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Metabolism of Palmitate in Perfused Rat Liver

EFFECT OF ETHANOL IN LIVERS FROM RATS FED ON A HIGH-FAT DIET WITH OR WITHOUT ETHANOL

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1. Rats were treated for 4 weeks with liquid diets that contained, on the basis of energy content, 35% fat, 18% protein and 47% carbohydrate (high-fat diet) or 35% fat, 18% protein, 11% carbohydrate and 36% ethanol (high-fat/ethanol diet). 2. The livers were perfused with 1 mM-[1-^{14}C]palmitate and with 0, 10 mM- or 80 mM-ethanol. The oxidation and esterification of palmitate was measured. Two subcellular pools of triacylglycerol were separated; one contained triacylglycerol from cytoplasmic lipid droplets and the other contained triacylglycerol from the endoplasmic reticulum and Golgi apparatus. 3. In the presence of ethanol, liver from rats fed on the high-fat diet esterified about 70% of the [1-^{14}C]palmitate taken up compared with 90% in liver from rats fed chow (containing 11% fat on the basis of energy content). Compared with chow diet the high-fat diet did not potentiate the effect of ethanol on storage of [1-^{14}C]palmitate in hepatic triacylglycerol. The relation between the fat content of the diet and the degree of fatty liver induced by by ethanol [Lieber & DeCarli (1970) *Am. J. Clin. Nutr.* **23**, 474–478] is discussed. 4. The ethanol-containing diet increased the hepatic content of triacylglycerol 4-fold and the increase was exclusively found in the fraction suggested to contain lipid from cytoplasmic lipid droplets. The ethanol-induced fatty liver, perfused with ethanol, esterified and oxidized palmitate at rates that were quite similar to the rates found in high-fat control livers perfused without ethanol. This suggests that the fatty liver had adapted to the presence of ethanol with respect to palmitate metabolism. 5. O_2 and ethanol uptake by the livers were not affected by the ethanol-containing diet.

Prolonged ethanol feeding causes triacylglycerol to accumulate in the liver and the fat content of the diet appears to be important for the degree of fatty liver induced. When ethanol, providing 30% of the energy, was given to rats in their drinking water, as a supplement to the ordinary chow diet, the triacylglycerol content of the liver was doubled after 35 days (Dobbins *et al.*, 1972). A doubling of hepatic triacylglycerol was also found after feeding rats the same amount of ethanol in a liquid diet containing approximately the same amount of fat as does chow; however, when the fat content of the diet was increased from 10% to 35–40% on the basis of energy content, ethanol induced a 4–5-fold increase in hepatic triacylglycerol (Lieber & DeCarli, 1970; Savolainen *et al.*, 1977). The purpose of the present study was to investigate whether the deposition of [1-^{14}C]palmitate in triacylglycerol in cytoplasmic lipid droplets in perfused liver from rats fed a high-fat liquid diet is more markedly affected by ethanol than it was in liver from chow-fed rats (Kondrup *et al.*, 1979*b*).

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To determine whether changes in the hepatic fatty acid metabolism can explain why the fatty liver ceases to progress (Lieber & DeCarli, 1970), [1-^{14}C]-palmitate metabolism in rats fed on ethanol for 4 weeks was also investigated. The effect of ethanol on the metabolism of 1 mM-[1-^{14}C]palmitate was investigated at concentrations of 10 mM- and 80 mM-ethanol, which cover the range of blood ethanol concentration in rats given 30% ethanol on the basis of energy content (Dobbins *et al.*, 1972).

Methods

Female Wistar rats were fed the liquid diets devised by Lieber & DeCarli (1970), as commercially available from Bio-Serv, Frenchtown, NJ, U.S.A. The rats (not litter-mates) weighed 125–130 g when switched to the diet. To prevent an initial weight loss in the ethanol group all rats were fed the control diet for 3 days and then, during 6 days, ethanol was gradually introduced into the control diet given to the ethanol group; then the proper ethanol diet was given for 19–21 days. When switched to the proper ethanol diet the ethanol-treated rats weighed 138 ± 8 g (mean ± S.D., $n = 15$) compared with 137 ± 5 g (mean ± S.D., $n = 15$) in the control group at the

same time. The rats were kept in individual wire-bottom cages and the control rats were pair-fed as devised by Lieber & DeCarli (1970) to ensure that the two groups received the same amount of energy. The average consumption was about 160kJ/rat per day, which in the ethanol-fed rats corresponded to an intake of about 14g of ethanol/day per kg of rat. The composition of the liquid diets is shown in Table 1. The carbohydrate was dextrin/maltose, the protein was casein and the fat was 21wt.-% of corn oil, 72wt.-% of olive oil and 7wt.-% of ethyl linoleate. The rats were fed every day at 15:00h and weighed twice a week. The food was withdrawn at 07:00h on the day of experiment and perfusions were started at about 13:00h. Of 20 ethanol-fed rats, 16 rats markedly hyper-reacted to handling. The rats were given 20mg of sodium pentothal by intraperitoneal injection, but to suppress the toe-pad reflex, ten of the ethanol-treated rats had to be given 30–50% higher doses of the anaesthetic. Blood ethanol concentrations in samples of caval blood from the ethanol-treated rats before perfusion ranged from 0.1 to 0.3mM. All other methods have been described elsewhere (Kondrup, 1979; Kondrup *et al.* 1979*b*). The flow was kept near to 3ml/min per 100g body wt. by the use of a roller pump. The mean flow for all experiments was 4.96 ± 0.27ml/min per liver (mean ± S.D.). The maximum difference between the mean flow of each group was 7%.

Results

Table 1 shows some effects of the liquid diets. The data from rats given the normal chow diet are also shown for comparison. The growth rates were calculated from the slopes of linear regression analysis of the weights, excluding the period when ethanol was gradually introduced. The rats given the high-fat diet grew at nearly the same mean rate as rats fed on chow and kept singly in wire-bottom cages. The ethanol-fed rats gained weight at a 30% lower rate, which is in accordance with the original

Table 1. *Comparison of dietary treatments*

The rats were pair-fed on the liquid diets for 28 days as described in the text. The composition of the diets is expressed as a percentage on the basis of energy content. The results are expressed as means ± S.D.

Dietary treatment	Chow	Liquid diet	
Dietary composition (%)			
Fat	11	35	35
Carbohydrate	73	47	11
Protein	16	18	18
Ethanol			36
Number of experiments	6	5	5
Growth rate (g/day)	1.66 ± 0.78*	1.59 ± 0.28†	1.09 ± 0.38† §§
Body weight when perfused (g)‡	168 ± 4	166 ± 7	156 ± 10
Perfusion pressure (cm of water)‡	7 ± 1	6 ± 2	6 ± 1
Liver weight after perfusion (g)‡	6.75 ± 0.64	5.89 ± 0.48§	5.75 ± 0.54‖
Liver weight (g/100g body wt.)‡	4.01 ± 0.42	3.56 ± 0.30	3.69 ± 0.27
O_2 uptake			
(μmol/min per g wet wt.)‡	2.83 ± 0.20	2.84 ± 0.60	2.84 ± 0.30
(μmol/min per 100g body wt.)‡	10.98 ± 0.84	10.16 ± 2.51	10.46 ± 2.51
Ethanol elimination			
10mM-Ethanol treatment (μmol/min per g wet wt.)	1.93 ± 0.42	1.89 ± 0.24	1.52 ± 0.26‖‖
10mM-Ethanol treatment (μmol/min per 100g body wt.)	8.06 ± 1.96	6.31 ± 0.75	5.66 ± 1.11§
80mM-Ethanol treatment (μmol/min per g wet wt.)	3.07 ± 0.36‡‡	2.40 ± 0.60	2.36 ± 0.86††
80mM-Ethanol treatment (μmol/min per 100g body wt.)	13.74 ± 2.02‡‡	8.31 ± 2.23**	8.79 ± 2.62¶ ††

* $n = 12$.
† $n = 15$.
‡ Perfusion without ethanol.
§ $P < 0.05$, compared with value for rats fed on chow.
‖ $P < 0.02$, compared with value for rats fed on chow.
¶ $P < 0.01$, compared with value for rats fed on chow.
** $P < 0.005$, compared with value for rats fed on chow.
†† $P < 0.05$, compared with value for perfusion of the same group with 10mM-ethanol.
‡‡ $P < 0.001$, compared with value for perfusion of the same group with 10mM-ethanol.
§§ $P < 0.001$, compared with value for rats fed on the high-fat diet.
‖‖ $P < 0.05$, compared with value for rats fed on the high-fat diet.

observation of Lieber *et al.* (1965). The perfusion pressure was not affected by the diets. The weights of the livers after perfusion from rats fed on the liquid diets were lower than those of livers from rats fed on ordinary chow. Ethanol feeding did not affect the liver weight. The O_2 uptake in the presence of 1 mM-palmitate was not affected by the diets. The rate of ethanol oxidation was lower after treatment with the liquid diets. In all groups the rate of ethanol elimination was higher at 80 mM, although the difference was not statistically significant in the group fed on the high-fat diet ($P<0.1$). Prolonged ethanol feeding did not increase the rate of ethanol elimination.

Table 2 shows the chemically estimated contents of neutral acylglycerol (equivalent to alkali-labile glycerol in acylglycerol) and phospholipid (equivalent to lipid P_i) in the subcellular fractions. The ethanol diet had no effect on the lipid content of the low-speed pellet, but increased the content of phospholipid and neutral acylglycerol in the two other fractions. The ethanol diet caused an almost 4-fold increase in the mean content of acylglycerol in the diethyl ether fraction. However, the effect was highly

Table 2. *Contents of glycerolipids in the subcellular fractions isolated from perfused rat liver*
The subcellular fractions were isolated as described by Kondrup (1979). The results from all animals in each dietary group are shown. The results are means ± S.D. ($n = 15$).

Subcellular fraction	Diet	Content (μmol/g wet wt.) Acylglycerol	Phospholipid
Low-speed pellet	High-fat	1.36 ± 0.39	10.90 ± 2.77
	High-fat+ethanol	1.56 ± 0.57	10.70 ± 1.91
High-speed pellet	High-fat	4.47 ± 0.68	32.86 ± 4.05
	High-fat+ethanol	5.48 ± 1.09*	41.45 ± 5.18†
Diethyl ether	High-fat	10.66 ± 4.60	0.40 ± 0.10
	High-fat+ethanol	40.19 ± 22.59*	0.82 ± 0.29†

* $P<0.01$, compared with value for high-fat group.
† $P<0.001$, compared with value for high-fat group.

Table 3. *Uptake, esterification and oxidation of* 1 *m*M-[1-^{14}*C*]*palmitate in perfused liver*
The radioactive label was introduced after a 26 min equilibration period and its metabolism was followed for 21 min. See Kondrup (1979) for experimental details. The results are means ± S.D. for five experiments in each group.

	Diet	Rate (μmol/min per liver) Without ethanol	10 mM-Ethanol	80 mM-Ethanol
Uptake of [1-^{14}C]palmitate	High-fat	3.57 ± 0.47	3.58 ± 0.16	3.51 ± 0.23
	High-fat+ethanol	3.18 ± 0.40	3.11 ± 0.62	3.44 ± 0.20
Total esterification of [1-^{14}C]-palmitate	High-fat	2.08 ± 0.38	2.49 ± 0.26	2.08 ± 0.26
	High-fat+ethanol	1.28 ± 0.27*	1.94 ± 0.21‖	2.15 ± 0.14¶
Total oxidation of [1-^{14}C]palmitate	High-fat	1.65 ± 0.35	1.12 ± 0.13§	1.17 ± 0.17§
	High-fat+ethanol	1.82 ± 0.26	1.26 ± 0.18‖	1.21 ± 0.11¶
Water-soluble products in the liver	High-fat	0.194 ± 0.009	0.142 ± 0.035‡	0.124 ± 0.018§
	High-fat+ethanol	0.166 ± 0.034	0.157 ± 0.024	0.157 ± 0.031
Water-soluble products in the perfusate	High-fat	1.388 ± 0.386	0.961 ± 0.055	1.000 ± 0.164
	High-fat+ethanol	1.560 ± 0.204	1.077 ± 0.161‖	1.030 ± 0.114¶
CO_2 in the perfusate	High-fat	0.114 ± 0.032	0.031 ± 0.010¶	0.031 ± 0.010¶
	High-fat+ethanol	0.089 ± 0.029	0.029 ± 0.006¶	0.032 ± 0.005¶

* $P<0.005$, compared with value for rats fed the high-fat diet.
† $P<0.05$, compared with value for perfusion without ethanol.
‡ $P<0.01$, compared with value for perfusion without ethanol.
§ $P<0.02$, compared with value for perfusion without ethanol.
‖ $P<0.005$, compared with value for perfusion without ethanol.
¶ $P<0.001$, compared with value for perfusion without ethanol.

Table 4. *Incorporation of [1-^{14}C]palmitate into lipid classes in subcellular fractions isolated from perfused rat liver*
The experiments are the same as those presented in Table 3. Results are means ± S.D. for five experiments in each group.

			Esterification (nmol/min per liver)		
Subcellular fraction	Lipid class	Diet	Without ethanol	10mM-Ethanol	80mM-Ethanol
High-speed pellet	Phospholipid	High-fat	332±72	373±25	293±34
		High-fat+ethanol	256±74	328±70	301±70
	Diacylglycerol	High-fat	83±17	116±12‖	91±25
		High-fat+ethanol	62±18	85±12‡	110±43‡
	Triacylglycerol	High-fat	742±195	736±247	640±105
		High-fat+ethanol	337±35†	486±200	670±140¶
Diethyl ether	Phospholipid	High-fat	5±1	6±3	6±1
		High-fat+ethanol	5±3	7±1	6±2
	Diacylglycerol	High-fat	20±4	35±14‡	31±14
		High-fat+ethanol	24±13	42±10‡	48±13‡
	Triacylglycerol	High-fat	584±91	722±121‡	618±91
		High-fat+ethanol	390±172*	648±227‡	657±71§

* $P<0.05$, compared with value for rats fed on the high-fat diet.
† $P<0.001$, compared with value for rats fed on the high-fat diet.
‡ $P<0.05$, compared with value for rats perfused without ethanol.
§ $P<0.02$, compared with value for rats perfused without ethanol.
‖ $P<0.01$, compared with value for rats perfused without ethanol.
¶ $P<0.001$, compared with value for rats perfused without ethanol.

Table 5. *Secretion of triacylglycerol into the perfusate*
The experiments are the same as those presented in Table 3. The results are means ± S.D. for five experiments in each group.

	Triacylglycerol (nmol/min per liver)		
Diet	Without ethanol	10-mM-Ethanol	80mM-Ethanol
High-fat	137±41	225±88	144±44
High-fat+ethanol	129±48	147±38	186±52

variable: in all ethanol-treated rats the range was 12–100μmol/g wet wt. By t.l.c. it was found that triacylglycerol accounted for about 95% of total acylglycerol in the diethyl ether fraction.

The rates of uptake, esterification and oxidation of [1-^{14}C]palmitate are shown in Table 3. The recovery of labelled palmitate taken up was 100±7% (mean ± S.D. for all experiments). In the high-fat group perfused with 80mM-ethanol the recovery was only 93±1% (mean ± S.E.M.).

Total esterification was measured as incorporation of [1-^{14}C]palmitate into total lipids in all subcellular fractions; 90% was recovered as esters after t.l.c., with no difference between the groups. In the high-fat group 10mM-ethanol failed to increase the total esterification statistically significantly and 80mM-ethanol apparently had no effect at all. Since the uptake and total oxidation of [1-^{14}C]palmitate were similar at the two ethanol concentrations the radioactivity not recovered in the 80mM group may have been associated with an ester that was lost in our extraction procedure. In the fatty liver the esterification in the absence of ethanol was lower than in the high-fat group. With ethanol present the esterification increased to a rate that was comparable with the rate found in the high-fat group perfused without ethanol.

Total oxidation is the sum of production of radioactively labelled water-soluble products in the liver and in the perfusate and of CO_2 in the perfusate. Irrespective of the ethanol concentration, or the dietary treatment, ethanol decreased the total oxidation to the same degree. CO_2 production was decreased by about 60% and the production of water-soluble products was decreased by about 30%. Table 4 shows the rates of incorporation of palmitate into individual lipid classes in the two major subcellular fractions. Perfusion with ethanol had no significant effect on the incorporation into phospholipid in either dietary group. Small effects were seen on the incorporation into diacylglycerol.

The incorporation of [1-^{14}C]palmitate into triacylglycerol in the high-speed pellet in the high-fat group was not affected by ethanol, whereas incorporation into triacylglycerol in the diethyl ether fraction was slightly increased by 10mM-ethanol. In the fatty liver ethanol increased the incorporation into triacylglycerol in both subcellular fractions.

In the high-fat group, 10mM- and 80mM-ethanol increased the total incorporation into phospholipid

Table 6. *Contents and relative specific radioactivity of di- and tri-acylglycerol in the high-speed pellet*
The experiments are the same as those presented in Table 3. See Kondrup (1979) for experimental details. The relative specific radioactivity (r.s.a.) of fatty acids in di- and tri-acylglycerol is expressed relative to the specific radioactivity of the perfused [1-^{14}C]palmitate (=100). The results are means ± S.D. for five experiments in each group.

Diet	[Ethanol] (mM)	Diacylglycerol		Triacylglycerol	
		(μmol/liver)	(r.s.a.)	(μmol/liver)	(r.s.a.)
High-fat	0	4.73 ± 0.63	19 ± 6	17.90 ± 1.91	26 ± 5
	10	4.27 ± 0.46	30 ± 4¶	13.43 ± 2.81§	39 ± 11†
	80	4.30 ± 0.81	24 ± 6	15.02 ± 2.24	30 ± 3
High-fat+ethanol	0	4.64 ± 1.29	15 ± 6	12.30 ± 2.46*	18 ± 3†
	10	4.25 ± 1.05	22 ± 6	11.97 ± 2.23	23 ± 4‡
	80	4.25 ± 0.53	26 ± 7‡	20.07 ± 3.86‖	23 ± 6

* $P<0.005$, compared with value for rats fed on the high-fat diet.
† $P<0.05$, compared with value for rats fed on the high-fat diet.
‡ $P<0.05$, compared with value for perfusion without ethanol.
§ $P<0.02$, compared with value for perfusion without ethanol.
‖ $P<0.01$, compared with value for perfusion without ethanol.
¶ $P<0.001$, compared with value for perfusion without ethanol.

di- and tri-acylglycerol in the low-speed pellet [control, 111 ± 16 nmol/min per liver (mean ± S.D., $n = 5$); 10 mM-ethanol treatment, 268 ± 55 nmol/min per liver (mean ± S.D., $n = 5$; value significantly different from control at $P<0.001$); 80 mM-ethanol treatment, 195 ± 47 nmol/min per liver (mean ± S.D.; $n = 5$; value significantly different from control at $P<0.01$)].

Table 5 shows that the secretion of triacylglycerol was not affected by the ethanol diet and addition of ethanol to the perfusion medium had no statistically significant effect.

Table 6 shows the contents and relative specific radioactivities of di- and tri-acylglycerol in the high-speed pellet. In the high-fat groups di- and tri-acylglycerol accounted for about 80% of total acylglycerol, as a mean (cf. Table 2), whereas in the groups with fatty liver these compounds accounted for only 60%. In monoacylglycerol only negligible radioactivity was found and no attempt was made to characterize the remainder of acylglycerol. The content of triacylglycerol in the fatty liver was similar to that in the high-fat group. Perfusion with ethanol increased the relative specific radioactivity of the acylglycerols in all groups, although the difference was not always statistically significant.

Discussion

Palmitate metabolism in liver from rats fed on a high-fat diet

The uptake of palmitate at 1 mM in liver from rats fed on the high-fat liquid diet was similar to the uptake in liver from rats fed on chow (Kondrup *et al.*, 1979*b*). However, in the high-fat group total esterification was less and total oxidation was larger than in liver from chow-fed rats ($P<0.01$ and $P<0.001$ respectively). Incorporation of [1-^{14}C]palmitate into phospholipid and triacylglycerol in the high-speed pellet was about the same in the high-fat group as in the chow-fed group. Less esterification was seen in diacylglycerol in the high-speed pellet and in ether triacylglycerol ($P<0.01$ and $P<0.01$ respectively). The secretion of triacylglycerol was increased more than 3-fold in the high-fat group compared with the chow-fed group ($P<0.001$). These results suggest that a high-fat diet increases the β-oxidation and leads fatty acids esterified in the endoplasmic reticulum to be secreted rather than deposited in the cytoplasmic lipid droplets. After a high-fat dietary treatment Fellenius & Kiessling (1973) found the perfused liver to have a markedly increased ketogenesis, estimated chemically.

In the high-fat group perfusion with 10 mM-ethanol increased the secretion of triacylglycerol about 60%, although the effect was of low statistical significance ($P<0.08$, Table 5). Diacylglycerol in the high-speed pellet was proposed as the precursor for triacylglycerol in the cytoplasmic lipid droplets (Kondrup *et al.*, 1979*a*); since 10 mM-ethanol increased the relative specific radioactivity of diacylglycerol in the high-speed pellet relatively more than it increased the incorporation of [1-^{14}C]palmitate into triacylglycerol in the diethyl ether fraction (Tables 4 and 6), it is likely that ethanol did not increase the deposition of fatty acids in the cytoplasmic lipid droplets. The decreased oxidation of [1-^{14}C]palmitate in the presence of ethanol may reflect decreased β-oxidation or increased dilution of the radioactive isotope with endogenous fatty acids, as discussed in the preceding paper (Kondrup *et al.*, 1979*b*).

Induction of fatty liver

From our results, ethanol *in vitro* has only small effects on the incorporation of [1-^{14}C]palmitate into triacylglycerol in cytoplasmic lipid droplets. However, ethanol increased the incorporation of [1-^{14}C]-palmitate into other glycerolipids from which fatty acids may be transferred to the cytoplasmic lipid droplets at later time points. The decreased oxidation of [1-^{14}C]palmitate cannot be taken to reflect a similar decrease in β-oxidation and therefore the observed effect of ethanol on total oxidation and total esterification of [1-^{14}C]palmitate may be looked on as maximum possible effects of ethanol on oxidation and deposition of palmitate in the isolated liver. As an average for the chow-fed and high-fat-fed groups perfused with 1 mM-palmitate, ethanol increased the fraction of the [1-^{14}C]palmitate uptake that was esterified by 20%. A minimum effect of ethanol can be calculated on the assumptions that β-oxidation was unaffected by ethanol, that the relatively larger decrease in $^{14}CO_2$ production (compared with the decrease in appearance of water-soluble products in the perfusate) was due to inhibition of the tricarboxylic acid cycle and that the amount of [1-^{14}C]palmitate not oxidized in the tricarboxylic acid cycle was stored in the liver. This would increase the fraction of the [1-^{14}C]palmitate uptake that was stored by 1–2%. Even such a small effect of ethanol could contribute to the formation of fatty liver in experiments with prolonged ethanol feeding.

In addition, the dietary fat content determines the degree of fatty liver induced (see the introduction). This may be due to an increased hepatic uptake of dietary fatty acids caused by the increased fat content of the diet. An increased fatty acid uptake is indirectly suggested by the finding that, when perfused with the same concentrations of [1-^{14}C]-palmitate and ethanol, the liver from rats given the high-fat diet disposed of fatty acids by oxidation and triacylglycerol secretion at rates that were severalfold higher than those of liver from chow-fed rats. If, *in vivo*, the hepatic fatty acid uptake were the same in rats given ethanol in a high-fat diet as in rats given ethanol in a low-fat diet, an inverse relationship between the dietary fat content and the degree of fatty liver would be expected.

However, an increased hepatic uptake of dietary fat cannot alone be responsible for the increased accumulation of triacylglycerol. The higher fat content of the fat diet would be expected to increase the hepatic fatty acid uptake similarly whether or not ethanol was given in addition. But in the high-fat control group the content of triacylglycerol in the diethyl ether fraction was only doubled, compared with chow-fed controls, whereas in the group given ethanol in addition, the content of triacylglycerol in the diethyl ether fraction was increased 7-fold, compared with chow-fed controls. An increased hepatic fatty acid uptake caused by the higher fat content of the diet, and an increased fractional esterification of the fatty acids taken up by the liver caused by ethanol could explain the fact that the excess absolute amount of dietary fatty acids deposited in the liver increases with an increased fat content of the diet.

The rats given the high-fat diets consumed about 50 mmol of triacylglycerol during the feeding period. The amount of triacylglycerol that accumulated in the liver of rats fed ethanol in addition accounted for 0.33% of the amount consumed. During the same period of time rats given a diet with 10% fat on the basis of energy content would consume (50/3.5) = about 15 mmol of triacylglycerol. It would then be expected that rats given ethanol in this low-fat diet would accumulate (15.00 × 0.33%) = about 50 μmol of triacylglycerol/liver. Our chow-fed rats had a content of 58 μmol of triacylglycerol/liver and therefore ethanol feeding would be expected to double the content of hepatic triacylglycerol in rats given the low-fat diet. This is the effect that has been found experimentally by other workers (see the introduction). This suggests that the fat content of the diet plays no other role for the formation of fatty liver than by determining the amount of fat on which ethanol acts by increasing the fractional deposition in the liver. The increased fractional deposition in the liver of dietary fat may be exerted by the increase in the fractional esterification of the hepatic fatty acid uptake, but the indirect effect of ethanol discussed in the preceding paper (Kondrup *et al.*, 1979*b*) may contribute in addition. However, it is not likely that increased peripheral lipolysis plays a significant role for the formation of fatty liver during prolonged ethanol treatment, since the composition of fatty acids accumulated in hepatic triacylglycerol resembles dietary fat much more than the fat in adipose tissue (Lieber *et al.*, 1966) and since the plasma concentration of non-esterified fatty acids is not increased by prolonged ethanol treatment (Savolainen *et al.*, 1977).

Palmitate metabolism in the fatty liver

Ethanol feeding caused triacylglycerol to accumulate exclusively in the diethyl ether fraction, which was proposed to contain lipid from the cytoplasmic lipid droplets (Tables 2 and 6). Ethanol also increased the phospholipid content of the high-speed pellet, which probably reflects the increase in microsomal phospholipid reported by Ishii *et al.* (1973).

In the fatty liver, perfused with 10 mM- or 80 mM-ethanol, the rates of oxidation and esterification of [1-^{14}C]palmitate were quite similar to those in the high-fat group perfused without ethanol (Table 3). This is a relevant comparison for the situation *in vivo* when the fatty liver has been formed. Also, the

incorporation of [1-^{14}C]palmitate into lipids in the subcellular fractions was similar in the three groups (Table 4). This similarity in initial esterification of [1-^{14}C]palmitate may be a contributory cause to the cessation of triacylglycerol accumulation observed after 4 weeks of ethanol treatment (Lieber & DeCarli, 1970).

In the fatty liver perfused without ethanol total oxidation of palmitate tended to be higher and total esterification was lower than in the high-fat group. Incorporation of [1-^{14}C]palmitate into triacylglycerol in both subcellular fractions was lower. This probably reflects decreased triacylglycerol synthesis, since the relative specific radioactivity of diacylglycerol in the high-speed pellet was about the same in the two groups. Therefore, the increase in glycerophosphate acyltransferase found after prolonged ethanol treatment (Joly *et al.*, 1973) does not correlate with an increased synthesis of triacylglycerol. β-Oxidation was increased in mitochondria isolated from ethanol-induced fatty liver (Cederbaum *et al.*, 1975), which is in accordance with our finding of an increased total oxidation. The increased rate of oxidation of palmitate in fatty liver compared with non-fatty liver from rats fed on the high-fat diet may help to explain how the fatty livers recover within a few days after removal of ethanol from the diet (Joly *et al.*, 1973; Gordon, 1973).

Baraona *et al.* (1973) investigated lipoprotein production in intact rats after a similar prolonged ethanol regime and proposed that the liver from ethanol-treated rats had an increased capacity for lipoprotein formation. In the isolated liver secretion of triacylglycerol was unaltered (Table 5).

The rate of ethanol elimination in the isolated liver was not increased by prolonged ethanol feeding (Table 1). This is in contrast with experiments with isolated hepatocytes from ethanol-treated rats (Cederbaum *et al.*, 1978). The rate of ethanol elimination in our perfused fatty liver was quite similar to the reported rate in isolated hepatocytes from fatty liver. In the control hepatocytes the elimination rate was only half the value observed in our control livers. No explanation can be offered for this discrepancy.

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