# Metabolism of hepatic and plasma triglycerides in rabbits given ethanol or ethionine

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ABSTRACT The formation and transport of hepatic triglyceride fatty acids (TGFA) were studied after intravenous administration of palmitate-1-$^{14}$C or palmitate-9,10-$^{3}$H in rabbits pretreated with ethanol or ethionine.

Administration of ethanol produced significant hypertriglyceridemia without consistent accumulation of hepatic fat. The isotopic studies suggest that plasma free fatty acids were the major precursors of TGFA in d < 1.006 lipoproteins and that fatty acids synthesized in the liver were not the source of the hypertriglyceridemia in the ethanol-treated animals.

Administration of ethionine resulted in an increased concentration of TGFA in the liver, a decreased level of TGFA in d < 1.006 lipoproteins and a very low specific activity in this plasma fraction. These findings suggest that the development of fatty liver after administration of ethionine is in part accompanied by impaired release of TGFA from the liver.

KEY WORDS long-term · short-term · ethanol · ethionine · rabbit · hepatic triglyceride fatty acid · hypertriglyceridemia · plasma free fatty acid · d < 1.006 lipoproteins · fatty liver

A NUMBER OF investigators have shown that administration of ethanol produces fatty liver in rats (1–6). This effect may be associated with increased mobilization of free fatty acids (FFA) (5), increased lipogenesis within the liver (7), or preferential incorporation of FFA into hepatic triglyceride fatty acids (TGFA) rather than into phospholipid fatty acids (PLFA) (8, 9). Nikkila and Ojala (10) reported increased hepatic $\alpha$-glycerophosphate levels in ethanol-treated animals. Hypertriglyceridemia also accompanies ingestion of ethanol in humans, dogs, and rats (2, 11, 12, 13). The relation between fatty liver and hypertriglyceridemia is not clear, since most workers have measured triglycerides in liver or plasma, but not both. Horning, Knox, and Mani, however, found elevated triglyceride levels in both liver and plasma of rats 6–8 hr after ethanol (6). Studies in the dog and rat have shown that administration of ethionine also produces fatty liver (14–17) and a decrease in plasma triglyceride concentration (16, 17), possibly by inhibiting the production of protein needed to transport lipid, in the form of lipoprotein, from the liver into the blood (18). Decreased oxidation of lipid by the liver may also play a role (19).

Since a fraction of the FFA taken up by the liver is esterified and released as TGFA into the plasma (20, 21), the metabolism of TGFA in liver and plasma can be investigated in animals injected with labeled FFA. The results of such a study on normal rabbits were reported by Havel, Felts, and Van Duyne (21). In the present experiments, we used similar techniques to investigate the formation of hepatic TGFA and their transport from the liver to plasma in rabbits treated with ethanol or ethionine.

## MATERIALS AND METHODS

### *Experimental Animals*

New Zealand white rabbits, weighing 2–3 kg and maintained on Nunes rabbit pellets, were used. They were divided into four groups: A, *Long-term ethanol treatment.* Three male rabbits were given 40% ethanol instead of drinking water for periods ranging from 6 months to 1.5

Abbreviations: FFA, free fatty acids; TGFA, triglyceride fatty acids; PLFA, phospholipid fatty acids.

* This work was done during the tenure of an Advanced Research Fellowship of the American Heart Association.

‡ Fellow of the Life Insurance Medical Research Fund of Australia and New Zealand.

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yr. During the 3 days prior to the experimental procedure they consumed about 24 g of 40% ethanol daily, but received no solid food. During the period of long-term ethanol treatment the animals appeared well and maintained their weight. There was no overt evidence of ethanol intoxication. B, *Short-term ethanol treatment*. Eight male rabbits fasted for 3 days received daily by stomach tube a single dose of 5.7 g of ethanol per kg of body weight. The ethanol was given as a 40% solution in water. About 30 min after receiving the ethanol the animals became ataxic and then comatose for a period of about 2–3 hours. Preliminary studies showed that this dose of ethanol was lethal in about one-third of the animals. The rabbits lost about 10% of their initial body weight at the end of the 3 day period of fasting. The isotopic studies were performed 12 hr after the final dose of ethanol. C, *Ethionine treatment*. Three male and three female rabbits, fasted for 3 days, were given ethionine in 0.15 M sodium chloride (25 mg/ml) in a dose of 250 mg/kg body weight intraperitoneally on the 2nd and 3rd day of their fast. They were studied 24 hr after the last injection. D, *Control group*. Eight male rabbits and one female rabbit, fasted for 3 days, served as controls. The rabbits were considered to be in the fasting state only after 3 days without food, since food remains in the stomach for at least 2 days. Drury (22) showed that the respiratory quotient does not fall to 0.7 until rabbits have been fasted for 3 days. The rabbits lost about 10% of their initial body weight at the end of the 3 day period of fasting.

### *Preparation of Radioactive Palmitate for Injection*

Palmitic acid-1-$^{14}$C or palmitic acid-9,10-$^{3}$H (New England Nuclear Corp., Boston, Mass.) was converted to the sodium salt and complexed with crystalline bovine albumin (Armour Pharmaceutical Co., Chicago, Ill.) (23). The amount of radioactivity given varied between 30 and 70 $\mu$c. In all calculations, the quantity of isotope administered was corrected to $10^6$ cpm/kg of body weight.

### *Experimental Procedure*

Experiments were carried out as described in detail elsewhere (21). All rabbits were anesthetized with a 1% aqueous solution of chloralose, given intravenously. The control animals received 55 mg of chloralose per kg of body weight; the experimental animals, which were more easily narcotized, received 25 mg/kg. Blood samples from the control group D and short-term ethanol group B were taken from the ear of unanesthetized animals each day. On the 3rd day, a final blood sample, taken to measure d < 1.006 lipoproteins, was also obtained by intracardiac puncture 1.5 hr after the animals received chloralose. Blood was obtained prior to administration of ethanol and placed in chilled tubes containing a small amount of heparin. The d < 1.006 lipoproteins were separated from plasma by ultracentrifugation and extracted in chloroform–methanol 2:1 (v/v). After they were anesthetized, all other rabbits were given an intravenous injection of albumin-bound palmitic acid-1-$^{14}$C or palmitic acid-9,10-$^{3}$H. Blood samples were collected at intervals after the injection and handled as noted above.

During the course of each experiment, four or five small samples of liver were obtained through an abdominal incision. These samples were first homogenized, then separated into subcellular fractions by ultracentrifugation. At the end of the experiment, the liver was removed and weighed. All liver samples were extracted in chloroform–methanol 2:1 (v/v). $^{131}$I-labeled albumin (E. R. Squibb & Sons, N. Y.) was used to make appropriate corrections for contamination of liver samples by radioactivity in plasma FFA (21).

### *Analytical Methods*

The blood samples were processed, and the lipid classes were separated as described elsewhere (21).

Homogenates of liver were prepared as previously described (21), except that a loosely packed "fluffy layer" was separated from the mitochondrial pellet by gentle washing with 1 ml of 0.15 M sodium chloride.

Glyceride glycerol was determined by the method of Carlson (24). FFA were titrated as described by Dole (25). Lipid phosphorus was measured by the method of Stewart and Hendry (26). Tritium and $^{14}$C contents of lipids were determined in a Packard liquid scintillation spectrometer with 0.3% diphenyloxazole in toluene as scintillator. Protein-bound $^{131}$I-activity of the proteins precipitated by chloroform–methanol was determined in a crystal scintillation well counter. The fractional turnover rate of FFA was calculated from the rate of disappearance of radioactivity in plasma lipids between 2 and 6 min after injection of the radioactivity. The turnover rate of FFA was calculated from the product of the fractional turnover rate, the concentration of FFA, and the plasma volume. All calculations were done as previously described (21).

## RESULTS

### *Concentrations of TGFA and PLFA in Plasma and Liver*

Tables 1, 2, and 3 show the concentration of lipids in the plasma and liver of the experimental and control animals. For more accurate comparisons, the mean TGFA and PLFA concentrations obtained in another study of a group of normal male rabbits in our laboratory (21) are included in Tables 2 and 3.

TABLE 1 CONCENTRATION OF FREE FATTY ACIDS AND TRIGLYCERIDE FATTY ACIDS IN

| Rabbit No. | Sex | Free Fatty Acids: Whole Plasma† Fed | Day of Fast 1 | 2 | 3 |
|---|---|---|---|---|---|
| | | | | *μmole/ml* | |
| *Control* | | | | | |
| 1D | M | <0.05 | 0.43 | 0.10 | 0.41 |
| 2D | M | 0.20 | 0.33 | 0.30 | 0.56 |
| 3D | M | 0.07 | 0.73 | 0.48 | 0.62 |
| 4D | M | <0.05 | 0.46 | 0.33 | 0.44 |
| 5D | M | <0.05 | 0.73 | 0.51 | 0.38 |
| 6D | M | <0.05 | 0.60 | 0.33 | 0.52 |
| | | — | 0.55 ± 0.07 | 0.34 ± 0.06 | 0.49 ± 0.04 |
| *Short-term ethanol* | | | | | |
| 1B | M | 0.10 | 0.40 | 0.24 | 0.29 |
| 2B | M | 0.12 | 0.46 | 0.42 | 0.89 |
| 3B | M | 0.09 | 0.51 | 0.58 | 0.24 |
| 4B | M | 0.15 | 0.40 | 0.29 | 0.43 |
| 5B | M | 0.12 | 0.51 | 0.42 | 0.74 |
| | | 0.12 ± 0.007 | 0.46 ± 0.02 | 0.39 ± 0.06 | 0.52 ± 0.13 |

* Mean values ±SEM.
† Blood was taken in unanesthetized rabbits.
‡ Blood was taken about 1.5 hr after chloralose.
§ $P$ = <0.05
‖ $P$ = <0.01
¶ $P$ = >0.2

*Ethanol-Treated Animals*

As shown in Table 1, the concentrations of TGFA in whole plasma and in d < 1.006 lipoproteins were significantly higher in the ethanol-treated animals than in the control group on the 2nd and 3rd day of treatment. The concentration of FFA was similar in the two groups on each day. There was no significant difference in hepatic concentration of TGFA of the ethanol-treated animals as compared to the control group.

As shown in Table 2, the concentrations of TGFA and PLFA in the d < 1.006 lipoproteins were appreciably higher in four out of six (rabbits 1,3,4, and 5) of the other ethanol-treated animals than in the control animals. The plasma lipid concentrations were comparable in the rabbits treated with ethanol for long and short periods. The concentrations of TGFA in the whole liver and TGFA and PLFA in the subcellular fractions in the ethanol-treated rabbits were within the normal range in all but one rabbit (No. 2, Table 3).

*Ethionine-Treated Animals*

The concentrations of TGFA and PLFA in the d < 1.006 lipoproteins were within the normal range in three of the animals treated with ethionine but were below normal in the remaining three (Nos. 8, 10, and 11, Table 2). As shown in Table 3, the hepatic content of PLFA was normal in all animals in this group. The concentration of TGFA in the liver, however, was increased in all animals but one (No. 11). The increased concentration of TGFA in the liver was reflected in the floating fat fraction of the liver homogenates. The concentrations of TGFA and PLFA in the other subcellular fractions were unchanged.

*Hepatic Metabolism of Palmitate*

The percentage of injected radioactivity in the hepatic lipids determined 2 min after the injection of labeled palmitate is shown in Table 4. In general, the results obtained in the ethanol-treated and ethionine-treated groups did not differ appreciably from those in the control group, except that the hepatic floating fat contained approximately three times as much radioactivity in the ethionine-treated animals as that of the control and ethanol-treated animals.

Two minutes after the injection of labeled palmitate, the specific activity of hepatic TGFA was highest in the microsomes, fluffy layer, and mitochondria in the ethanol-treated and control groups (Table 5). In the rabbits treated with ethionine, the TGFA of the microsomes and fluffy layer had a higher specific activity than TGFA of the mitochondria. The specific activity of the floating fat fraction was low in all groups.

Serial samples of liver and blood were taken during a 3–4 hr period after injection of the isotope, and the specific activities of the hepatic and plasma TGFA were determined. In the control animals, the specific activities of TGFA in all hepatic subcellular fractions and in d < 1.006 lipoproteins were similar after 1 hr and remained so during the course of the experiment (Fig. 1). Similar

WHOLE PLASMA, PLASMA LIPOPROTEINS, AND LIVER OF TREATED AND CONTROL RABBITS*

| Triglyceride Fatty Acids | | | | | |
|---|---|---|---|---|---|
| Whole Plasma† | | | | d < 1.006 Lipoprotein‡ | Liver |
| | Day of Fast | | | | |
| Fed | 1 | 2 | 3 | 3 | 3 |
| | | *μmoles/ml* | | | *μmoles/g* |
| 1.58 | 2.16 | 3.24 | 3.38 | 1.24 | 30.3 |
| 3.00 | 3.53 | 4.60 | 5.99 | 3.13 | 79.0 |
| 4.30 | 4.15 | 8.68 | 12.60 | 6.68 | 131.0 |
| 1.18 | 3.20 | 5.86 | 6.30 | 3.93 | 46.6 |
| 2.06 | 2.68 | 3.48 | 4.01 | 1.35 | 38.0 |
| 1.40 | 1.76 | 3.10 | 2.59 | 1.21 | 74.7 |
| 2.25 ± 0.49 | 2.91 ± 0.37 | 4.84 ± 0.89 | 6.48 ± 1.49 | 2.92 ± 0.89 | 66.6 ± 15.1 |
| 4.70 | — | 11.80 | 27.93 | 23.21 | 150.0 |
| 4.22 | 5.56 | 17.01 | 16.41 | 10.90 | 78.8 |
| 2.86 | 1.86 | 11.62 | 19.80 | 14.01 | 206.2 |
| 1.91 | 2.63 | 6.55 | 11.33 | 7.52 | 37.1 |
| 2.57 | 2.16 | 5.70 | 11.92 | 8.33 | 81.0 |
| 3.24 ± 0.52 | 3.09 ± .97 | 10.53 ± 2.06§ | 17.48 ± 3.04‖ | 12.79 ± 2.85‖ | 110.6 ± 28.5¶ |

studies were performed on rabbits given long-term and short-term treatment with ethanol. As shown in Fig. 2, the specific activity of the TGFA remained considerably lower in the hepatic floating fat than in the other subcellular fractions in one rabbit treated with ethanol for a prolonged period (No. 2). In a second rabbit treated for a long period (No. 1) and in two rabbits treated for short periods (Nos. 4 and 5), the specific activities of TGFA in the floating fat and subcellular fractions were similar at the end of the experiment (Fig. 2). In all ethanol-treated rabbits, the specific activity of plasma TGFA in d < 1.006 lipoproteins reached a maximum 1–1.5 hr after the injection of labeled palmitate (Fig. 2).

The incorporation of radioactivity into the TGFA and PLFA of liver and plasma lipoproteins at intervals after the injection of labeled palmitate is shown in Table 6. A higher-than-normal percentage of the injected radioactivity appeared in the d < 1.006 lipoproteins of three of the ethanol-treated animals. This was accompanied by decreasing activity in total hepatic TGFA, while that of the floating fat changed little during the course of the experiments. In two of the rabbits in the ethionine group (Nos. 8 and 10), the results differed widely from those in the control and ethanol-treated animals. In these animals, the specific activities of the TGFA in all hepatic subcellular fractions including the floating fat were comparable and changed little during the course of the study (Fig. 3). The specific activity of TGFA in d < 1.006 lipoproteins was very low and reached equilibrium with the hepatic subcellular fractions in only one of the animals (No. 10, Fig. 3). The pattern observed in two other ethionine-treated rabbits was comparable to that obtained in the control animals, except for a delay in isotopic equilibration between the hepatic floating fat and the other subcellular fractions. The radioactivity in the TGFA of d < 1.006 lipoproteins was less in all ethionine-treated rabbits than in the control and ethanol-treated animals (Table 6).

## DISCUSSION

Ethanol, whether administered for 3 days or for as long as 1.5 yr, produced significant hypertriglyceridemia, but no consistent accumulation of hepatic fat. This dissociation has not been observed by other workers who administered ethanol to rats (2, 3, 6). Since rabbits have not been used by other workers, it is possible that this represents a special response of rabbits. The hepatic metabolism of TGFA in these animals did seem to differ from that of control animals (see below). The hypertriglyceridemia was associated with increased incorporation of radioactivity from plasma FFA into TGFA of d < 1.006 lipoproteins. The esterification of FFA undoubtedly occurred in the liver (21).

Rabbits fasted for 3 days were used as controls for this study. Carbohydrate could have been given to provide a caloric intake similar to that received by ethanol-treated animals. However, such treatment may have provided a

TABLE 2 VALUES FOR FREE FATTY ACIDS, TRIGLYCERIDE FATTY ACIDS, AND PHOSPHOLIPID FATTY ACIDS IN PLASMA LIPOPROTEINS OF TREATED AND CONTROL RABBITS

| Rabbit No. | Sex | Free Fatty Acids: Concentrations | Free Fatty Acids: Turnover Rate | Triglyceride Fatty Acids: d < 1.006 Lipoprotein | Triglyceride Fatty Acids: d > 1.006 Lipoprotein | Phospholipid Fatty Acids: d < 1.006 Lipoprotein | Phospholipid Fatty Acids: d > 1.006 Lipoprotein |
|---|---|---|---|---|---|---|---|
| | | *μmoles/ml* | *μmoles/min* | *μmoles/ml* | | *μmoles/ml* | |
| *Long-term ethanol** | | | | | | | |
| 1 | M | 1.47 | 52.8 | 14.76 | 1.60 | 2.65 | 1.50 |
| 2 | M | 1.10 | 39.7 | 4.42 | 1.06 | 1.05 | 1.40 |
| 3 | M | 1.60 | | 7.59 | 0.95 | 1.15 | 1.02 |
| *Short-term ethanol* | | | | | | | |
| 4 | M | 0.56 | 34.9 | 30.43 | 1.34 | 5.23 | 1.36 |
| 5 | M | 0.96 | 46.5 | 7.32 | 0.49 | 1.35 | 0.88 |
| 6 | M | 1.01 | | 3.84 | 1.42 | 0.80 | 2.10 |
| *Ethionine* | | | | | | | |
| 7 | M | 1.32 | | 3.21 | 1.02 | 0.71 | 1.11 |
| 8 | M | 1.60 | | 0.61 | 0.50 | 0.17 | 1.32 |
| 9 | M | 1.18 | | 1.27 | 0.83 | 0.40 | 2.95 |
| 10 | F | 2.79 | | 0.25 | 0.58 | 0.08 | 1.04 |
| 11 | F | 2.39 | | 0.54 | 0.79 | 0.22 | 2.39 |
| 12 | F | 1.17 | | 1.60 | 1.69 | 0.55 | 1.15 |
| *Control* | | | | | | | |
| 13 | M | 0.55 | 29.1 | 2.50 | 1.70 | 0.95 | 1.40 |
| 14 | M | 0.94 | | 1.44 | 0.87 | 0.24 | 0.92 |
| 15 | F | 0.52 | | 2.27 | 1.32 | 0.37 | 1.02 |
| Normal† | | | | 2.07 ± 0.71 | 1.17 ± 0.69 | | |

* Rabbits 1 and 3 received ethanol for 6 months; rabbit 2 received ethanol for 1.5 yr.
† Mean values ± SD for 11 normal male rabbits fasted 3 days, taken from Havel et al. (21).

TABLE 3 CONCENTRATIONS OF TRIGLYCERIDE FATTY ACIDS AND PHOSPHOLIPID FATTY ACIDS IN WHOLE LIVER AND SUBCELLULAR FRACTIONS OF TREATED AND CONTROL RABBITS

| Rabbit No. | Sex | Triglyceride Fatty Acids: Whole Liver | Floating Fat | Supernatant | Microsomes | Mitochondria | Fluffy Layer | Phospholipid Fatty Acids: Whole Liver | Supernatant | Microsomes | Mitochondria | Fluffy Layer |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *μmoles/g tissue* | | | | | | *μmoles/g tissue* | | | | |
| *Long-term ethanol* | | | | | | | | | | | | |
| 1 | M | 92.3 | 71.9 | 6.5 | 1.8 | 8.4 | 3.7 | 82.9 | 5.0 | 6.9 | 62.4 | 8.6 |
| 2 | M | 219.9 | 198.2 | 5.1 | 1.5 | 15.1* | | 56.5 | 2.3 | 3.8 | 50.4* | |
| 3 | M | 127.0 | 107.0 | 4.9 | 1.0 | 9.3 | 4.8 | 60.2 | 3.1 | 5.6 | 31.8 | 19.7 |
| *Short-term ethanol* | | | | | | | | | | | | |
| 4 | M | 52.1 | 31.9 | 6.2 | 1.2 | 10.3 | 2.5 | 93.7 | 2.9 | 7.8 | 67.4 | 15.6 |
| 5 | M | 115.9 | 93.9 | 5.5 | 1.5 | 11.7 | 3.3 | 96.9 | 4.4 | 7.5 | 68.2 | 16.8 |
| 6 | M | 95.8 | 83.5 | 2.1 | 0.9 | 7.6 | 1.7 | 85.0 | 2.6 | 7.1 | 63.6 | 11.7 |
| *Ethionine* | | | | | | | | | | | | |
| 7 | M | 206.3 | 186.8 | 1.8 | 0.8 | 12.4 | 4.5 | 66.8 | 1.3 | 4.7 | 43.9 | 16.9 |
| 8 | M | 229.2 | 212.1 | 3.8 | 2.7 | 8.5 | 2.1 | 58.6 | 2.0 | 7.8 | 38.6 | 10.2 |
| 9 | M | 187.6 | 173.0 | 1.7 | 1.4 | 6.9 | 4.6 | 64.6 | 3.6 | 8.1 | 34.0 | 18.9 |
| 10 | F | 323.8 | 297.6 | 2.2 | 2.3 | 15.6 | 6.1 | 82.9 | 2.8 | 6.9 | 48.9 | 24.3 |
| 11 | F | 133.9 | 110.4 | 2.2 | 1.8 | 12.7 | 6.8 | 76.0 | 1.4 | 6.8 | 50.4 | 17.4 |
| 12 | F | 374.3 | 350.2 | 5.7 | 1.0 | 14.4 | 3.0 | 56.8 | 3.2 | 5.3 | 36.7 | 11.6 |
| *Control* | | | | | | | | | | | | |
| 13 | M | 62.6 | 35.7 | 7.0 | 1.5 | 13.5 | 4.9 | 79.3 | 2.5 | 6.0 | 53.8 | 17.0 |
| 14 | M | 36.2 | 21.3 | 2.9 | 1.3 | 8.1 | 2.6 | 65.1 | 1.4 | 6.3 | 44.0 | 13.4 |
| 15 | F | 34.3 | 22.5 | 2.1 | 0.9 | 6.9 | 1.9 | 60.0 | 1.0 | 3.7 | 46.0 | 9.3 |
| Normal† | | 75 ± 37 | | | | | | 75.9 ± 10 | | | | |

* Including fluffy layer.
† Mean values ± SD for 14 normal male rabbits fasted for 3 days, taken from Havel et al. (21).

TABLE 4 Radioactivity in Whole Liver and Subcellular Fractions in Treated and Control Rabbits 2 Min after Intravenous Administration of Labeled Palmitate

| Rabbit No. | Sex | Dose Injected* | % of Radioactivity Administered: Whole Liver Lipid | Whole Liver TGFA | Floating Fat | Whole Liver PLFA |
|---|---|---|---|---|---|---|
| | | *cpm* | | | | |
| *Long-term ethanol* | | | | | | |
| 3 | M | $3.3 \times 10^6$ | 15 | 11 | 3 | 4 |
| *Short-term ethanol* | | | | | | |
| 6 | M | $3.3 \times 10^6$ | 20 | 15 | 3 | 5 |
| *Ethionine* | | | | | | |
| 9 | M | $2.5 \times 10^6$ | 16 | 14 | 9 | 2 |
| 12 | F | $1.9 \times 10^6$ | 21 | 19 | 11 | 2 |
| *Control* | | | | | | |
| 14 | M | $3.6 \times 10^6$ | 22 | 17 | 2 | 5 |
| 15 | F | $2.5 \times 10^6$ | 29 | 24 | 4 | 5 |
| Normal† | | | 21–39 | | | |

Abbreviations used: TGFA, triglyceride fatty acids; PLFA, phospholipid fatty acids.
* Corrected to $10^6$ cpm/kg body weight.
† Range in four normal male rabbits fasted 3 days (21).

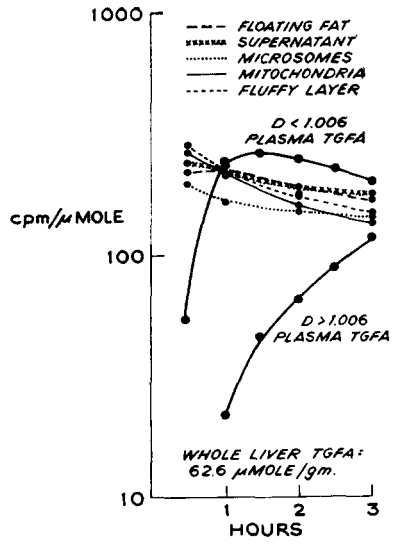


Fig. 1. Specific activities of TGFA of subcellular fractions of liver and plasma lipoproteins after intravenous injection of labeled palmitate in a fasted control rabbit (No. 13). The values were corrected to a dose of $10^6$ cpm/kg of body weight.

less suitable control, for it is well known that carbohydrate feeding has profound effects on insulin secretion, lipoprotein lipase activity, and mobilization of FFA which are different from those expected after ingestion of ethanol. If carbohydrate had been fed there would probably have been an even greater difference between the TGFA levels in plasma of ethanol-treated and carbohydrate-fed rabbits, since the studies of Havel et al. (21) have shown that the TGFA concentration in d < 1.006 lipoproteins is lower in refed than in fasted rabbits. A significant difference might have appeared in the hepatic TGFA concentration of the two groups under these circumstances, since refed rabbits have a lower and less variable concentration of hepatic TGFA than fasted animals (21).

Using the isotopic data and the flux of plasma FFA, we calculated the extent to which plasma TGFA in d < 1.006 lipoproteins were derived from circulating FFA.[1] The con-

[1] $\text{TGFA} = \frac{M}{D}\int_{t^0}^{t^\infty} \text{TGFA}'\, dt$, where $M$ is the turnover of plasma FFA in a steady state, TGFA′ is the quantity of radioactivity in TGFA at any time $t$, and $D$ is the dose of radioactive fatty acid. The integral expression is equal to the area under the curve obtained by plotting radioactivity in TGFA of d < 1.006 lipoproteins (cpm/ml) against time on a linear scale (27). Since a considerable amount of radioactivity remained in the TGFA of d < 1.006 lipoproteins at the end of the experiments, the curve used to obtain the area of the integral was extrapolated until 5% of the maximum activity was present.

TABLE 5 Specific Activities of Triglyceride Fatty Acids and Phospholipid Fatty Acids in Subcellular Fractions of Liver 2 Min after Intravenous Administration of Labeled Palmitate in Treated and Control Rabbits*

| Rabbit No. | Sex | Floating Fat TGFA | Supernatant TGFA | Microsomes TGFA | Microsomes PLFA | Mitochondria TGFA | Mitochondria PLFA | Fluffy Layer TGFA | Fluffy Layer PLFA |
|---|---|---|---|---|---|---|---|---|---|
| *Long-term ethanol* | | | | | | | | | |
| 3 | M | 14 | 98 | 275 | 45 | 219 | 33 | 250 | 24 |
| *Short-term ethanol* | | | | | | | | | |
| 6 | M | 15 | 133 | 414 | 15 | 418 | 11 | 447 | 18 |
| *Ethionine* | | | | | | | | | |
| 9 | M | 12 | 126 | 264 | 9 | 51 | 33 | 149 | 9 |
| 12 | F | 10 | 88 | 134 | 13 | 84 | 8 | 150 | 20 |
| *Control* | | | | | | | | | |
| 14 | M | 44 | 91 | 604 | 35 | 500 | 23 | 665 | 32 |
| 15 | F | 63 | 136 | 561 | 35 | 675 | 22 | 692 | 28 |

Abbreviations used: TGFA, triglyceride fatty acids; PLFA, phospholipid fatty acids.
* Specific activity expressed as cpm/μmole.

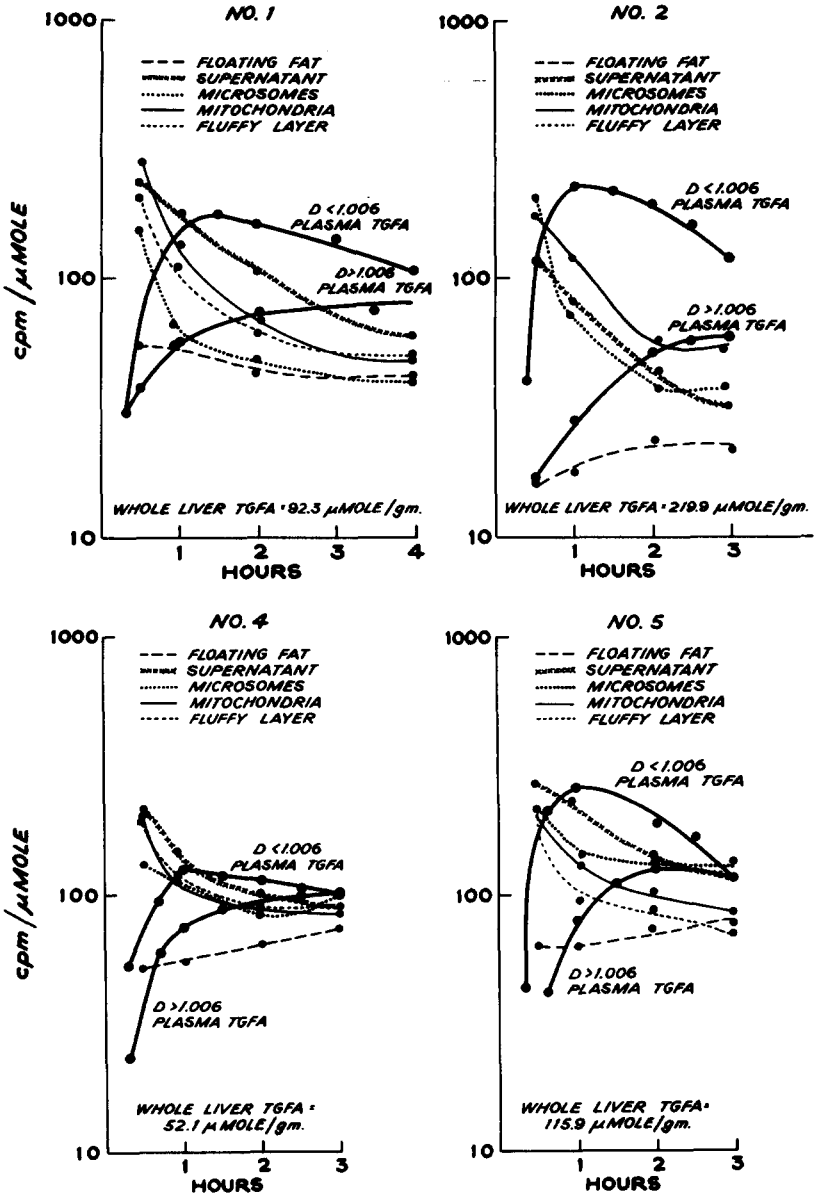


FIG. 2. Specific activities of TFGA of subcellular fractions of liver and plasma lipoproteins after intravenous injection of labeled palmitate in rabbits treated with ethanol for long periods (Nos. 1 and 2) and short periods (Nos. 4 and 5). Values corrected as in Fig. 1.

centration of TGFA in d < 1.006 lipoproteins calculated in this way agreed well with the observed values in the three ethanol-treated rabbits with marked hypertriglyceridemia. The calculated values were 16.41, 29.63, and 7.35 μmoles/ml respectively, and the observed values were 14.76, 30.43, and 7.32 μmoles/ml. In a fourth ethanol-treated rabbit, the observed concentration of TGFA in d < 1.006 lipoproteins was 4.42 μmoles/ml and the calculated value was 2.92 μmoles/ml. In a control rabbit, the calculated value was 2.20 μmoles/ml, and the observed value 2.50 μmoles/ml. The close agreement suggests that plasma FFA were the major precursors of TGFA in d < 1.006 lipoproteins. Thus, fatty acids newly synthesized in the liver were not the source of the hypertriglyceridemia in ethanol-treated animals.

The hypertriglyceridemia was not associated with preferential incorporation of FFA into hepatic TGFA rather than into PLFA, as found in rats given ethanol (8, 9). It should be pointed out that we did not measure the concentration of plasma FFA during the period of acute intoxication with ethanol. Since the hepatic concentration of TGFA was not significantly and consistently elevated,

TABLE 6 RADIOACTIVITY IN TRIGLYCERIDE FATTY ACIDS AND PHOSPHOLIPID FATTY ACIDS IN PLASMA LIPOPROTEINS AND LIVER OF TREATED AND CONTROL RABBITS AT INTERVALS AFTER THE INJECTION OF LABELED PALMITATE

| Rabbit No. | Sex | Dose Injected* | Time | % of Radioactivity Administered: TGFA d < 1.006 Lipoprotein | Whole Liver Lipid | Whole Liver TGFA | Floating Fat | Whole Liver PLFA |
|---|---|---|---|---|---|---|---|---|
| | | *cpm* | *min* | | | | | |
| *Long-term ethanol* | | | | | | | | |
| 1 | M | $2.6 \times 10^6$ | 30 | 5.3 | 28 | 21 | 9 | 7 |
| | | | 60 | 12 | 23 | 17 | 10 | 6 |
| | | | 90 | 14 | | | | |
| | | | 120 | 13 | 18 | 12 | 8 | 6 |
| | | | 180 | 11 | | | | |
| | | | 240 | 9 | 16 | 10 | 7 | 6 |
| 2 | M | $3.1 \times 10^6$ | 30 | 2.6 | 18 | 14 | 7 | 4 |
| | | | 60 | 5 | 14 | 10 | 7 | 4 |
| | | | 90 | 4.8 | | | | |
| | | | 120 | 4.6 | 13 | 10 | 10 | 3 |
| | | | 150 | 3.5 | | | | |
| | | | 180 | 2.5 | 16 | 12 | 8 | 4 |
| *Short-term ethanol* | | | | | | | | |
| 4 | M | $2.6 \times 10^6$ | 30 | 12 | 27 | 20 | 6 | 7 |
| | | | 60 | 19 | 20 | 14 | 6 | 6 |
| | | | 90 | 18 | | | | |
| | | | 120 | 18 | 20 | 13 | 7 | 7 |
| | | | 150 | 16 | | | | |
| | | | 180 | 15 | 22 | 14 | 8 | 8 |
| 5 | M | $2.4 \times 10^6$ | 30 | 4.2 | 35 | 28 | 15 | 7 |
| | | | 60 | 10 | 29 | 23 | 15 | 6 |
| | | | 90 | 9.3 | | | | |
| | | | 120 | 7.3 | 29 | 23 | 18 | 6 |
| | | | 150 | 6.9 | | | | |
| | | | 180 | 6 | 32 | 26 | 20 | 6 |
| *Ethionine* | | | | | | | | |
| 7 | M | $2.6 \times 10^6$ | 30 | 1.3 | 40 | 36 | 30 | 4 |
| | | | 60 | 2.2 | 41 | 38 | 33 | 3 |
| | | | 120 | 1.3 | 40 | 36 | 32 | 4 |
| | | | 180 | 1.1 | 31 | 28 | 25 | 3 |
| 8 | M | $2.1 \times 10^6$ | 30 | 0.02 | 43 | 41 | 37 | 2 |
| | | | 60 | 0.03 | 45 | 43 | 39 | 2 |
| | | | 90 | 0.04 | | | | |
| | | | 120 | 0.05 | 43 | 41 | 38 | 2 |
| | | | 150 | 0.05 | | | | |
| | | | 180 | 0.05 | 38 | 36 | 33 | 2 |
| 10 | F | $2.7 \times 10^6$ | 30 | 0.02 | 33 | 31 | 25 | 2 |
| | | | 60 | 0.01 | 28 | 25 | 23 | 3 |
| | | | 90 | 0.03 | | | | |
| | | | 120 | 0.02 | 30 | 28 | 25 | 2 |
| | | | 180 | 0.03 | | | | |
| | | | 240 | 0.05 | 33 | 30 | 28 | 3 |
| 11 | F | $2.5 \times 10^6$ | 30 | 0.29 | 27 | 23 | 16 | 4 |
| | | | 60 | 0.37 | 23 | 19 | 13 | 4 |
| | | | 120 | 0.26 | 27 | 23 | 17 | 4 |
| | | | 150 | 0.26 | | | | |
| | | | 180 | 0.24 | 30 | 26 | 20 | 4 |
| *Control* | | | | | | | | |
| 13 | M | $2.4 \times 10^6$ | 30 | 0.92 | 58 | 54 | 29 | 4 |
| | | | 60 | 4.0 | 50 | 45 | 29 | 5 |
| | | | 90 | 4.5 | | | | |
| | | | 120 | 4.4 | 44 | 40 | 26 | 4 |
| | | | 150 | 4.4 | | | | |
| | | | 180 | 4.1 | 39 | 35 | 22 | 4 |

Abbreviations used: TGFA, triglyceride fatty acids; PLFA, phospholipid fatty acids.
* Corrected to a dose of $10^6$ cpm/kg body weight.

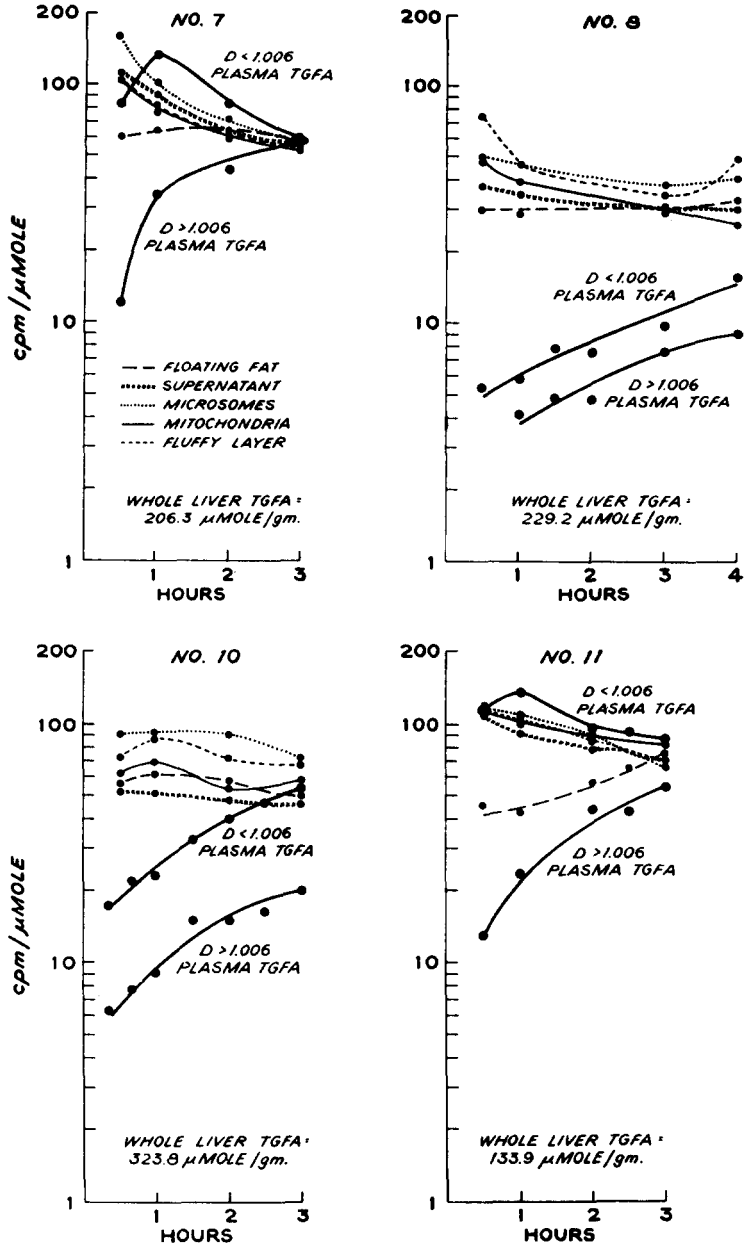


FIG. 3. Specific activities of TGFA of subcellular fractions of liver and plasma lipoproteins after intravenous injection of labeled palmitate in four rabbits treated with ethionine (Nos. 7, 8, 10, and 11). Values corrected as in Fig. 1.

it is unlikely that the hypertriglyceridemia was related to increased influx of FFA into the liver, since the results of many studies suggest that prolonged, increased mobilization of fat from adipose tissue generally results in accumulation of TGFA in the liver (28–32). Our data provide no information concerning the possibilities that the hypertriglyceridemia in the ethanol-treated rabbits might be related to impaired hepatic catabolism of fatty acids or impaired removal of TGFA from the blood. When compared with results obtained in untreated rabbits (No. 13) (21), there was a delay in isotopic equilibration between the hepatic floating fat and the other subcellular fractions of the liver in three of the four ethanol-treated animals (Fig. 2). In two of these animals the level of hepatic TGFA was in the normal range for fasted animals. On the basis of these results, it can be speculated that a large portion of the hepatic TGFA derived from plasma FFA may pass from the liver to the plasma of ethanol-treated animals without first traversing the floating fat. Should this be the case, a greater-than-normal fraction of this

hepatic TGFA might be delivered into d < 1.006 lipoproteins. This could account for the development of hypertriglyceridemia without an elevation in the concentration of hepatic TGFA. One might also consider the possibility that under certain conditions the hepatic floating fat is composed of more than one pool with different turnover rates.

Whereas ethanol produced hypertriglyceridemia without consistent elevation in hepatic levels of TGFA, ethionine resulted in hypotriglyceridemia in three animals, associated with increased concentrations of hepatic TGFA. In two of the ethionine-treated group, the specific activity of the TGFA in d < 1.006 lipoproteins did not reach a maximum within 1 hr of the injection of the isotope, as it did in the control and ethanol-treated groups, but continued to rise slowly over a 3–4 hr period. Radioactivity in TGFA of d < 1.006 lipoproteins was also decreased, and the specific activity of the hepatic TGFA in the subcellular fractions remained constant.

The variability in the effect of ethionine in these studies may be related in part to differences in the response of the rabbits to this agent, to differences in the degree of ethionine intoxication present when the animals were studied, or both. On the whole, the animals with the lowest level of TGFA in d < 1.006 lipoproteins and the highest concentration of TGFA in the liver showed the clearest impairment in transfer of radioactivity from hepatic TGFA to TGFA of d < 1.006 lipoproteins. These results are compatible with the results obtained by Olivecrona (33) in rats and with the conclusion of Robinson and Harris (18) that ethionine results in decreased synthesis of lipoprotein–protein and consequent decrease in the release of lipids from the liver.

This work was supported by PHS Research Grants HE-06285 and H-2554 from the National Institutes of Health, U. S. Public Health Service.

*Manuscript received 4 August 1965; accepted 19 November 1965.*

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