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Origin of hepatic triglyceride fatty acids: quantitative estimation of the relative contributions of linoleic acid by diet and adipose tissue in normal and ethanol-fed rats

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Abstract The present study demonstrates that the rat liver obtains most of its triglyceride fatty acids from dietary sources. The dietary and adipose tissue contributions of linoleic acid for hepatic triglyceride esterification were shown to be 50.42 and 13.85 μmoles, respectively, during a 4-day period. When ethanol provided 40% of the caloric intake, fatty liver developed and hepatic triglyceride content increased threefold. Under these conditions, the dietary and adipose tissue contributions of linoleic acid were estimated at 192.85 and 10.73 μmoles, respectively. This increase in dietary fatty acid utilization was sufficient to account for the entire increase in esterified hepatic linoleic acid.

Any explanation of these observations must include the high dietary fatty acid utilization in both control and ethanol-treated animals. One possibility is that most dietary lipids first enter a rapidly turning over pool in adipose tissue from which most hepatic triglyceride fatty acids are derived. Another is that dietary fatty acids, incorporated into chylomicrons, are stored separately and used preferentially by the liver as compared with lipids derived from adipose tissue and bound to albumin. The pros and cons of these possibilities are discussed.

Supplementary key words rat liver · rapidly turning over pool · chylomicrons · albumin

It was previously believed (1–3) that adipose tissue was the principal source of esterified fatty acids which accumulated in the ethanol-induced fatty liver. However, this has recently been challenged (4–6). Lieber, Spritz and DeCarli (6), using fatty acids not commonly present in mammalian diets, were able to label rat adipose tissue fatty acids. Subsequently, by dietary manipulation they showed that the triglyceride fatty acids which accumulated as a result of chronic ethanol feeding more closely resembled the dietary fatty acids than those of the adipose tissue, thus differentiating the primary source of the triglyceride fatty acids. The turnover rates or physiological half-lives of hepatic triglycerides, though varying with the animal species under observation as well as with the metabolic and nutritional conditions of the animals, can be expressed in terms of a few days, while those for adipose tissue are in terms of weeks or months (7, 8). This marked difference in the rate of triglyceride turnover forms the physiological basis which permitted this differentiation.

The current study was designed to determine the relative contributions of diet and adipose tissue to the fatty acid pool utilized for hepatic triglyceride formation in both normal and ethanol-treated animals. Linoleic acid was selected as the fatty acid for study since it is a common fatty acid in mammalian diets but cannot be synthesized by mammalian tissue (9). Hence, the origin of this fatty acid in mammalian liver must be either fatty acid tissue stores (adipose tissue) or the diet.

METHODS

General procedure

Male Holtzman rats, 4–6 months of age and weighing 350–450 g, were divided into two groups and pair-fed in order to maintain comparable body weights between treated and control animals (mean differential of ± 0.7%

between the pairs). As previously reported (10), the administered synthetic diet was nutritionally adequate and a mean weight gain of 8% was observed over the treatment period.

Animals in group 1 served as controls and were fed the synthetic diet as outlined in Table 1. Group 2 contained the alcohol-fed animals; 95% ethanol was substituted isocalorically for carbohydrate so as to provide 40% of the total calories, assuming 7 kcal/g of ethanol. The fatty acids supplied in the diet were of two types: predominantly long-chain unsaturated (>85%) esterified fatty acids from corn oil, and medium-chain saturated (>89%) esterified fatty acids from coconut oil. The compositions of these oils are listed in Table 2. 18% of the total calories of each diet was derived from the corn oil or coconut oil. Animals in both groups were maintained on this dietary program for 46 days. At the time of the experiment, the animals were decapitated, the livers were perfused in cold saline, and both liver and mesenteric adipose tissue were quick-frozen in liquid nitrogen for subsequent analysis.

Lipid analysis

Lipids were extracted from tissue by homogenization with chloroform–methanol 2:1 (v/v) according to the method of Folch, Lees, and Sloane Stanley (11). From the lipid extract, triglycerides were quantitated by the method of Van Handel and Zilversmit (12). Triglycerides were isolated for fatty acid analysis and radioactivity measurements by preparative thin-layer chromatography, using glass plates coated (0.2 mm) with silica gel G (Merck) in a system of petroleum ether–diethyl ether 90:10 (v/v). From the lipid-insoluble residues, protein nitrogen (Kjeldahl method) (13) and DNA (14) were determined.

TABLE 1. Composition of diet[a]

	Control	Ethanol
	g/liter	*g/liter*
Dextrose (corn)	250.1	93.0
Ethanol	0.0	89.8
Corn oil or coconut oil	30.9	30.9
Vitamin-free casein	75.6	75.6
Salts and minerals (mixture USP XIV)	7.6	7.6
Vitamin and cofactor mixture[b]	1.7	1.7
Cod-liver oil	3.2	3.2
Powdered tragacanth gum	14.0	14.0
Water	697.7	697.7

[a] Diet contained 1.58 kcal/ml. Approximately 50 ml daily was required for maintenance of a constant body weight.

[b] The vitamin and cofactor mixture contained (g): thiamine hydrochloride, 0.25; riboflavin, 0.50; pyridoxine hydrochloride, 0.25; calcium pantothenate, 2.00; niacinamide hydrochloride, 1.00; choline chloride, 100.00; biotin, 0.01; vitamin B_{12}, 0.02; folic acid, 0.10; inositol, 30.00; and vitamin-free casein, 258.48.

TABLE 2. Fatty acid composition of corn oil and coconut oil

Fatty Acid	Corn Oil	Coconut Oil
	% of total fatty acids	
8:0[a]		39.9
10:0		22.1
12:0	<1	12.0
14:0	<1	3.8
16:0	10.4	10.5
18:0	1.2	4.0
18:1	26.0	5.7
18:2	60.0	2.1
>18:2	2.4	

[a] Number of carbon atoms: number of double bonds.

Since both the amount of hepatic protein as well as the weights of the livers were noted to increase concomitantly with the increase in total triglycerides, these values were felt to be poor reference parameters. However, as shown in Table 3, total hepatic DNA was the same in both groups, indicating a similar, unaltered cell number. Hence, it was deemed simplest, and justifiable, to express all values as μmoles present in the entire liver.

In all experiments, each result was compared with the corresponding control, and the differences between the means were tested for significance by the Student t test (15).

Hepatic triglyceride turnover was determined using 52 animals that had been on the diets previously described and in which lipids were derived from corn oil. At the end of 42 days, each rat was given 120 μCi of [2-^{3}H]glycerol by intraperitoneal injection. The animals were divided into five subgroups using a random number technique, and they were sacrificed at precisely 48, 72, 96, 120, and 144 hr after isotope injection. Lipids and triglycerides were isolated and quantitated by the procedures outlined previously.

Labeled glycerol was selected as the triglyceride precursor because it has been shown to undergo minimal recycling as compared with fatty acids (16). To reduce the significance of differences in hepatic uptake of

TABLE 3. Effect of ethanol on hepatic weight and on total protein and DNA content[a]

	Control	Ethanol	*P*
Liver weight (g)	11.3 ± 0.53	14.1 ± 0.66	0.01
Protein (g)	1.4 ± 0.12	2.3 ± 0.22	0.01
DNA (mg)	16.9 ± 1.41	17.2 ± 3.55	NS[b]

[a] Values are presented as means ± SE. Liver weights were obtained for all 10 rats in each group. Samples from seven rats in each group were analyzed for protein; samples from five rats in each group were analyzed for DNA.

[b] NS indicates difference is not significant at the 5% level.

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glycerol which are known to be induced by ethanol (17), the triglyceride disappearance curves were evaluated 48–144 hr after the isotope injection. At these time periods no free [2-^{3}H]glycerol could be detected in plasma, indicating that complete uptake and removal of the precursor had occurred. Thus, determinations of half-times could be in error only because of hepatic recycling of the [2-^{3}H]glycerol-labeled triglycerides or conversion of the labeled glycerol into other compounds such as fatty acids and then into the triglycerides. Since kinetic studies indicate nonrecycling of hepatic triglycerides (16), and since a significant dilution effect would result and would greatly diminish any contribution derived from the interconversion of labeled glycerol into an intermediate prior to its incorporation into the triglycerides, the half-times thus determined were felt to be valid estimates for hepatic triglyceride turnover.

Radioactivity in the triglyceride fraction was determined using a Nuclear-Chicago model 701 liquid scintillation counter. Samples were dissolved in 10 ml of toluene containing 2,5-diphenyloxazole (PPO) and 1,4-bis-(2-5-phenyloxazolyl)benzene (POPOP). Quenching was estimated by using both an internal standard and the channel-ratio method. Appropriate corrections were made and all radioactivity measurements were expressed as disintegrations per minute (dpm). Total and specific radioactivities for the triglycerides were plotted against time, and a regression analysis was performed to determine half-time values.

Fatty acid composition

Two groups of rats (20 control and 20 ethanol-treated) were each equally divided into four subgroups, A, B, C, and D, with five animals in each group. Groups A and B were fed for 46 days a diet in which corn oil and coconut oil, respectively, were the sole sources of fat. Group C received coconut oil for 42 days and then corn oil for 4 days. Group D received corn oil for 42 days and coconut oil for 4 days. After the animals were killed, triglycerides were isolated from liver and mesenteric adipose tissue using the procedures already described. Samples of triglycerides from each group were treated with boron trifluoride in methanol (Applied Science Laboratories Inc., State College, Pa.) according to the method of Morrison and Smith (18). The fatty acid methyl esters derived from this reaction were quantitated by gas–liquid chromatography, using a Barber-Colman model 5000 gas–liquid chromatograph, with a 6-ft, U-shaped glass column and a ^{90}Sr detector. The column was packed with Chromosorb W, 80–100 mesh, coated with 15% diethylene glycol succinate (Applied Science). Separations were performed at a column temperature of 172°C, an injector temperature of 192°C, and a detector temperature of 182°C, using argon (64 ml/min at a pressure of 13.0 psi) as carrier gas. Individual peaks recorded during the separation were identified by comparison with retention times of standard methyl esters (Applied Science). The area under each fatty acid peak was estimated by triangulation and expressed as a percentage of the total fatty acids present.

Calculations

The following algebraic model was used to estimate the μmoles and % of hepatic linoleic acid (18:2) derived from adipose tissue and from diet for hepatic triglyceride synthesis.

X = the fraction or % of esterified hepatic 18:2 derived from the diet.

Y = the fraction or % of 18:2 derived from adipose tissue stores. Then, since no endogenous synthesis can occur,

$$X + Y = 1.00$$

A = the μmoles of 18:2 in hepatic triglycerides of animals in group A; it represents the maximum 18:2 content in livers of animals fed a corn oil diet.

B = the μmoles of 18:2 in hepatic triglycerides of animals in group B; it represents the maximum 18:2 content in livers of animals fed a coconut oil diet.

We may now calculate the fraction of fatty acid arising from diet and from adipose tissue of animals fed for 42 days on coconut oil followed by 4 days on corn oil (group C) from the following equation:

$$RB + XA + YB = C$$

where R = the fraction or % of 18:2 deposited in the liver as a result of the initial 42 days on a coconut oil diet and not replaced during the terminal 4 days on a corn oil diet. The value of R is calculated from the half-lives of hepatic triglycerides (see above).

C = the μmoles of 18:2 in the liver after such dietary manipulation. This value was obtained experimentally from group C.

RB represents the μmoles of residual fatty acids not turned over during the terminal 4-day period; YB represents the μmoles derived from adipose tissue. YB is used rather than some point between YB and XA because the half-life of adipose tissue is so long that any slight change in 18:2 content over the terminal 4-day period may be considered negligible.

RESULTS

In Table 4 are shown the amounts of triglycerides in livers from rats fed for 6 wk on diets containing 40% of the calories as ethanol. A 2–3-fold increase was observed in triglycerides in all the groups irrespective of

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TABLE 4. Effect of diet on content of hepatic triglycerides

Dietary Lipids	Total Hepatic Triglycerides[a]	
	Control	Ethanol
Group A (corn oil)	94.13 ± 7.47	338.02 ± 27.7
Group B (coconut oil)	134.76 ± 7.61	296.95 ± 10.75
Group C (coconut oil then corn oil)	119.65 ± 5.05	292.66 ± 14.76
Group D (corn oil then coconut oil)	114.74 ± 4.91	282.76 ± 28.26

[a] Values are in µmoles of triglycerides per liver ± SEM.

the type of dietary lipid. This represented a significant increase over the controls ($P < 0.001$).

Table 5 gives the fatty acid compositions of liver triglycerides from rats fed each of the four dietary regimens. It should be noted that the fatty acids which predominated in the diet during the terminal days prior to sacrifice represented the principal fatty acids in the hepatic triglycerides. Thus, when coconut oil was fed for the entire period (group B), a tenfold increase in esterified medium-chain triglyceride fatty acids was observed in ethanol-fed animals, while 18:2 was relatively unchanged (22.57 vs. 21.67 µmoles). However, when corn oil was substituted for coconut oil during the terminal 4 days (group C), the content of 18:2 rose from 21.67 to 208.36 µmoles in the ethanol-treated animals and from 22.57 to 66.80 µmoles in the control group. Thus, in both groups 18:2, which was the predominant fatty acid of the diet, underwent the most significant increase.

In like manner, when animals were fed a corn oil diet (group A), esterified medium-chain fatty acids constituted only a small fraction of the liver triglycerides, and the amount was altered only slightly by the addition of ethanol to the diet (3.00 µmoles in the controls and 6.83 µmoles in ethanol-treated rats). However, when coconut oil replaced corn oil for the terminal 4 days (group D), these acids increased to 24.15 and 120.86 µmoles, respectively.

At the same time, 18:2 in adipose tissue triglycerides remained relatively unchanged, as seen in Table 6. A corn oil diet (group A) resulted in adipose tissue triglycerides which contained 27.7% and 32.9% 18:2 in control and ethanol-treated animals, respectively. In group D, these percentages were 25.1% and 32.6%, respectively. Conversely, the values for Group B (coconut oil feeding for 46 days) and those for Group C (coconut oil for 42 days followed by corn oil for the terminal 4 days) for medium-chain triglycerides were 43.8% and 41.8%, respectively, for the controls and 65.5% and 54.8%, respectively, for the alcohol-treated rats. The only exception to this was in Group D in which, during the terminal 4 days, a moderate increase in esterified medium-chain fatty acids was noted. There was no obvious explanation for this observation.

The estimation of hepatic triglyceride turnover was determined by a regression analysis of the data given in Table 7. As previously reported (19), using [2-^{3}H]-glycerol-labeled triglycerides the half-life of hepatic triglycerides was found to be 1.25 days for the controls and 1.79 days for the ethanol-treated rats, with a turnover rate of 49.8 mg/day and 61.0 mg/day, respectively. Although mathematical studies suggest the existence of more than one subcellular pool for hepatic triglycerides similar to that proposed for free fatty acids (20), the present study does not attempt to differentiate these. The estimated half-lives represent a mean composite turnover for all existing hepatic pools. Thus, assuming a random turnover in 4 days, 88.8% of hepatic triglyc-

TABLE 5. Effect of ethanol and diet on content of individual triglyceride fatty acids in liver[a]

Fatty Acid	Group A (Corn Oil)[b]		Group B (Coconut Oil)[b]		Group C (Coconut Oil then Corn Oil)[c]		Group D (Corn Oil then Coconut Oil)[d]	
	Control	Ethanol	Control	Ethanol	Control	Ethanol	Control	Ethanol
8:0 to 14:0	3.00 ± 0.37	6.83 ± 0.52	33.84 ± 8.52	318.34 ± 82.28	6.53 ± 1.06	24.96 ± 4.31	24.15 ± 4.22	120.86 ± 50.17
16:0	158.64 ± 11.94	265.41 ± 10.49	115.29 ± 14.41	426.11 ± 53.06	142.74 ± 14.99	330.99 ± 32.91	139.36 ± 9.11	305.52 ± 44.34
16:1	13.31 ± 3.09	26.11 ± 10.17	10.79 ± 2.35	53.47 ± 10.82	17.61 ± 1.70	34.20 ± 6.23	12.09 ± 0.76	55.68 ± 8.33
18:0	7.75 ± 1.73	19.27 ± 10.29	9.17 ± 1.26	12.04 ± 0.95	8.38 ± 1.04	25.13 ± 9.29	40.22 ± 9.20	32.46 ± 5.44
18:1	90.14 ± 9.33	191.34 ± 15.85	90.72 ± 13.84	182.44 ± 13.18	116.89 ± 7.36	254.35 ± 12.71	67.04 ± 2.10	218.70 ± 43.44
18:2	130.45 ± 19.17	381.88 ± 17.18	22.57 ± 4.43	21.67 ± 2.73	66.80 ± 4.14	208.36 ± 23.12	61.25 ± 4.05	115.07 ± 17.85

[a] Values are µmoles of esterified fatty acids ± SEM.
[b] Diets containing either corn oil or coconut oil were fed for 46 days.
[c] Diet containing coconut oil was fed for 42 days, after which corn oil was fed for 4 days.
[d] Diet containing corn oil was fed for 42 days, after which coconut oil was fed for 4 days.

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TABLE 6. Effect of ethanol and type of dietary fat on fatty acid composition of triglycerides in mesenteric adipose tissue[a]

Fatty Acid	Group A (Corn Oil)[b]			Group B (Coconut Oil)[b]			Group C (Coconut Oil then Corn Oil)[c]			Group D (Corn Oil then Coconut Oil)[d]		
	Control	Ethanol	*P*	Control	Ethanol	*P*	Control	Ethanol	*P*	Control	Ethanol	*P*
8:0 to 14:0	2.2 ± 0.14	1.6 ± 0.09	0.05	43.8 ± 3.31	65.5 ± 4.55	NS[e]	41.8 ± 7.59	54.8 ± 1.29	NS	20.9 ± 1.44	17.2 ± 2.16	NS
16:0	33.2 ± 1.21	25.5 ± 1.04	0.01	22.7 ± 1.88	16.0 ± 0.94	NS	22.8 ± 2.19	15.6 ± 1.63	0.05	24.0 ± 1.08	20.2 ± 1.17	0.05
16:1	6.4 ± 0.47	4.1 ± 0.67	0.05	6.9 ± 0.37	2.5 ± 2.19	NS	4.9 ± 0.67	2.8 ± 0.35	0.05	3.7 ± 0.50	3.3 ± 0.38	NS
18:0	2.6 ± 0.43	3.2 ± 0.56	NS	2.0 ± 0.30	1.3 ± 0.58	NS	2.2 ± 0.28	1.6 ± 0.27	NS	2.3 ± 0.18	1.9 ± 0.22	NS
18:1	27.8 ± 0.59	32.4 ± 1.89	NS	21.7 ± 1.28	12.9 ± 2.14	0.05	20.9 ± 3.26	17.8 ± 0.90	NS	24.0 ± 0.86	25.2 ± 0.74	NS
18:2	27.7 ± 1.63	32.9 ± 1.16	0.05	2.8 ± 0.37	1.5 ± 0.92	NS	7.4 ± 2.56	6.5 ± 1.53	NS	25.1 ± 2.38	32.6 ± 1.51	0.05

[a] Values are percentages of total triglyceride fatty acids ± SEM.
[b] Diets containing either corn oil or coconut oil were fed for 46 days.
[c] Diet containing coconut oil was fed for 42 days, after which corn oil was fed for 4 days.
[d] Diet containing corn oil was fed for 42 days, after which coconut oil was fed for 4 days.
[e] NS indicates difference is not significant at the 5% level.

TABLE 7. Radioactivity in hepatic triglycerides labeled with [2-^{3}H]glycerol[a]

Time	n[b]	Control	n[b]	Ethanol
hr		*dpm*		*dpm*
48	6	36,303 ± 1,276	5	30,465 ± 5,882
72	5	16,249 ± 4,762	5	19,449 ± 5,556
96	4	12,233 ± 3,115	5	12,781 ± 6,667
120	6	5,806 ± 754	6	8,391 ± 681
144	4	3,406 ± 300	6	6,164 ± 670

[a] Values represent mean dpm ± SEM in the hepatic triglycerides at 24 hr-intervals after injection of labeled [2-^{3}H]glycerol.
[b] Number of animals.

TABLE 8. Origin of linoleic acid in hepatic triglycerides[a]

	Control		Ethanol	
	%	*μmoles*	%	*μmoles*
Diet	75.5	50.42	92.6	192.85
Adipose tissue	20.7	13.85	5.1	10.73
Residual	3.8	2.53	2.3	4.79
Total		66.80		208.37

[a] Calculations were performed on data from animals fed a diet containing coconut oil (high in medium-chain triglycerides and low in linoleic acid) for 42 days and then a diet containing corn oil (high in linoleic acid) for 4 days.

erides are replaced in the controls and 77.9% in alcohol-treated rats. This leaves a residual of 11.2% and 22.1%, respectively. These represent the *R* fractions used in the mathematical calculations. The final results of these calculations are shown in Table 8.

Note that these results demonstrate mathematically that even in the control animals diet serves as the predominant source for esterified linoleic acid in liver triglycerides, representing 75.5%. However, when alcohol is chronically fed, this percentage increases to 92.6%. The effect of ethanol on utilization of adipose tissue fatty acids appears negligible, since both controls and ethanol-treated animals utilized comparable amounts from this source, 13.85 and 10.73 μmoles, respectively. However, because of the much greater amount of hepatic triglyceride fatty acids present in the ethanol-treated animals, the percentage of fatty acid derived from adipose tissue fell from 20.7% in the controls to 5.1% in the ethanol-treated animals, thus representing a relative rather than an absolute decrease.

One test for the validity of the mathematical model might be its ability to predict the amount of 18:2 one would find if the sequence of dietary manipulations were reversed and corn oil were fed for 42 days followed by coconut oil for 4 days. This would require the additional assumption that the fraction or percentage of 18:2 in hepatic triglycerides from diet and adipose tissue during the terminal 4 days is independent of the type of lipid supplied in the diet so long as the total content of dietary lipid is constant and nutrition is maintained.

The equation then becomes:

$$X + Y = 1.00; \text{ and } RA + XB + YA = D$$

Solving for *D* in the controls we obtain a predicted value of 58.79 μmoles. When animals were fed diets in this sequence and then killed, we observed an actual value of 61.25 μmoles. This represents an error of less than 5%. For the ethanol-treated rats, the predicted value was 124.15 μmoles and the actual value was 115.1 μmoles. Although this is slightly higher than the observed value, it is within 1 SEM of the observed values.

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DISCUSSION

In the present study, using the essential fatty acid 18:2 as a model, a quantitative estimation is made of the relative contributions of fatty acids derived from diet and adipose tissue for hepatic triglyceride esterification. This study indicates that dietary fatty acids are the principal source of fatty acids in hepatic triglycerides for both controls and ethanol-treated animals. With chronic ethanol ingestion, increased triglyceride synthesis results in an increase in the amount of hepatic triglyceride fatty acids from this dietary fatty acid pool. This then corroborates the work of Lieber et al. concerning the source of hepatic triglyceride fatty acids in the alcoholic (6).

By means of simple algebra we have demonstrated that even in the control animals 75.5% of the 18:2 is derived from diet. When ethanol was administered, the percentage increased to 92.6%. In absolute numbers this represented an increase from 50.42 to 192.85 μmoles and was sufficient to account for the entire increase in esterified 18:2 which occurred due to alcohol ingestion.

Initial studies dealing with fat absorption and hepatic chylomicron uptake (6) failed to demonstrate changes which could account for this increased utilization of dietary fatty acids. Hence, if we are to rely on these studies, we must conclude that there is an alteration within the hepatic cell resulting in preferential esterification of fatty acids derived from diet rather than fat stores.

Several explanations are available. One possibility that has been advanced by Dole (21) is that a small, but very active, compartment exists within adipose tissue, communicating with both serum and the much larger, but relatively inactive, adipose storage compartment. Thus, dietary fats could initially be taken up by adipose tissue, enter the very active compartment, and then be rapidly released into plasma as free fatty acids. Subsequently, they could be transported to liver for triglyceride formation.

The existence of such a small, rapidly turning over pool would explain the relative absence of any alteration in adipose tissue fatty acid composition, but would allow for adipose tissue to represent the immediate source of hepatic triglyceride fatty acids both in normal and ethanol-treated animals. If this is correct, then the plasma free fatty acids should reflect dietary lipids which have traveled through this active pool prior to incorporation into hepatic lipids. To evaluate such a possibility, the plasma free fatty acid composition as well as circulating triglyceride fatty acids were examined in four animals in group D. In each instance, the esterified fatty acids of triglycerides resembled the diet in that linoleic acid was less than 5% (mean 2.33% $\pm$ 0.76), while the free fatty acids more closely resembled adipose tissue (mean 13.40% $\pm$ 0.89). This is strong evidence against Dole's theory.

Also against this theory is the fact that in spite of several well-planned studies, no direct experimental evidence has been found to substantiate the existence of such a rapidly turning over compartment. Indeed, in vitro, all fat cells appear to be metabolically active (21). In addition, Crouse et al. have shown that ethanol inhibits fatty acid release from adipose tissue (22) at a time when the requirement for triglyceride synthesis is increased.

Another possibility is that at least two distinct pools of fatty acids exist within the hepatocyte: one derived predominantly from adipose storage depots and another of dietary origin. Under normal conditions the fatty acids might be stored separately within the liver, with those derived from the diet supplying 75.5% of the fatty acids needed for hepatic triglyceride esterification. Irrespective of which theory is correct, it appears that in times of increased need, i.e., during chronic ethanol ingestion, there is an exaggeration of the normal process, with increased utilization of up to 92.6% from the fatty acid types derived from dietary lipids, but with minimal utilization of fatty acid types present in adipose tissue.

Most recently in preliminary studies, we have found evidence which suggests that the earlier studies related to ethanol's effect on dietary fat absorption were incorrect, and that ethanol does exert a direct effect, increasing both triglyceride (23) and free fatty acid absorption.[1]

Thus, altered dietary absorption may contribute, at least in part, to the increased utilization of dietary fatty acids used for hepatic triglyceride formation in the fatty livers of ethanol-treated animals. This does not, however, explain the fact that 75.5% of fatty acids are preferentially derived from the diet in control animals.

Thus far, we have considered only the essential fatty acid 18:2. However, it is well known that ethanol stimulates de novo fatty acid synthesis (1, 3, 24) and that this may contribute a considerable amount to the observed esterified nonessential fatty acids. Although the exact quantitative contributions from de novo synthesis cannot be determined from this present study, inferences can be made regarding its relative role.

Since both diets contained identical amounts of lipid (18% of total calories) and identical amounts of palmitic acid (16:0), an estimate can be made of the effect of dietary fatty acid composition alone and in the presence of chronic ethanol ingestion on endogenous fatty acid synthesis. It is noteworthy that in the controls, diets high in long-chain polyunsaturated or medium-chain saturated fatty acids did not result in a significant change in the amount of 16:0 in hepatic triglycerides. How-

[1] Julian, D., and C. L. Mendenhall. Unpublished results.

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ever, when ethanol was chronically administered, a significant increase in 16:0 was observed in hepatic triglycerides in animals maintained on a high medium-chain fatty acid diet compared with those on long-chain polyunsaturated fatty acids (426.1 vs. 265.4 μmoles, respectively; $P < 0.001$). One might suspect that the observed changes in 16:0 content are the result of increased de novo synthesis induced by ethanol rather than increased preferential utilization from dietary or storage depots. The greater amount of 16:0 found in animals fed a high medium-chain fatty acid diet probably reflects the fact that medium-chain fatty acids can serve as precursors for 16:0. This is in agreement with the findings of Lieber et al. (6) in which endogenous synthesis was stimulated by ethanol and resulted in increased palmitate formation when diets high in carbohydrate or medium-chain triglycerides were fed. It would appear that the amount of ethanol-induced fatty acid synthesis is dependent upon the availability of dietary precursors, and the relative contributions of these individual dietary precursors will vary with the dietary lipid composition.

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