Effect of ethanol on cholesterol and bile acid metabolism

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Abstract Ethanol feeding increased significantly levels of hepatic esterified cholesterol and serum free and esterified cholesterol in rats. Incorporation of intraperitoneally administered [^{14}C]acetate into cholesterol was significantly increased. Labeling of cholesterol was also enhanced in liver slices from animals pretreated with ethanol and incubated with [^{14}C]-acetate.

Ethanol consumption prolonged the half-excretion time of labeled cholic or chenodeoxycholic acids, increased slightly the pool size, and decreased daily excretion. By contrast, supplementation of the diet with cholesterol shortened the half-excretion time, did not modify pool size, and increased daily excretion.

When ethanol and cholesterol feeding were combined, the effects of ethanol prevailed and there was suppression of the adaptive changes in bile acid metabolism induced by cholesterol feeding. There was also a greater accumulation of esterified cholesterol in the liver than that produced by cholesterol alone, ethanol administration alone, or the summation of both effects.

Thus, cholesterol accumulation produced by ethanol feeding is associated with both enhanced cholesterogenesis and decreased bile acid excretion. Both mechanisms may play a role, but the latter is probably predominant in these studies in which cholesterol accumulation was markedly enhanced by the addition of cholesterol to the ethanol-containing diet.

Supplementary key words cholic acid · chenodeoxycholic acid · mevalonate

CHRONIC ADMINISTRATION of ethanol was found to produce an accumulation of esterified cholesterol in the liver as well as an increase in cholesterol concentration in the blood (1, 2). Since the diet given with the ethanol was devoid of cholesterol, it can be assumed that simultaneous accumulation of cholesterol in the blood and in the liver resulted mainly from increased synthesis or decreased cholesterol disposition, or both, although transfer of cholesterol from one pool to another has been suggested in some conditions (3, 4). The present paper represents an attempt to define the effects of ethanol feeding on hepatic cholesterogenesis and on the excretion of bile acids which represent more than 90% of the pathways for cholesterol disposition in the rat (5).

METHODS AND PROCEDURES

Experimental design

Cholesterol synthesis. Male rats of a Sprague-Dawley strain (CD) were purchased from Charles River Breeding Laboratories (North Wilmington, Mass.) in groups of weanling littermates. They were fed Purina Laboratory Chow and tap water ad lib. until they reached the weight of 120–150 g. They were then housed in individual wire-bottomed cages and were pair-fed daily, in groups of two littermates each, the liquid diet previously described (2). During the 24 hr preceding the killing of the rats, the diet was given in two or three rations, to equalize both the amount and the rate of food intake.

At the end of 10 or 22–24 days of pair-feeding, the animals were killed by decapitation, blood was obtained from the neck veins, and livers were quickly excised. In some experiments, [1-^{14}C]acetate (16.6 mCi/mmole; 200 μCi/kg) or [2-^{14}C]mevalonate (4.1 mCi/mmole; 200 μCi/kg) was administered intraperitoneally 2 hr prior to killing.

In other experiments, the liver was quickly removed after the rat was killed and slices were prepared in the cold with a Stadie-Riggs slicer, as described previously (6). The slices were then randomly distributed in flasks (with center wells) containing 3–5 ml of isotonic Krebs-Ringer bicarbonate buffer, pH 7.4, with glucose (5 μmoles/ml) and sodium [1-^{14}C]acetate at a concentration of 10.0 μmoles/ml (0.8 to 0.9 μCi/ml). In some experiments [5-^{3}H]mevalonate was also used (6 μCi/ml).

The flasks were gassed with a mixture of O_2 (95%) and CO_2 (5%) for 5 min and then shaken in a Dubnoff incubator at 37°C for 1, 2, and 3 hr. The incubation was stopped by the addition of 0.2 ml of 10 N sulfuric acid, and the CO_2 released was collected in 1 ml of 0.5 M Hyamine hydroxide introduced into the center well. A known amount of Hyamine was dissolved in toluene containing 2,5-diphenyloxazole (4 g/liter) and *p*-bis(2-[5-phenyloxazolyl])-benzene (50 mg/liter) (7) and counted in a liquid scintillation counter. Suitable corrections were made for background and quenching.

The slices were homogenized and lipids were extracted (8). Aliquots of the extract were chromatographed by thin-layer chromatography, and free and esterified cholesterol was eluted as described previously (1). Incorporation of the labeled precursor into cholesterol was measured after 1, 2, and 3 hr of incubation. At the concentration of acetate used (10 μmoles/ml), incorporation was found to be linear over the first 2 hr and these data were used to calculate the rates of incorporation. Preliminary experiments also indicated that, under our conditions, incorporation rates are proportional to the amount of tissue present. In some studies, [^{3}H]acetate and [2-^{14}C]mevalonate were used. All isotopes were purchased from New England Nuclear Corp., Boston, Mass. Their purity was determined by radiochromatography.

Bile acid metabolism. Since the feeding of a synthetic diet is capable, in itself, of modifying bile acid metabolism (9), our basic liquid diet (1), containing 18% of total calories as protein, 35% as fat, and 47% as carbohydrates (generously supplied as Dextri-Maltose by Dr. H. P. Sarett, Mead Johnson & Co., Evansville, Ind.), was first given ad lib. to every rat for an equilibration period of 3 wk.

For the next 22 days, the experimental diets were fed to the animals in groups of four. Two rats received the carbohydrate-containing diet and two were fed a diet in which carbohydrates were isocalorically replaced by ethanol, up to 36% of total calories, as described previously (1). One rat in each subgroup received a supplement of cholesterol, incorporated into the diet in the amount of 3 mg/kcal. The caloric value of the cholesterol was considered to be negligible. This dietary cholesterol was given to suppress endogenous synthesis in the liver (10), and thereby to dissociate the possible effects of ethanol on cholesterol degradation (to bile acids) from its action on hepatic cholesterol synthesis.

During the experimental period, 48 rats (12 for each diet) were injected intraperitoneally with a tracer dose of [^{14}C]cholic acid (24 rats) or [^{14}C]chenodeoxycholic acid (24 rats) (0.8–1.5 μCi/100 g; 10–36 μg/100 g of body weight). In some experiments (16 additional rats), the animals were given both [^{14}C]chenodeoxycholic and [^{3}H]cholic acids, prepared according to Hofmann (11).[1] Rats were injected 11 days before killing. In those experiments in which the bile acid turnover was known to be accelerated, the labeled bile acid was given 4 days before killing.

To minimize coprophagia, from the time of injection until the end of the experiment, rats were housed in wire-bottomed cages with wide orifices through which the feces dropped into collecting pans. The cages were inspected several times a day to verify that no feces were retained at the bottoms of the cages.

The rats were killed by decapitation, blood was collected from the neck veins and livers, and small and large intestines were excised for further analysis. Liver slices obtained from 16 rats given the four diets (with and without ethanol and cholesterol) were incubated with [1-^{14}C]-acetate, as described for the experiments concerning cholesterol synthesis.

Chemical determinations and calculations

Free and esterified cholesterol levels were determined in the blood by the method of Searcy and Bergquist (12). In the liver, lipids were extracted according to the procedure of Folch, Lees, and Sloane Stanley (8). An aliquot of the total lipid extract, containing approximately 20 mg of fat, was evaporated under nitrogen to a volume of about 0.5 ml, applied to a 0.5-mm-thick silica gel chromatoplate (13), and developed in hexane–diethyl ether–acetic acid 83:16:1. The cholesteryl esters and free cholesterol were eluted by the method of Goldrick and Hirsch (14) and were determined by the method of Searcy and Bergquist (12). Incorporation of labeled precursors into total hepatic free and esterified cholesterol was determined by scintillation counting as previously described (6).

Feces were dried at 110–120°C, pulverized in a mortar, and extracted by refluxing with boiling 80% (v/v) ethanol for 6 hr. An aliquot of the ethanolic extract was dissolved in Bray's solution (15), and its radioactivity was determined in a liquid scintillation counter with corrections for background and quenching.

Liver bile salts were extracted with lipids (8), and small and large intestines were treated as described for the feces. This enabled us to measure the total radioactivity remaining in the rat at the time of killing, since the bile acid pool is known to be restricted to the intestinal tract, the portal circulation, and the hepatobiliary system (16).

Aliquots of the small intestine extract were further hydrolyzed in 2 N NaOH at 115°C for 4 hr in order to deconjugate the bile acids. The nonsaponifiable material

[1] We are thankful to Dr. A. F. Hofmann for providing us with methyl-3-keto-7α,12α-dihydroxy-5β-cholanoate needed for the preparation of tritiated cholic acid.

was removed with ether from the alkaline medium and the latter was acidified to free the bile acids, which were extracted with ether (17). Bile acids were subsequently separated and measured according to Gaenshirt, Koss, and Morianz (18). The separation was performed on silica gel plates, using as solvent the upper phase of a toluene–acetic acid–water 5:5:1 solution. The bile acids were eluted with a mixture of ethanol–acetone–water 5:5:1, reacted with sulfuric acid, and measured spectrophotometrically (18). Their radioactivity was determined and the specific activity was calculated.

Thin-layer chromatography with the solvents described by Siegfried and Elliott (19) did not reveal any muricholic acids, as expected in rats fed synthetic diets (20). Moreover, even if some traces of muricholic acids undetectable by thin-layer chromatography were present, they should not influence our results; we found indeed that β-muricholic acid[2] does not give any colored complex with sulfuric acid when used according to Gaenshirt et al. (18).

Since bile acids represent the end products of cholesterol metabolism and are continuously recirculated through the enterohepatic cycle or excreted in the feces, their excretion time was determined by plotting the cumulative fecal excretion of radioactivity, as described by Portman and Murphy (21). Before being excreted, primary bile acids are partially degraded by the intestinal flora into a number of components which are not readily measurable by chemical methods but are still radioactive if the primary bile acid has been labeled. Therefore, the rate of appearance of the labeling in the stools does not represent the turnover of the primary bile acid, in the restricted sense of the term, but rather reflects both processes of biodegradation and excretion of the corresponding bile acid, which is not to be confused with the bile acid half-life derived from the die-away curve of the specific activity of cholic acid in the bile. To avoid any confusion, the term "half-excretion time" will be used to characterize the rate of appearance of the bile acid label in the feces.

The bile acid pool at the time of killing was calculated from the total radioactivity and from the specific activity of the primary bile acid at that time. Although the label traverses a number of catabolic pools before being excreted, it must be pointed out that, at any given time, the bile acid fraction recirculating through the enterohepatic cycle represents more than 95% of the total pool (22) and, therefore, dividing the total radioactivity by the specific activity of the primary bile acid can be considered to be an acceptable estimation of the pool size. Theoretically, however, one could raise the following objection to this method. During the experimental period after the injection of the labeled primary bile acid, its specific activity continuously decreases; thus, that of the degradation products derived from the primary bile acid will be higher in the earlier part of the experimental period than later. If one divided total radioactivity remaining in the animal by the specific activity of the primary bile acid determined at the end of the experimental period, the calculated pool size would be too high. Furthermore, the magnitude of this artifact would not be constant, but would be larger under experimental circumstances where the half-excretion time was shorter. Actually, the data obtained in Tables 2 and 3 indicate that the increase in pool size calculated using these methods was associated with a prolongation rather than a shortening of the half-excretion time; therefore, the objection does not invalidate the conclusions reached in this study.

The methods we applied for assessing cholic and chenodeoxycholic acid pool sizes and half-excretion times were introduced by Lindstedt (23) as early as 1957 and were subsequently used by various investigators (24–26).

In those experiments with greatly accelerated bile acid degradation (such as the chenodeoxycholic acid excretion in cholesterol-fed rats), it was difficult to accurately measure both pool size and half-excretion time in the same animal. These parameters were assessed in two groups of rats fed the same diet, but for the measurement of half-excretion time, the labeled substrate was injected 4 days before killing, whereas for the assessment of pool size, it was administered only 24 hr before the end of the experiment.

In all experiments, each result was compared with the corresponding control and the means of the individual differences were tested for significance using Student's *t* test (27).

RESULTS

Modifications of cholesterol concentrations in blood and liver

As observed previously (1), replacement of carbohydrate by ethanol caused a significant increase in hepatic esterified cholesterol after 22 days (Table 1). Addition of cholesterol to the diet produced a 10-fold accumulation of esterified cholesterol, as reported by others (28). The simultaneous feeding of cholesterol and ethanol resulted in a 20-fold increase of esterified cholesterol, an effect much more striking than that produced by the feeding of ethanol alone or cholesterol alone, or than the sum of the two effects. By contrast, free cholesterol concentrations in the liver were not significantly modified by cholesterol or ethanol feeding, or both (Table 1).

[2] We are thankful to Dr. E. Mosbach for providing us with β-muricholic acid.

TABLE 1. Effects of ethanol and cholesterol feeding (for 22 days) on blood and liver cholesterol fractions

		Diets			
		Without Cholesterol		With Cholesterol	
	Cholesterol Fractions	Control	With Ethanol	Control	With Ethanol
Serum	Total (mg/100 ml)	119 ± 13	141 ± 8	155 ± 21	150 ± 12
Liver	Free (mg/100 g)	189 ± 25	193 ± 24	189 ± 21	216 ± 22
	Esterified (mg/100 g)	68 ± 25	176 ± 60	665 ± 158	1310 ± 185

The effects of these various diets upon blood cholesterol concentrations, reported in Table 1, were much less striking than those observed in the liver. Ethanol feeding and dietary cholesterol slightly increased blood cholesterol levels, but the combination of ethanol and exogenous cholesterol resulted in effects similar to those obtained by cholesterol feeding alone or by ethanol alone.

Effect of ethanol feeding on incorporation of labeled acetate and mevalonate into hepatic cholesterol in vivo

In 15 pairs of rats, incorporation of [^{14}C]acetate into free cholesterol was markedly and significantly ($P < 0.02$) enhanced by 24 days of ethanol feeding (6884 ± 1900 dpm/g of liver in the alcohol-fed rats vs. 2831 ± 666 in the controls). Though the incorporation into the cholesterol moiety of the esterified cholesterol was not as pronounced, there was a significant difference between the controls (779 ± 507 dpm/g) and the alcohol-fed rats (1501 ± 522; $P < 0.01$). Similar findings were observed after 10 days of ethanol feeding in a group of 11 pairs of rats. No such effect, however, was observed when labeled mevalonate was used instead of acetate.

Effect of ethanol feeding on incorporation of labeled acetate and mevalonate into cholesterol in liver slices

Increased incorporation of [1-^{14}C]acetate into cholesterol was also found in liver slices, obtained from 12 animals pretreated with ethanol in vivo for 10 days, incubated with [1-^{14}C]acetate in vitro: 9034 ± 3963 dpm/hr/g of liver were incorporated into free cholesterol and 538 ± 281 into esterified cholesterol, compared with 2977 ± 443 and 157 ± 32, respectively, in the controls ($P < 0.02$). By contrast, ethanol pretreatment did not produce any significant effect on the conversion by liver slices of [1-^{14}C]acetate into labeled CO_2 (2541 ± 445 × 10^3 dpm/hr/g of liver vs. 3012 ± 193 × 10^3 in controls). No enhanced incorporation into cholesterol was observed when labeled mevalonate was used instead of acetate.

In vitro incubation of liver slices obtained from the different groups of animals showed that ethanol administration did not suppress the inhibitory effect of dietary cholesterol upon cholesterol synthesis. In the liver of carbohydrate-fed rats, supplementation with dietary cholesterol decreased the incorporation of labeled acetate into free cholesterol by 70–90%; comparable results were observed in the ethanol-treated rats.

Effects of ethanol on bile acid metabolism

As shown in Table 2, chronic administration of ethanol markedly prolonged the half-excretion time of cholic acid from 7.9 ± 1.5 days in the control animals to 15.9 ± 2.7 days in the ethanol-treated rats ($P < 0.01$). By contrast, feeding of cholesterol produced an opposite effect and decreased the half-excretion time to 3.6 ± 1.0 days ($P < 0.01$). When cholesterol and ethanol were fed simultaneously, the ethanol effect predominated and the half-excretion time was prolonged up to 15.3 ± 2.5 days ($P < 0.01$).

Table 2 shows that the cholic acid pool size was not significantly modified by adding cholesterol to the diets. Substitution of carbohydrates by ethanol, however, increased the cholic acid pool size from 8.8 ± 2.2 to 11.9 ± 2.2 mg/100 g of body weight ($P < 0.05$) in the rats not given cholesterol, and from 7.4 ± 1.6 to 13.6 ± 2.8 mg/100 g of body weight ($P < 0.05$) in the animals which

TABLE 2. Effects of ethanol and cholesterol feeding (for 22 days) on cholic acid metabolism

	Diets			
	Without Cholesterol		With Cholesterol	
Cholic Acid	Control	With Ethanol	Control	With Ethanol
Half-excretion time (days)	7.9 ± 1.5	15.9 ± 2.7	3.6 ± 1.0	15.3 ± 2.5
Pool (mg/100 g body weight)	8.8 ± 2.2	11.9 ± 2.2	7.4 ± 1.6	13.6 ± 2.8
Daily excretion (mg/100 g body weight)	0.82 ± 0.16	0.56 ± 0.10	1.54 ± 0.20	0.65 ± 0.06

received cholesterol supplementation. This ethanol effect on cholic acid pool size was already observed after 11 days, since the cholic acid pool size measured at that time was 8.1 ± 1.0 mg/100 g of body weight in the animals fed carbohydrates (with or without cholesterol) and 11.0 ± 1.2 ($P < 0.05$) in the ethanol-treated rats. Thus, the pool size had achieved a new steady state before the label was injected for the determination of the half-excretion time.

The effects of ethanol or cholesterol, or both, on the cholic acid half-excretion times were much more striking than their effects on the pool size and, therefore, the daily excretion of cholic acid and its derivatives, calculated from the pool and from the rates of excretion, followed the general pattern of the half-excretion time (Table 2). Administration of ethanol decreased the daily excretion of cholic acid and its derivatives from 0.82 ± 0.16 to 0.56 ± 0.10 mg/100 g of body weight ($P < 0.02$), while dietary cholesterol doubled the excretion to 1.54 ± 0.20 mg/100 g of body weight ($P < 0.02$). These results are in keeping with previous observations made by Wilson (29) and Beher et al. (30). When ethanol and cholesterol were fed simultaneously, the ethanol effect prevailed again, as shown by a reduced daily production of cholic acid and its derivatives of 0.65 ± 0.06 mg/100 g of body weight; in effect, ethanol abolished the increase of cholic acid excretion normally produced by cholesterol feeding.

To illustrate the effects of ethanol or cholesterol feeding, or both, on the appearance of the labeled derivatives of the cholic acid in the feces, some of the results from Table 2 have been depicted in Fig. 1.

With each diet (Table 3), the half-excretion time of chenodeoxycholic acid was found to be shorter than that for cholic acid. The effect of ethanol or dietary cholesterol, or both, on chenodeoxycholic acid half-excretion times was similar to that observed with cholic acid. Dietary cholesterol shortened the half-excretion time from 3.2 ± 0.3 days to 1.7 ± 0.2 days ($P < 0.01$), while ethanol

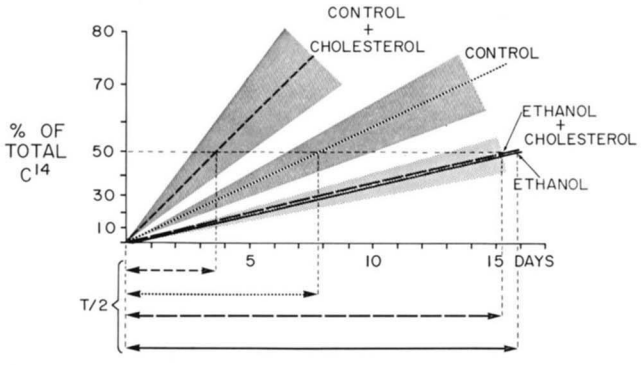

FIG. 1. Cumulative excretion in feces of labeled bile acids following an intraperitoneal injection of [^{14}C]cholic acid. Shaded areas represent SEM.

increased the half-excretion time to 6.0 ± 0.6 days ($P < 0.01$). Again, when cholesterol and ethanol were combined, the effect of the latter largely predominated, resulting in a prolonged half-excretion time of 6.1 ± 0.9 days ($P < 0.02$).

As is also indicated in Table 3, ethanol administration increased the chenodeoxycholic acid pool from 3.5 ± 0.8 to 5.3 ± 1.1 mg/100 g of body weight, but dietary cholesterol had a similar effect (an increase to 5.2 ± 1.0 mg) and the combination of ethanol and cholesterol almost doubled the chenodeoxycholic acid pool to 6.5 ± 1.3 mg/100 g of body weight. The results observed for chenodeoxycholic acid were more variable than those obtained for cholic acid; for instance, daily cholic acid excretion was significantly decreased by ethanol ($P < 0.02$) (see above), whereas that of chenodeoxycholic acid was not (0.75 ± 0.11 mg/100 g of body weight in the control animals compared with 0.62 ± 0.09 mg/100 g of body weight after ethanol). Daily chenodeoxycholic acid excretion was significantly increased to 2.18 ± 0.36 mg/100 g of body weight by dietary cholesterol ($P < 0.01$), but when ethanol and cholesterol were fed simultaneously, no significant change was observed (0.93 ± 0.15 mg/100 g of body weight). Thus, as was the case for cholic acid, ethanol administration abolished the increase of chenodeoxycholic acid excretion produced by cholesterol feeding.

DISCUSSION

The present study confirms our previous reports (1, 2) that isocaloric substitution of ethanol for dietary carbohydrate results in accumulation of cholesteryl ester in the liver. This effect could be due to a variety of mechanisms, including a shift in distribution between liver and circulating cholesterol, enhanced cholesterol synthesis, and/or decreased conversion of cholesterol to bile acid.

Though the distribution of cholesterol among the liver, blood, and other tissues was not specifically assessed in this study, the fact that hypercholesterolemia was observed in association with cholesterol accumulation in rats in the present investigation (Table 1) (and in volunteers given alcohol as shown previously [2]) suggests that a block in transfer of cholesterol from the liver into the blood stream does not play a major role. The second mechanism, namely an enhanced cholesterol synthesis, is theoretically possible, since chronic ethanol consumption is associated with proliferation of the smooth endoplasmic reticulum (31–33), which is the site of major steps in cholesterol synthesis (34–36). The proliferation of the smooth endoplasmic reticulum induced by ethanol may be linked to the fact that the hepatic microsomes which comprise the smooth endoplasmic reticulum contain a microsomal ethanol-oxidizing system that

TABLE 3. Effects of ethanol and cholesterol feeding (for 22 days) on chenodeoxycholic acid metabolism

Chenodeoxycholic Acid	Diets			
	Without Cholesterol		With Cholesterol	
	Control	With Ethanol	Control	With Ethanol
Half-excretion time (days)	3.2 ± 0.3	6.0 ± 0.6	1.7 ± 0.2	6.1 ± 0.9
Pool (mg/100 g body weight)	3.5 ± 0.8	5.3 ± 1.1	5.2 ± 1.0	6.5 ± 1.3
Daily excretion (mg/100 g body weight)	0.75 ± 0.11	0.62 ± 0.09	2.18 ± 0.36	0.93 ± 0.15

increases in activity upon ethanol feeding (37). It is noteworthy that the activity of this system, as well as the microsomal synthesis of cholesterol, can be inhibited by CO (38), which binds to the microsomal cytochrome P-450, a heme protein involved in oxygen activation. This suggests that microsomal ethanol oxidation and cholesterol synthesis depend on some common microsomal components. It is tempting to speculate that the increased incorporation of [1-^{14}C]acetate into cholesterol consequent to ethanol consumption results from the proliferation of the endoplasmic reticulum. This view is supported by the fact that other drugs (such as phenobarbital), which are also metabolized by CO-sensitive microsomal enzymes and can induce proliferation of the smooth endoplasmic reticulum (39), were also shown to enhance incorporation of [1-^{14}C]acetate into cholesterol (40–42).

It must be emphasized that the significance of the increased incorporation of [1-^{14}C]acetate into cholesterol in the animals fed alcohol is enhanced by the fact that the metabolism of ethanol itself results in a large production of acetate (43, 44) which can be expected to dilute the pool of labeled acetate much more than in the control animals. The dilution of the isotope should therefore have resulted in decreased incorporation of the label into cholesterol, but the opposite was found; this strengthens the conclusion that alcohol may indeed increase the incorporation of the precursor into cholesterol through an effect on cholesterol synthesis. A similar change was observed in vitro in liver slices obtained from animals pretreated with ethanol in vivo. Again, incorporation of [1-^{14}C]acetate into cholesterol was increased by ethanol pretreatment; if this were due to decreased dilution of the [1-^{14}C]acetate by an unlabeled pool, one would expect a similar difference of incorporation into other metabolites such as CO_2, but this was not the case.

The observation of a change in cholesterol labeling in liver slices incriminates an effect of ethanol feeding on hepatic cholesterol metabolism itself, independent of any possible disturbances in cholesterol transport. The increased incorporation of [1-^{14}C]acetate into cholesterol in liver slices of animals pretreated with ethanol was observed in the absence of added ethanol, which indicates that in ethanol-pretreated rats, ethanol itself need not be present for the demonstration of increased incorporation of [1-^{14}C]acetate into cholesterol. This is consistent with the postulated role of the proliferation of the smooth endoplasmic reticulum induced by ethanol feeding.

The lack of increase in cholesterol labeling when mevalonate was used instead of acetate as a precursor for cholesterol synthesis suggests that the action of ethanol may be located at the steps of cholesterol synthesis preceding mevalonate. It also indicates that the increased incorporation of acetate into cholesterol cannot be attributed merely to decreased cholesterol breakdown, which ought to enhance cholesterol labeling from both mevalonate and acetate.

Though the present study reveals evidence in favor of stimulation of cholesterol synthesis by ethanol, the question still remains whether this mechanism is responsible for the cholesterol accumulation in the liver. It must be pointed out that though both phenobarbital and ethanol have now been found to enhance incorporation of [1-^{14}C]-acetate into cholesterol and to produce proliferation of the smooth endoplasmic reticulum, hepatic cholesterol accumulation of any significance has been observed only after alcohol feeding, and not after administration of phenobarbital (40, 42). This suggests that the enhanced cholesterol synthesis produced by either phenobarbital or ethanol may not be sufficient, by itself, to produce hepatic cholesterol accumulation and that additional factors, such as decreased catabolism of cholesterol to bile acid, may play a role. Actually, feeding ethanol to rats for 3 wk decreased the daily excretion of bile acids and suppressed the increase of bile acid excretion normally produced by cholesterol feeding.

Bile acids and their derivatives are excreted or continuously recirculated through the enterohepatic cycle; therefore, the daily excretion corresponds to the daily transformation of cholesterol into bile acids, in so far as a steady state is achieved. Ethanol administration tended to expand the bile acid pool, but this increase occurred at the beginning of the ethanol administration period and a new equilibrium had already been reached when the labeled material was injected. Chenodeoxycholic acid pool sizes measured in our animals are somewhat higher than some data published by others with methods similar to ours (45), but the rats were fed another diet, which may readily explain the difference in pool sizes (5).

Another finding of the present studies was the suppres-

sion, after chronic administration of ethanol, of the increase of bile acid excretion normally produced by cholesterol feeding (29, 30); this was accompanied by a striking increase in the concentration of esterified cholesterol in the liver. The mechanism by which ethanol affects cholesterol transformation into bile acids is not clear at the present time. Ethanol could act directly at the hepatocyte level and decrease the enzymatic degradation of cholesterol into bile acids. 7α-Hydroxylase, an enzyme known to be involved in bile acid formation, can be induced by drug administration (46). Ethanol consumption acts as an inducer for microsomal drug-metabolizing enzymes (47), and its presence also inhibits various enzymes (47). Assuming that ethanol inhibits hepatic bile acid formation, the bile acid pools should have decreased if the intestinal reabsorption remained unchanged. If the decreased formation of bile acid was compensated for by an increased intestinal reabsorption, the bile acid pools would have remained stable, but, actually, they increased. Moreover, some preliminary experiments were performed in bile fistula rats fed ethanol or isocaloric amounts of carbohydrates;[3] they showed that chronic ethanol administration does not suppress the rise in bile acid formation which has been demonstrated to occur after bile flow diversion and bile acid loss (48).

An alternate explanation for our findings is that chronic ethanol administration alters bile acid metabolism in the intestinal tract itself. It is conceivable that ethanol acts directly upon the bacterial flora of the intestine, which plays a large role in bile acid metabolism (49). In germ-free rats, for instance, bile acid half-life is markedly prolonged (50), whereas cholesterol accumulation in the liver produced by dietary cholesterol is much more striking in these animals than in conventional ones (51). It is also possible that ethanol affects properties of the intestinal wall or even the intestinal motility in such a way that bile acids are more easily and more completely reabsorbed or less easily excreted. This would then result in an increased pool and, secondarily, in a decreased production of bile acids at the hepatic level, as a result of the feedback mechanism whereby an increase in bile acid pool reduces bile acid formation (52).

In conclusion, our data show that chronic feeding of ethanol to rats increases the incorporation of labeled acetate into hepatic cholesterol and decreases the degradation of cholesterol into bile acids. The respective roles of these two mechanisms in the accumulation of hepatic esterified cholesterol observed after ethanol consumption have not yet been established. In rats fed dietary cholesterol, however, which suppresses cholesterol synthesis (10), accumulation of hepatic cholesterol was much more striking in the rats fed ethanol than in the rats given carbohydrates. One can thus conclude that, when ethanol is fed with a cholesterol-supplemented diet, cholesterol degradation to bile acids is impaired, and this plays a major role in the accumulation of cholesterol in the liver.

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[3] A. Lefèvre and C. S. Lieber. Unpublished observations.

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