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Robert H. Schapiro, ... , Yoshitaka Shimizu, Kurt J. Isselbacher

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Studies on the Pathogenesis of the Ethanol-induced Fatty Liver. II. Effect of Ethanol on Palmitate-1-C¹⁴ Metabolism by the Isolated Perfused Rat Liver *

ROBERT H. SCHAPIRO,† GLADYS D. DRUMMEY, YOSHITAKA SHIMIZU, AND
KURT J. ISSELBACHER

(From the Department of Medicine, Harvard Medical School, and the Medical Services [Gastrointestinal Unit], Massachusetts General Hospital, Boston, Mass.)

In man and in the experimental animal, a fatty liver can result from acute or chronic ethanol ingestion. In the rat an increase in liver lipid occurs either after a single intoxicating dose of ethanol or after the chronic administration of this drug (2, 3). The lipid that accumulates in the liver under these conditions is predominantly in the form of triglycerides (4).

The production of a fatty liver would appear to involve one or more of five possible mechanisms: *a*) increased mobilization of fatty acids from the peripheral adipose depots to the liver, *b*) increased *de novo* synthesis of fatty acids by the liver, *c*) decreased hepatic catabolism (e.g., oxidation) of fatty acids, *d*) increased or preferential esterification of fatty acids to triglycerides, or *e*) a decreased release of lipid from the liver. In considering these mechanisms, it should be noted that studies by Mallov (5) and by Brodie and Maickel (4) have demonstrated an increased mobilization of plasma free fatty acids after relatively large doses of ethanol; this effect is apparently mediated through hypophyseal-adrenal pathways and the sympathetic nervous system (4). Furthermore, the fatty liver produced by ethanol contains large amounts of linoleic acid that is stored in the adipose tissue but not synthesized by mammalian liver (6). Lieber and Schmid (7) have observed that ethanol stimulates

the synthesis of fatty acids by the liver, but the quantitative importance of this finding remains to be determined. Under conditions of decreased mobilization of fatty acids (e.g., cordotomy) *de novo* hepatic fatty acid synthesis is still increased after ethanol administration, but a fatty liver does not develop (8). Decreased oxidation of fatty acids to CO₂ results after ethanol administration, but this effect occurs also with agents such as glucose and sorbitol, which do not produce a fatty liver (8).

Within the liver the free fatty acids are esterified primarily to triglycerides (9), and these are then returned to the plasma in the form of lipoproteins (10). Several laboratories have presented evidence indicating that hepatotoxins such as carbon tetrachloride and ethionine interfere with this triglyceride release (11-14), and it has been suggested that the production of fatty liver by these agents is largely due to a decreased release of triglycerides from the liver (12).

We have attempted to determine whether ethanol also exerts an effect on the release of triglycerides by the liver. Experiments were therefore designed to test the direct effects of ethanol on hepatic lipid metabolism. In the present paper studies are reported on the metabolism of palmitic acid-1-C¹⁴ by the isolated rat liver. We found that in the presence of ethanol there is a decreased rate of appearance of C¹⁴ in perfusate triglycerides and a concomitant accumulation of C¹⁴-triglycerides in the liver.

Materials and Methods

Palmitate-1-C¹⁴ (SA, 7.2 to 11.4 × 10⁶ cpm per μmole)¹ was purified as described previously (15). Al-

¹ New England Nuclear Corp., Boston, Mass.

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A preliminary report of these observations has appeared previously (1).

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bumin-palmitate solutions were prepared (15) using a 20% solution of crystalline bovine albumin.² Hyamine-10X was prepared according to the method of Passman as modified by Eisenberg (16). Female albino rats³ weighing 275 to 450 g were used as liver donors, and male albino rats weighing greater than 450 g were used as blood donors.

Liver perfusion technique

Under light ether anesthesia the bile duct and portal and hepatic veins of female rats fed ad lib were cannulated according to the method of Brauer, Pessotti, and Pizzolato (17) except that no anticoagulant was injected into the donor rat. The interval between portal vein cannulation and onset of perfusion averaged 9 minutes. The isolated liver was placed in a continuous perfusion apparatus described by Brauer and colleagues (17), which is a modification of the technique of Miller, Bly, Watson, and Bale (18). This apparatus was enclosed in a cabinet maintained at 37.5° C. Oxygenation was accomplished by passing 100% oxygen over a thin flowing layer of the perfusate. CO₂ was trapped by passing the gas under a slight vacuum through a sintered glass tower containing Hyamine. The perfusion pressure in the portal vein was maintained at 12 cm of blood. The flow of perfusate through the liver varied between 4.2 and 9.0 ml per minute. Experiments were discarded if this rate could not be maintained or if congested areas appeared in the liver.

The initial experiments were conducted using small amounts of added palmitate-1-C¹⁴ (hereafter referred to as "low" palmitate experiments). The perfusion fluid was prepared from 70 to 90 ml of blood obtained by cardiac puncture from male rats fasted overnight under light anesthesia. Heparinized syringes were used, but care was taken not to inject heparin into the donor rats. This procedure was considered acceptable, since lipoprotein lipase is not released when heparinized blood is perfused through the isolated liver (19, 20). After the blood had been diluted with Ringer's solution containing 45 mg heparin and the substrates had been added, the final volume of perfusion ranged between 93 and 120 ml. The hematocrit of this solution averaged 35%.

Ten minutes after the onset of perfusion, sufficient 25% (vol/vol) ethanol was added to the reservoir to make a final concentration in the perfusing fluid of 400 mg per 100 ml. An equal volume of Ringer's solution was used in control experiments. Fifteen minutes later 5 ml of the albumin-palmitate solution, containing 8 mg of palmitate-1-C¹⁴ (8×10^6 cpm), was injected slowly into the portal vein cannula. The perfusion was continued for an additional 3-hour period, during which serial samples of perfusate were removed from the reservoir for analysis. At the end of the perfusion the liver was rinsed free of blood and then homogenized with 20 vol of chloroform-methanol (2/1, vol/vol).

In other experiments (hereafter referred to as "high"

palmitate experiments), an increased amount of palmitic acid was added to the perfusion fluid in an attempt to simulate a state of increased fatty acid mobilization. Because of the limited ability of albumin to bind free fatty acid, the amount of albumin used was increased sevenfold to accommodate the added palmitic acid. Tracer amounts of palmitate-1-C¹⁴ were added to an albumin solution as described previously (15). Extra unlabeled palmitic acid was dissolved in 0.1 N hot KOH, neutralized to pH 7 with 1.0 N HCl, and added rapidly to the solution, which was adjusted to a final albumin concentration of 20%. The preparation was discarded if there was any turbidity. Thirty-five ml of this albumin-C¹⁴ palmitate solution (containing 100 mg of palmitic acid and 20×10^6 cpm of C¹⁴) was added to the reservoir 25 minutes after the onset of liver perfusion. The perfusate otherwise consisted of 90 to 95 ml of blood from fasting rats, 120 mg heparin, and sufficient Ringer's solution to make a final volume of 150 ml. The average hematocrit of the perfusate was 29%. In appropriate experiments 3.0 ml of 25% ethanol was added to the reservoir 10 minutes after the onset of perfusion in order to make the initial ethanol concentration of the perfusate 400 mg per 100 ml. Otherwise, the high palmitate experiments were conducted in the same manner as the low palmitate studies.

Analytical procedures: extraction of tissues and determination of radioactivity

1) *Plasma*. Perfusate samples were immediately refrigerated, and plasma was separated by centrifugation. One-ml fractions of the plasma specimens were extracted with chloroform-methanol (2/1, vol/vol) according to the technique of Folch, Lees, and Sloane Stanley (21). After separation of the layers, the chloroform fraction was evaporated to dryness under a stream of air at 40° C, redissolved in 10 ml of petroleum ether, and a 1-ml portion taken for counting. Free fatty acids (and monoglycerides) were extracted from the remainder with 0.25 N NaOH in 50% ethanol according to the method of Borgström (22), and residual neutral fats were again counted. Thin-layer chromatography (*vide infra*) revealed the label to be predominantly in triglyceride. Radioactivity was assayed as previously described (15) in a Packard Tri-Carb liquid scintillation spectrometer using toluene as the solvent with diphenyloxazole as primary phosphor and *p*-bis-1,2-(phenyloxazolyl)-1-benzene as secondary phosphor. All samples were corrected for quenching.

2) *Liver*. Lipids were extracted by homogenizing the whole liver in a Waring blender using 150 ml of chloroform-methanol. Subsequently 30 ml of 0.05 M KCl containing 0.05 N HCl was added as the aqueous phase. After removal of the upper aqueous-methanol layer, the chloroform fraction was taken to dryness as described above, and the lipids were redissolved in 60 ml of chloroform. Samples were removed, evaporated in counting vials, and taken up in petroleum ether for measurements of radioactivity as described (8). Thin-layer silicic acid chromatography was performed with

² Nutritional Biochemicals Corp., Cleveland, Ohio.

³ Charles River Laboratories, Boston, Mass.

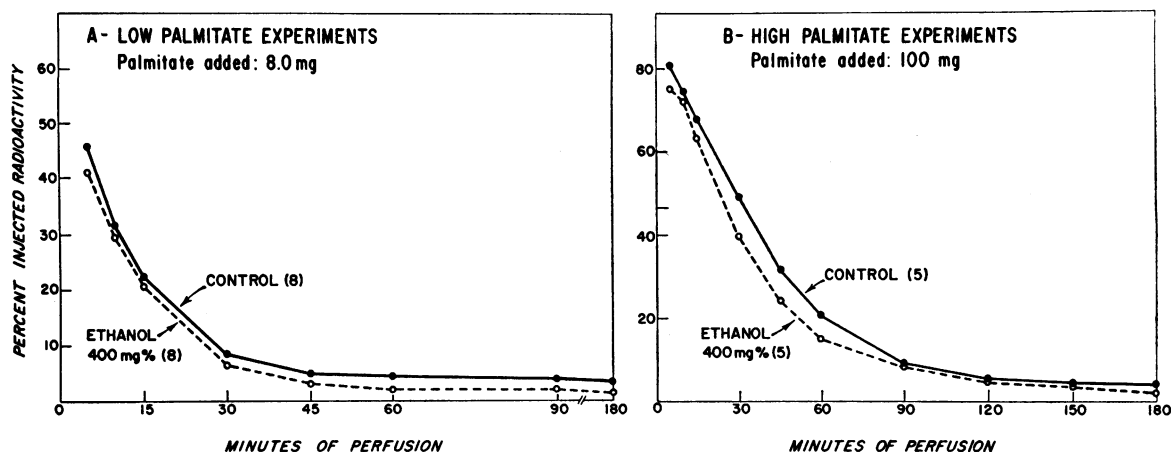


FIG. 1. DISAPPEARANCE OF C^{14} PALMITATE FROM PERFUSATE IN THE PRESENCE OF LOW (A) AND HIGH (B) AMOUNTS OF NONRADIOACTIVE PALMITATE. For details see Materials and Methods. Parentheses refer to the number of perfusions.

40- μ l samples of the chloroform solution. A solvent system consisting of 69% hexane, 30% anhydrous ether, and 1% glacial acetic acid was used to separate the lipids into the following fractions: phospholipids plus monoglycerides, diglycerides, cholesterol, free fatty acids, triglycerides, and cholesterol esters. A solvent system consisting of 49% hexane, 50% anhydrous ether, and 1% glacial acetic acid was used to separate monoglycerides from phospholipids. After brief development of the pattern in iodine vapor the silicic acid in the regions corresponding to individual lipid fractions was scraped off the plates into separate counting vials, allowed to stand overnight in 5 ml of 95% ethanol, and then counted after addition of 10 ml of the toluene counting solution.

3) CO_2 . The $C^{14}O_2$ was trapped in Hyamine as described. One ml of Hyamine was added to 10 ml of the toluene counting solution for assay of radioactivity.

Other chemical measurements

Free fatty acids were measured on plasma samples by the method of Dole (23). Blood alcohol levels were determined on perfusate samples by the method of Sunshine (24). Plasma triglycerides were assayed according to the technique of Van Handel and Zilversmit (25).

Results

Effect of alcohol on the perfusion system. The addition of alcohol to the perfusing medium had no striking effect on the rate of circulation through the liver, either immediately or as measured over the entire perfusion period. The average flow rate in control experiments was 6.7 ± 0.4 ml per

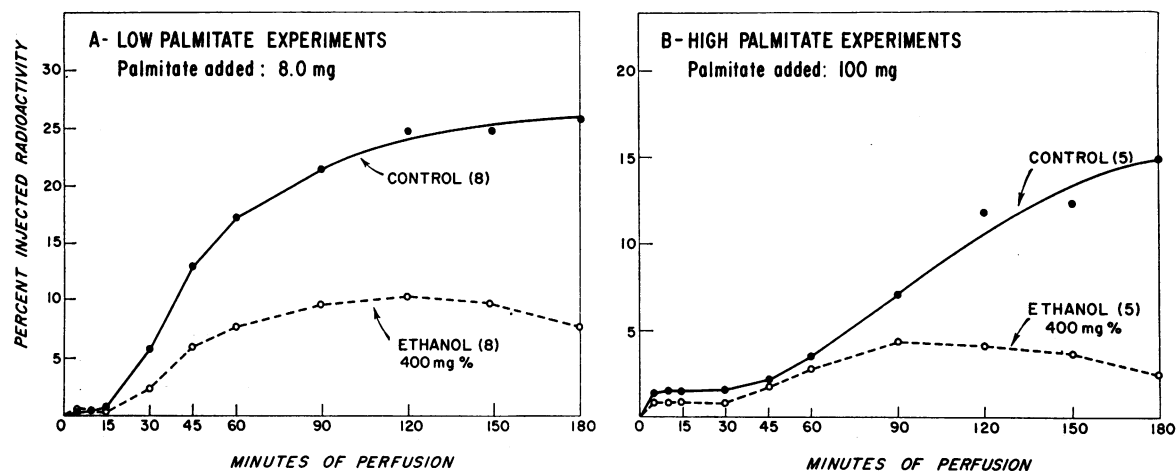


FIG. 2. APPEARANCE OF C^{14} IN NEUTRAL FAT (TRIGLYCERIDE) OF PERFUSATE IN THE PRESENCE OF LOW (A) AND HIGH (B) AMOUNTS OF NONRADIOACTIVE PALMITATE. Parentheses refer to the number of perfusions.

TABLE I
Fate of injected C¹⁴-palmitate after 3 hours of liver perfusion*

Labeled products	Low palmitate experiments (8 mg C ¹⁴ -palmitate added)		High palmitate experiments (100 mg C ¹⁴ -palmitate added)	
	Control	Ethanol	Control	Ethanol
	% of injected radioactivity			
Perfusate neutral fat	25.6 ± 1.6 (8)	7.5 ± 2.2 (8)	14.7 ± 3.8 (5)	2.4 ± 1.6 (5)
	p < 0.001		p < 0.001	
Liver lipids	55.4 ± 3.1 (8)	76.2 ± 4.0 (8)	59.9 ± 10.6 (5)	75.3 ± 13.0 (5)
	p < 0.005		p < 0.4	
C ¹⁴ O ₂	9.7 ± 1.2 (8)	3.9 ± 0.6 (8)	5.0 ± 1.8 (5)	3.1 ± 0.6 (5)
	p < 0.001		p < 0.4	
Water soluble products	2.9 ± 0.5 (6)	3.1 ± 0.6 (6)	3.1 ± 0.4 (4)	3.9 ± 1.7 (4)
	p < 0.40		p < 0.6	

* All values are expressed as means of determinations ± SE; probability values are derived by Student's *t* test. Number of experiments is in parentheses.

minute (mean ± SE), and in the ethanol experiments, 6.0 ± 0.4 ml per minute. Bile flow from the liver averaged 2.1 ± 0.2 ml per 3-hour perfusion in the control experiments and was reduced to 1.2 ± 0.5 ml per perfusion in the presence of ethanol. The added ethanol was rapidly metabolized by the liver; the initial concentration of 400 mg per 100 ml decreased to a mean of 77 mg per 100 ml at the end of the perfusion.

Disappearance of palmitate-1-C¹⁴ and reappearance of label in perfusate neutral lipid: 1) *Low palmitate experiments.* As seen in Figure 1A, the rate of disappearance of the labeled palmitate was quite rapid, about 50% of the C¹⁴-palmitate being removed in 5 minutes. By 60 minutes, the liver had removed essentially all of the labeled fatty acid. There was no difference in rate of removal between control perfusions and those containing ethanol.

By contrast, there was a significant difference between the control and ethanol perfusions in the rate of labeling of the perfusate neutral lipids. As shown in Figure 2A, labeled neutral fat was first detected in the perfusate 30 minutes after the addition of the C¹⁴-palmitate to the system. In the ethanol group, the percentage of injected radioactivity that appeared was strikingly depressed. At the end of 3 hours (Table I) 7.5% of the label was in the perfusate neutral lipid compared to 25.6% in the control perfusions, in

which ethanol was replaced by an equal volume of Ringer's solution. These differences were statistically significant (*p* < 0.001).

2) *High palmitate experiments.* To observe the effects of increased supplies of fatty acids on the uptake and release of lipid by the liver, 100 mg of nonradioactive palmitate was added to the perfusion system in addition to the labeled fatty acid. This addition, together with the endogenous fatty acid in the plasma of the perfusate (average, 15.0 mg per 100 ml), represented a sixfold increase in the total free fatty acids of the perfusing medium. Under these conditions the uptake of palmitate-1-C¹⁴ by the liver (Figure 1B) was again unaffected by ethanol. However, since the palmitate was added to the reservoir of the system rather than injected directly into the portal vein, the rate of removal of free fatty acid was somewhat slower than in the low palmitate experiments. Figure 2B demonstrates that the appearance of label in the perfusate neutral lipids was depressed in the presence of ethanol, although this difference did not become evident until after 60 minutes. At the end of 3 hours (Table I), 2.4% of the label was in perfusate neutral lipid in the ethanol group as compared to 14.7% in the control group (*p* < 0.001).

Fate of injected palmitate-1-C¹⁴. As Table I illustrates, the decreased labeling of the perfusate neutral lipid by ethanol was accompanied by

TABLE II
*Triglyceride content of liver and perfusate**

Determinations	Low palmitate experiments (8 mg C ¹⁴ -palmitate added)		High palmitate experiments (100 mg C ¹⁴ -palmitate added)	
	Control	Ethanol	Control	Ethanol
Triglyceride content of perfusate at end of perfusion (3 hours), mg	41.4 ± 2.8 (9)	32.5 ± 4.3 (7)	42.8 ± 5.3 (5)	14.2 ± 3.0 (5)
	p < 0.1		p < 0.001	
Change in triglyceride content of perfusate during perfusion, mg	+26.7 ± 2.2 (5)	+20.9 ± 1.8 (5)	+25.9 ± 5.3 (3)	-1.2 ± 3.2 (4)
	p < 0.20		p < 0.001	
Triglyceride content of liver† at end of perfusion (3 hours), mg/g liver	4.6 ± 0.7 (4)	5.0 ± 0.8 (4)	9.4 ± 0.5 (4)	11.3 ± 0.8 (5)
	p < 0.70		p < 0.1	

* All values are expressed as means of determination ± SE; probability values derived by Student's *t* test. Number of experiments is in parentheses.

† Calculated as (final triglyceride concentration - initial triglyceride concentration) times final volume of perfusate. Mean triglyceride concentration at beginning of perfusions was 27.0 ± 7.4 mg per 100 ml.

an increased retention of label in liver lipids. In the low palmitate experiments, the amount of injected label present in liver lipids increased from 55.4 to 76.2%; in the high palmitate experiments it increased from 59.9 to 75.3%. There was also a slight but statistically significant decrease in C¹⁴O₂ production in the low palmitate perfusions when ethanol was present. Very little label appeared in water-soluble products, and this amount was not affected by ethanol. Approximately 83 to 94% of the injected radioactivity was accounted for in the four products shown in Table I. Most of the remainder was present in the samples removed for analysis.

Distribution of C¹⁴ in liver lipids. The liver lipids present at the end of the perfusion (3 hours) were separated by thin-layer chromatography in four of the experiments in each group. In the low palmitate experiments, the label was predominantly in the phospholipid fraction (which also contained traces of monoglyceride). Small quantities of label were found in diglycerides, fatty acids, cholesterol, and cholesterol esters. The rest of the lipid radioactivity was in triglycerides. When ethanol was present in the perfusion system, the increased amount of C¹⁴ retained in liver lipids was primarily in the triglyceride and phospholipid fractions. The radioactivity in triglycerides increased by 71%; that in phospholipids by 26%.

In the case of the high palmitate experiments (Figure 3), even in the absence of ethanol more

of the C¹⁴ appeared in hepatic triglycerides than in the low palmitate experiments. When ethanol was added to the perfusate, however, there was a still further increase of label in the triglyceride fraction, and in contrast to the low palmitate perfusions, there was no increased labeling of the phospholipid fraction.

Triglyceride levels of perfusate and liver. The triglyceride content of perfusate and livers was measured chemically, and the results are shown in Table II. In the low palmitate experiments the total triglyceride content of the perfusate (at the end of perfusion) was slightly but not signifi-

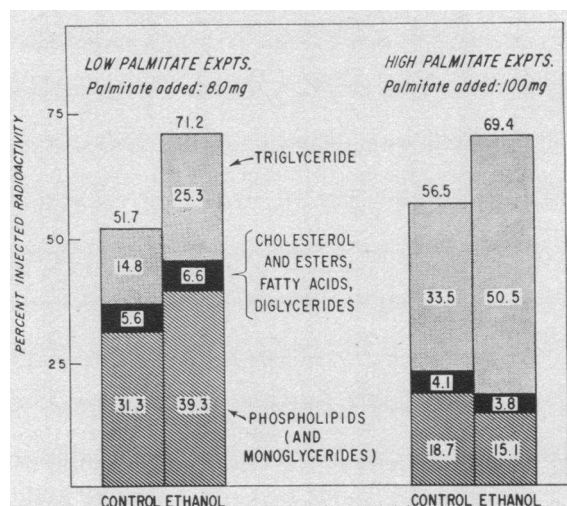


FIG. 3. DISTRIBUTION OF C¹⁴ IN LIVER LIPIDS AFTER 3 HOURS OF PERFUSION. Figures represent the mean values of four experiments in each group.

cantly decreased in the ethanol group as compared to the control (32.5 mg compared to 41.4 mg). In the high palmitate experiments, however, ethanol produced a statistically significant decrease in the triglyceride content of the perfusate (14.2 mg compared to 42.8 mg).

During the course of the perfusion of the isolated rat liver, the triglyceride content of the perfusing blood gradually increased. Table II shows that in both the low and high palmitate experiments there was a mean increase of triglyceride in the perfusate of approximately 26 mg. Ethanol did not affect this appreciably in the low palmitate group. On the other hand, in the high palmitate experiments, ethanol prevented the increase in the triglyceride content of the perfusate and, as seen in Table II, was about the same after 3 hours as at the beginning of the perfusion.

Direct chemical measurement established that the triglyceride content of the livers at the end of the high palmitate perfusions was approximately double that found in the livers at the end of low palmitate perfusions (9.4 mg per g liver as compared to 4.6 mg per g liver). The addition of ethanol to the perfusate caused a slight but not significant increase of hepatic triglyceride levels in the low palmitate perfusions and a somewhat greater increase (significant to the 10% level) in the high palmitate experiments.

The increases in hepatic triglyceride under the influence of ethanol were within the range expected from the conditions of the experiments. Thus, in the low palmitate experiments, with a 21% retention of label in the liver lipids at 3 hours (Table I) and a total free fatty acid level in the perfusate of approximately 21 mg (13 mg endogenous plus 8 mg added), one might only expect a 4-mg increase in liver lipid. With an average liver weight of 10 g, this would amount to 0.4 mg per g liver. As seen in Table II (bottom line), the observed increase was 0.4 mg per g liver. In the high palmitate experiments the total FFA content of the perfusate was about 115 mg (15 mg endogenous plus 100 added). A 15% retention of label in the liver (Table I) would lead to an increase in the liver of 17 mg or 1.7 mg per g. As shown in Table II, the observed mean increase of hepatic triglyceride was 1.9 mg per g of liver.

Discussion

The present experiments using the technique of isolated liver perfusion provide further evidence that ethanol exerts direct effects on hepatic metabolism. Under the conditions of our experiments, with ethanol present in the perfusing medium at an initial concentration of 400 mg per 100 ml, the uptake of C¹⁴-palmitate was rapid and not diminished by ethanol. The control data without ethanol compare closely to the results of Hillyard, Cornelius, and Chaikoff (20). On the other hand, there was a definite reproducible decrease in the rate of appearance of the C¹⁴-labeled neutral glycerides in the perfusate when ethanol was present in the perfusing medium. When the total amount of the fatty acids in the perfusate was relatively small (viz. about 23 mg), the decreased labeling of the perfusate glycerides was not accompanied by a significant change in total liver lipid. When the perfusing medium contained an increased amount of fatty acid (viz. about 115 mg), however, the decreased labeling of perfusate glycerides was accompanied by a suggestive increase in liver triglycerides. The perfusion system used differs from the situation *in vivo*, where high levels of fatty acids may be continuously presented to the liver.

The decreased labeling of the perfusate triglycerides in the presence of ethanol seems likely to reflect a decrease in the release or secretion of triglycerides from the liver. However, since triglyceride is also normally being continuously taken up by the liver from the perfusate (26), it could be argued that these results are due to an increased uptake of labeled glycerides in the form of chylomicrons or low density lipoproteins. Although the mechanisms of the uptake of fatty acids and triglycerides are probably different, the fatty acid uptake of the liver was not appreciably affected by ethanol under the conditions of these experiments.

In the high palmitate experiments there was a reasonably good correlation between the 84% decrease in labeling of perfusate glycerides (14.7% compared to 2.4%, Figure 2) and the 67% change in the triglyceride content of the perfusate (42.8 mg compared to 14.2 mg). In the low palmitate experiments, however, the agreement was not so good. The 67% decrease in labeling of the per-

fusate glycerides (25.6% compared to 7.5%) was accompanied by only a 20% reduction in the total perfusate triglycerides (41.4 mg compared to 32.5 mg). Although a number of explanations are possible, it seems reasonable that these results could readily be due to the existence of separate triglyceride pools in the liver. Gidez, Roheim, and Eder (27) have presented data in support of such a concept. If the exogenous fatty acids equilibrated with one of the triglyceride pools in the liver, one might expect that when the exogenous source in the plasma is large, as in the high palmitate experiments, the radioactive and chemical data would be quite comparable. On the other hand, in the low palmitate experiments, where the supply of isotopically labeled fatty acid is relatively small compared to the total amount of glycerides in the perfusate, some discrepancy between the isotopic and chemical results might be expected.

Of additional interest in these experiments are the effects of the palmitate load on the triglyceride content of liver and perfusate. An increase in the fatty acid content of the perfusate per se was associated with a doubling of liver triglyceride (Table II). This finding agrees with other reported studies. Feigelson, Pfaff, Karmen, and Steinberg (28) were able to produce an increase in hepatic triglyceride in the dog *in vivo* when fatty acid mobilization from adipose tissue was enhanced by the action of epinephrine and norepinephrine. Nestel and Steinberg (29) found similar results when they perfused livers with added palmitic acid and found that the majority of the fatty acids was retained in liver in the form of glycerides. These authors concluded that the amount of triglyceride synthesized by the liver exceeded the capacity of the liver to release triglyceride. Such an interpretation may also apply in the case of the high palmitate perfusions, where one might have expected more triglyceride to be released from the liver than in the low palmitate perfusions. Actually, in both groups, the triglyceride content of the perfusate increased to a similar extent during the course of the perfusions.

The isotopic data in our studies apparently indicate that whenever an increase in hepatic lipid occurred, the increase was predominantly in the

triglyceride fraction. Thus, as seen in Figure 3, when the fatty acid content of the perfusate was raised, a relative increase in labeling of the liver triglycerides occurred together with a relative decrease in the labeling of phospholipid. When the effect of ethanol was superimposed on the increased supply of fatty acids presented to the liver, there was a further diversion of label into triglyceride as compared to phospholipid. Even in the low palmitate experiments accumulation of label in hepatic triglyceride was favored in the presence of ethanol. In this connection, recent studies with liver homogenates in our laboratory have shown ethanol to lead to a preferential conversion of C¹⁴-palmitate into triglyceride rather than into phospholipid (30). Whether this is due to a direct stimulation of diglyceride acylase or to an inhibition of phospholipid synthesis remains to be elucidated. Nevertheless, from the perfusion data presented above, it would appear that in addition to favoring the conversion of fatty acids to triglyceride, ethanol at high concentrations enhances the accumulation of triglyceride by inhibiting its release from the liver.

The manner whereby triglycerides are normally released or secreted by the liver is still incompletely understood. Before their appearance in the plasma as low density lipoproteins, the triglyceride molecules evidently must somehow interact with a reasonably specific protein moiety in the liver. Theoretically, a fatty liver might be produced either by an interference with hepatic lipoprotein synthesis or by an inhibition of lipoprotein release or secretion into the plasma. In the rat, Robinson and Seakins have been able to produce a fatty liver together with decreased plasma lipoprotein levels by the administration of puromycin (31), an antibiotic known to inhibit protein synthesis. Smuckler, Iseri, and Benditt (32, 33) have shown that carbon tetrachloride causes a disruption of the endoplasmic reticulum and a reduction in protein synthesis. These latter findings, together with the concept of Recknagel, Lombardi, and Schotz (11, 12) that decreased hepatic triglyceride release is the cause of the carbon tetrachloride fatty liver, suggest that the decreased triglyceride release under these conditions may be the result of impaired lipoprotein synthesis. Experiments by Harris and Robinson

with ethionine (14) likewise have shown an association of fatty liver, decreased protein synthesis, and decreased plasma lipoprotein levels.

In the case of ethanol, preliminary observations have revealed a decreased labeling of perfusate low density lipoproteins when experiments similar to those reported above were performed with C¹⁴-leucine (34). However, no definite reduction in labeling of total liver proteins occurred. Similarly, in experiments with liver slices a decreased labeling of plasma lipoproteins has been observed without an apparent reduction of hepatic protein synthesis (34). Thus ethanol at high concentrations may well exert its effect on release or secretion of lipoproteins from the liver rather than on the synthesis of lipoproteins.

It still remains to be determined how applicable the present perfusion experiments may be in relation to the events that occur in the whole animal or in man after ethanol ingestion. Under certain conditions ethanol may lead to increases rather than decreases in plasma triglycerides. Maling, Wakabayashi, and Horning found significant increases in serum triglyceride after ethanol administration to rats (35), although Elko, Wooles, and DiLuzio reported no change in their experiments (36). In man, Zieve (37) was the first to draw attention to a syndrome of hyperlipemia and fatty liver in some alcoholics. In view of the evidence favoring the liver as the major source of circulating triglyceride in the fasting state (10), the occurrence of hyperlipemia might be considered as an argument against the concept that alcohol *in vivo* inhibits hepatic triglyceride release.

There are, however, several alternative explanations for this apparent lack of correlation between the *in vivo* and *in vitro* experiments: *a*) Ethanol *in vivo*, in addition to its effects on the liver, may lead to triglyceride elevations by inhibiting the removal or clearance of triglycerides from the circulation. In this regard Losowsky, Jones, Davidson, and Lieber (38) have recently observed lower serum lipoprotein lipase levels in some subjects given ethanol. *b*) In the face of increased mobilization and increased triglyceride formation in the liver, a small but definite interference of hepatic triglyceride release might still permit a relative elevation of plasma triglycer-

ides. Finally, *c*), the effect of ethanol on hepatic triglyceride release may be seen only at relatively high blood ethanol levels. Recent findings from our laboratory seem pertinent in this connection (39). In a group of alcoholics drinking increasing amounts of alcohol over a 25-day period, there was an initial increase in serum triglycerides when alcohol levels were less than 200 mg per 100 ml. However, there was a sudden and dramatic fall (in some instances to below initial triglyceride levels) when serum ethanol values rose above 300 mg per 100 ml. In most instances this fall in serum triglycerides occurred at times when serum free fatty acid levels were increased up to six times the normal values.

In speculating as to the mechanisms whereby ethanol ingestion leads to the development of a fatty liver, one needs to distinguish between causal or primary and contributory or secondary factors. The data presented above do not permit the conclusion that decreased hepatic triglyceride release is a primary factor, but they certainly suggest that at high ethanol concentrations, this mechanism contributes to triglyceride accumulation in the liver.

Summary

Experiments are presented concerning the effects of ethanol on the uptake and metabolism of palmitic acid-1-C¹⁴ by the isolated perfused liver. Palmitate was added to the perfusing medium in near physiological (8 mg) or approximately 10 times physiological (100 mg) amounts. All ethanol experiments were carried out at an initial concentration of 400 mg per 100 ml.

The uptake of C¹⁴-palmitate by the perfused liver was rapid and not diminished in the presence of ethanol. However, ethanol produced a significant decrease in the rate of appearance of C¹⁴ in the triglycerides of the perfusate. This decrease, which in all likelihood reflected a diminished release of triglyceride from the liver, was accompanied by an increased retention of C¹⁴ in the liver, mostly in triglycerides.

Chemical measurements of triglycerides revealed that in comparison to control experiments, the addition of ethanol was associated with a decrease in the triglyceride content of the perfusate at the end of 3 hours. This difference was statistically

significant in the high palmitate experiments. Despite the smallness of the increases in liver triglycerides in the ethanol studies, they were nevertheless within the range expected from calculations of the amount of label retained in the liver and the amount of fatty acids initially present in the perfusate.

Although the data from isolated liver perfusion are not necessarily applicable *in vivo*, ethanol, at high concentrations, is apparently able to exert a direct effect on the metabolism and transport of triglycerides by the liver.

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