Spring Valley, N.Y.), and fatty acid-free bovine albumin from Sigma Chemical Co. (St. Louis, Mo.).

Hepatic lipids were extracted, then fractionated by thin-layer chromatography, and the triglyceride was quantitated as described before (18).

In all experiments the values obtained in the treated animals were compared with those of their pair-fed controls, and the mean of the individual differences was tested by the Student *t* test (29). All values are expressed by their mean ± SEM. Values per gram of liver are given per gram of fat-free tissue.

RESULTS

The results from the first experimental group are shown in Table 1. In this group, ethanol administration for 6 wk resulted in a greater than twofold increase in total liver lipids and a sixfold increase in liver triglycerides. P-450

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TABLE 2. Cytochrome P-450, microsomal protein, and GPAT activity in microsomal subfractions of rats fed ethanol for 6 wk (group II)

			Rough Microsomes		Smooth Microsomes	
Cytochrome P-450 (nmoles)	Per mg microsomal protein	Alcohol	0.73 ± 0.07	$P < 0.02$	0.83 ± 0.06	$P < 0.001$
		Control	0.66 ± 0.06		0.47 ± 0.02	
	Per g liver	Alcohol	12.37 ± 1.10	P = NS	12.28 ± 1.60	$P < 0.001$
		Control	10.60 ± 0.90		5.71 ± 0.50	
Microsomal protein (mg/g liver)		Alcohol	15.64 ± 0.84	P = NS	14.88 ± 1.13	$P < 0.05$
		Control	17.30 ± 0.47		12.83 ± 0.80	
GPAT activity (nmoles CoA min^{-1})	Per mg microsomal protein	Alcohol	12.56 ± 1.30	$P < 0.02$	17.35 ± 1.60	$P < 0.02$
		Control	8.69 ± 1.20		13.14 ± 1.35	
	Per g liver	Alcohol	198.30 ± 24.90	P = NS	257.90 ± 35.50	$P < 0.01$
		Control	148.70 ± 19.00		166.10 ± 19.30	

Values are means ± SE.

content rose by 84% per gram of liver and by 54% per milligram of microsomal protein. Microsomal recovery was not different in ethanol-fed and control rats. Microsomal protein uncorrected for preparative losses was higher in ethanol-fed rats ($P < 0.05$) and remained so after correction. GPAT activity per milligram of microsomal protein was higher by 73% after chronic alcohol feeding ($P < 0.001$). There was an 8% decrease in deacylase activity in the ethanol-fed rats ($P < 0.05$). The increments in GPAT activity became 91% and 134% when values were expressed per gram of liver and per 100 g of body weight, respectively. Ethanol-fed animals exhibited a mean liver-to-body weight ratio 26% greater than that of the controls. This was only partially due to a 7% lesser mean body weight in the ethanol-fed rats. In the second experimental group (Table 2), in which microsomes were subfractionated, the distribution of GPAT activity was found to be greater in SM than in RM (when expressed per milligram of microsomal protein) in both control ($P < 0.02$) and alcohol-fed ($P < 0.05$) animals. Ethanol increased GPAT activity per milligram of microsomal protein by 32% in SM and 44% in RM. When expressed per gram of liver, however, the GPAT activity after ethanol feeding was increased by 55% in SM and 33% in RM.

In the third experimental group, ethanol was fed for only 10 days (Table 3). This resulted in a 65% increase in liver total lipids and a doubling of cytochrome P-450. When corrected for preparative losses, microsomal proteins were found to be significantly increased. GPAT activity showed no significant increase after ethanol feeding whether expressed per milligram of microsomal

TABLE 3. Hepatic lipid, microsomal components, and GPAT activity in rats fed alcohol for 10 days and their controls (group III) and in rats administered phenobarbital in drinking water for 22 days and their controls (group IV)[a]

					Cytochrome P-450			GPAT Activity		
	Body Wt	Liver Wt	Total Lipids	Tri-glycerides	Per g Liver	Per mg Micro-somal Protein	Microsomal Protein[b]	Per mg Micro-somal Protein	Per g Liver	Per 100 g Body Wt
	g	*g/100 g wt body*	*mg/g liver*		*nmoles*		*mg/g liver*	*nmoles CoA min^{-1}*		
Alcohol	128.2 ± 4.4[c]	4.64 ± 0.08	66.6 ± 5.1[d]		40.3 ± 12.3[d]	1.32 ± 0.40[d]	33.6 ± 0.9[e]	12.5 ± 3.2	428 ± 32	1980 ± 147
Control	137.4 ± 2.7	4.27 ± 0.14	40.3 ± 3.0		18.1 ± 4.7	0.66 ± 0.23	29.9 ± 0.9	13.1 ± 4.3	399 ± 43	1700 ± 180
Phenobarbital	228.3 ± 7.5[e]	6.53 ± 0.22	54.5 ± 4.2[c]	9.5 ± 1.3[c]	82.5 ± 6.6[d]	1.51 ± 0.12[d]	55.0 ± 2.9[d]	8.2 ± 0.8[d]	440 ± 41[e]	2857 ± 261
Control	268.8 ± 10.8	4.91 ± 0.03	34.1 ± 1.2	5.0 ± 0.9	32.3 ± 1.1	1.01 ± 0.02	32.1 ± 1.2	17.6 ± 0.8	569 ± 39	2796 ± 200

[a] Values are means ± SEM.
[b] Corrected for losses during preparation.
[c,d,e] $P < 0.02$, 0.001, and 0.05, respectively, when compared with respective controls.

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TABLE 4. Hepatic lipids, microsomal components, and GPAT activity in rats fed alcohol or a high fat diet for 24 days and their pair-fed controls (group V)[a]

	Body Wt	Liver Wt	Total Lipids	Cytochrome P-450: Per g Liver	Cytochrome P-450: Per mg Microsomal Protein	Microsomal Protein[b]	GPAT Activity: Per mg Microsomal Protein	GPAT Activity: Per g Liver	GPAT Activity: Per 100 g Body Wt
	g	*g/100 g body wt*	*mg/g liver*	*nmoles*	*nmoles*	*mg/g liver*	*nmoles CoA min^{-1}*	*nmoles CoA min^{-1}*	*nmoles CoA min^{-1}*
Alcohol	224.0 ± 17.9[c]	4.2 ± 0.1[c]	138.2 ± 22.0[c]	56.5 ± 6.6[d]	1.261 ± 0.146[d]	44.25 ± 0.74[c]	18.9 ± 2.7	970 ± 141[c]	4059 ± 575[d]
Control	247.3 ± 18.9	3.5 ± 0.3	39.4 ± 4.0	31.9 ± 1.6	0.834 ± 0.040	38.82 ± 0.97	14.0 ± 0.8	567 ± 140	1989 ± 215
High fat	260.2 ± 18.9[c]	3.1 ± 0.2[d]	51.2 ± 2.8	41.7 ± 5.2[c]	0.918 ± 0.060	45.25 ± 2.80[c]	18.3 ± 1.6[d]	884 ± 122[d]	2726 ± 443[c]

[a] Values are means ± SEM.
[b] Corrected for losses during preparation.
[c] Difference from controls $P < 0.05$.
[d] Difference from controls $P < 0.02$.

protein, per gram of liver, or per 100 g body weight. The fourth experimental group was given phenobarbital for 22 days in drinking water; the average daily intake for the group during the experimental period was 129.4 ± 7.8 mg/kg/day, which is more than the dose commonly administered intraperitoneally daily (75–100 mg/kg/day) to induce formation of liver microsomes. We observed an increase in hepatic total lipids of 60% and in triglycerides of 88%; triglycerides accounted for only 15% of the total lipids but they represented half of the total lipids accumulated after chronic ethanol feeding (group I, Table 1). P-450 per milligram of protein was 50% higher. When expressed per gram of liver the increase was 155%, due to a 72% rise in microsomal protein. GPAT activity (per gram of liver) was significantly lower than that of the controls, but when expressed per 100 g body weight the values were identical (Table 3).

In the fifth experimental group the effect of a high fat diet was studied (Table 4). Chronic feeding of this diet resulted in a much lower accumulation of liver lipids than the chronic administration of ethanol. Total P-450 content increased 30%, which is less than the 77% change after ethanol feeding. The increase in P-450 per milligram of protein was also less after high fat (10%) than after ethanol treatment (52%). Microsomal protein increased to the same extent both in animals fed the high fat diet and in alcohol-treated rats. GPAT activities per milligram of microsomal protein were similar after ethanol and high fat feeding. The values were significantly higher than those of the controls ($P < 0.02$). GPAT activities of the ethanol-fed group rose above those of the high fat-fed group when expressed per gram of liver and increased even more when expressed per 100 g of body weight. This was due to greater liver weight per 100 g of body weight in the ethanol group than in the group fed the high fat diet.

DISCUSSION

The present study reveals that the fatty liver produced by chronic alcohol feeding is associated with an increase in hepatic microsomal GPAT activity, whether expressed per gram of liver or per milligram of microsomal protein. Although the rise in activity per milligram of microsomal protein was slightly higher in RM, the SM fraction was found to be responsible for most of the increase in the total enzymatic activity observed.

The factors responsible for the enhancement of GPAT activity after chronic ethanol feeding are unknown. Ethanol administration increases the liver NADH/NAD ratio (8, 9) and the α-glycerophosphate concentration (10). Ethanol also decreases fatty acid oxidation (3–5) and enhances fatty acid synthesis (3, 4). Incorporation of glycerolipid precursors into liver lipid has been shown to be accelerated by acute ethanol pretreatment (30). In the present study, however, ethanol feeding for 10 days did not result in a rise in GPAT activity (per milligram of protein) but there was an increase after 24 days of ethanol administration. The mechanism for such a delayed response is not apparent. To test the possibility that the rise in GPAT activity occurred in response to a chronic increase in the supply of glycerolipid precursors, a high fat diet model was used which after 24 days was found to stimulate GPAT activity, expressed per milligram of microsomal protein. This contrasts with the changes in microsomal P-450, which, though increased, was much less stimulated by the high fat diet than after ethanol feeding. When expressed per 100 g of body weight, all parameters were much less affected by the high fat diet than by ethanol feeding.

It is not likely that enhancement of GPAT activity is related to the total lipid content of the liver per se, since

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the increase in GPAT activity in the group fed the high fat diet was observed in presence of much smaller concentrations of liver lipids than after 24 days of ethanol feeding. Furthermore, 10 days of ethanol feeding and 22 days of phenobarbital administration were both associated with a moderate increase in total liver lipids without any increase in GPAT activity. Moreover, in the subfractionation experiments in which the animals were fasted for 18 hr prior to being killed, we observed a maintenance of increased GPAT activity in ethanol-fed animals while the amount of total hepatic lipids was restored to normal by the fasting. Thus, no correlation was found between the concentration of liver lipid and GPAT activity.

After chronic ethanol feeding, the microsomal concentration increased by 19% and the liver size by 25%; these factors obviously play a role in the increased GPAT activity (expressed per 100 g body weight) observed after 6 wk of ethanol feeding. However, after 10 days of ethanol feeding we observed a moderate increase in both microsomal protein (10%) and liver mass (9%) without any absolute increase in total GPAT activity. Furthermore, after chronic phenobarbital treatment the striking increase in both microsomal concentration (71%) and liver size (33%) did not result in an increased total GPAT activity. An inhibition of GPAT activity by phenobarbital is unlikely since the experiments were performed 48 hr after the last dose of phenobarbital, at which time phenobarbital has been shown to have disappeared from the blood under similar conditions (31). Phenobarbital is known to be a potent microsomal inducer responsible for the increase in total microsomal protein and in the activities of many enzymes involved in drug metabolism (32). The former would artificially decrease the microsomal content in GPAT when expressed per milligram of microsomal protein, assuming no direct effect of phenobarbital on GPAT activity; such a phenomenon was indeed observed.

The physiological significance of the increased GPAT activity in livers of rats fed ethanol for at least 4 wk has not been elucidated. This change, however, could provide a ready avenue for the utilization of increased amounts of hepatic fatty acids and glycerophosphate in the synthesis of glycerolipids and may well participate in the pathogenesis of the persistence of alcoholic fatty liver and hyperlipemia.

In summary, chronic ethanol feeding leads to hepatic fat accumulation associated with an increase in total microsomal GPAT activity. This change, although partly explained by an increase in microsomal concentration and liver weight, cannot be merely attributed to the proliferation of the endoplasmic reticulum, since other microsomal inducers such as phenobarbital do not produce this change. The effect is mimicked, in part, by a high fat diet. It is suggested that the increased hepatic fatty acid esterifying activity associated with chronic administration of ethanol may contribute, to a certain extent, to the persistence of alcoholic fatty liver and hyperlipemia.

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REFERENCES

1. Lieber, C. S., and L. M. DeCarli. 1970. Quantitative relationship between amount of dietary fat and severity of alcoholic fatty liver. *Amer. J. Clin. Nutr.* **23:** 474–478.
2. Baraona, E., and C. S. Lieber. 1970. Effects of chronic ethanol feeding on serum lipoprotein metabolism in the rat. *J. Clin. Invest.* **49:** 769–778.
3. Lieber, C. S., and R. Schmid. 1961. The effect of ethanol on fatty acid metabolism; stimulation of hepatic fatty acid synthesis in vitro. *J. Clin. Invest.* **40:** 394–399.
4. Rebouças, G., and K. J. Isselbacher. 1961. Studies on the pathogenesis of the ethanol-induced fatty liver. I. Synthesis and oxidation of fatty acids by the liver. *J. Clin. Invest.* **40:** 1355–1362.
5. Lieber, C. S., A. Lefèvre, N. Spritz, L. Feinman, and L. M. DeCarli. 1967. Difference in hepatic metabolism of long- and medium-chain fatty acids: the role of fatty acid chain length in the production of the alcoholic fatty liver. *J. Clin. Invest.* **46:** 1451–1460.
6. Lieber, C. S., N. Spritz, and L. M. DeCarli. 1966. Role of dietary, adipose, and endogenously synthesized fatty acids in the pathogenesis of the alcoholic fatty liver. *J. Clin. Invest.* **45:** 51–62.
7. Lieber, C. S., and N. Spritz. 1966. Effect of prolonged ethanol intake in man: role of dietary, adipose, and endogenously synthesized fatty acids in the pathogenesis of the alcoholic fatty liver. *J. Clin. Invest.* **45:** 1400–1411.
8. Smith, M. E., and H. W. Newman. 1959. The rate of ethanol metabolism in fed and fasting animals. *J. Biol. Chem.* **234:** 1544–1549.
9. Forsander, O., N. Räihä, and H. Suomalainen. 1958. Alkoholoxydation und Bildung von Acetoacetate in normaler und glykogenarmer intakter Rattenleber. *Hoppe-Seyler's Z. Physiol. Chem.* **312:** 243–248.
10. Nikkilä, E. A., and K. Ojala. 1963. Ethanol-induced alterations in the synthesis of hepatic and plasma lipids and hepatic glycogen from glycerol-C^{14}. *Life Sci.* **2:** 717–721.
11. Stein, O., and Y. Stein. 1967. Lipid synthesis, intracellular transport, storage, and secretion. I. Electron microscopic radioautographic study of liver after injection of tritiated palmitate or glycerol in fasted and ethanol-treated rats. *J. Cell. Biol.* **33:** 319–339.
12. Wilgram, G. F., and E. P. Kennedy. 1963. Intracellular distribution of some enzymes catalyzing reactions in the biosynthesis of complex lipids. *J. Biol. Chem.* **238:** 2615–2619.
13. Iseri, O. A., C. S. Lieber, and L. S. Gottlieb. 1966. The ultrastructure of fatty liver induced by prolonged ethanol ingestion. *Amer. J. Pathol.* **48:** 535–555.

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14. Lane, B. P., and C. S. Lieber. 1966. Ultrastructural alterations in human hepatocytes following ingestion of ethanol with adequate diets. *Amer. J. Pathol.* **49:** 593–603.
15. Rubin, E., F. Hutterer, and C. S. Lieber. 1968. Ethanol increases hepatic smooth endoplasmic reticulum and drug-metabolizing enzymes. *Science.* **159:** 1469–1470.
16. Rubin, E., P. Bacchin, H. Gang, and C. S. Lieber. 1970. Induction and inhibition of hepatic microsomal and mitochondrial enzymes by ethanol. *Lab. Invest.* **22:** 569–580.
17. Joly, J.-G., L. Feinman, H. Ishii, and C. S. Lieber. 1970. Stimulation of L-α-glycerophosphate acyltransferase (GPAT) activity by ethanol consumption. *Clin. Res.* **18:** 679. (Abstr.)
18. DeCarli, L. M., and C. S. Lieber. 1967. Fatty liver in the rat after prolonged intake of ethanol with a nutritionally adequate new liquid diet. *J. Nutr.* **91:** 331–336.
19. Fallon, H. J., and E. L. Kemp. 1968. Effects of diet on hepatic triglyceride synthesis. *J. Clin. Invest.* **47:** 712–719.
20. Bergstrand, A., and G. Dallner. 1969. Isolation of rough and smooth microsomes from rat liver by means of a commercially available centrifuge. *Anal. Biochem.* **29:** 351–356.
21. Greim, H. 1970. Synthesesteigerung und Abbauhemmung bei der Vermehrung der mikrosomalen Cytochrome P-450 und b-5 durch Phenobarbital. *Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol.* **266:** 261–275.
22. Omura, T., and R. Sato. 1964. The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J. Biol. Chem.* **239:** 2370–2378.
23. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
24. Dallner, G., P. Siekevitz, and G. E. Palade. 1966. Biogenesis of endoplasmic reticulum membranes. I. Structural and chemical differentiation in developing rat hepatocyte. *J. Cell Biol.* **30:** 73–96.
25. Mejbaum, W. 1939. Über die Bestimmung kleiner Pentosemengen, insbesondere in Derivaten der Adenylsäure. *Hoppe-Seyler's Z. Physiol. Chem.* **258:** 117–120.
26. Eibl, H., E. E. Hill, and W. E. M. Lands. 1969. The subcellular distribution of acyltransferases which catalyse the synthesis of phosphoglycerides. *Eur. J. Biochem.* **9:** 250–258.
27. Brandes, R., J. Olley, and B. Shapiro. 1963. Assay of glycerol phosphate acyltransferase in liver particles. *Biochem. J.* **86:** 244–247.
28. Fallon, H. J., and R. G. Lamb. 1968. Acylation of *sn*-glycerol 3-phosphate by cell fractions of rat liver. *J. Lipid Res.* **9:** 652–660.
29. Snedecor, G. W., and W. G. Cochran. 1967. *In* Statistical Methods. 6th ed. Iowa State Univ. Press, Ames, Iowa. 91–119.
30. Horning, M. G., M. Wakabayashi, and H. M. Maling. 1961. Biochemical processes involved in the synthesis, accumulation and release of triglycerides by the liver. *In* Effects of Drugs on Synthesis and Mobilization of Lipids. Vol. 2. E. C. Horning, editor. Pergamon Press, Oxford. 13–27.
31. Lieber, C. S., and L. M. DeCarli. 1972. The role of the hepatic microsomal ethanol oxidizing system (MEOS) for ethanol metabolism *in vivo*. *J. Pharmacol. Exp. Ther.* **181:** 279–287.
32. Conney, A. H. 1967. Pharmacological implications of microsomal enzyme induction. *Pharmacol. Rev.* **19:** 317–366.

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