Metabolism of Palmitate in Perfused Rat Liver

EFFECT OF LOW AND HIGH ETHANOL CONCENTRATIONS AT VARIOUS CONCENTRATIONS OF PALMITATE IN THE PERFUSION MEDIUM

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1. The effect of ethanol on the metabolism of [1-¹⁴C]palmitate in rat liver was investigated in a single-pass perfusion system at concentrations of 10 mM- or 80 mM-ethanol and 0.2 mM- or 1 mM-palmitate. 2. After the perfusion the hepatic lipid was isolated in subcellular fractions. The two major fractions contained triacylglycerol from cytoplasmic lipid droplets and from endoplasmic reticulum plus Golgi apparatus respectively. 3. In experiments with 0.2 mM-palmitate perfusion with 10 mM- or 80 mM-ethanol did not measurably increase the esterification, and the oxidation was markedly decreased and the fatty acid uptake was not affected. 4. Perfusion with ethanol, at 1 mM-palmitate, increased the fatty acid uptake, increased esterification and decreased oxidation. The effects of 10 mM- and 80 mM-ethanol were similar. The incorporation of [1-¹⁴C]palmitate into triacylglycerol in cytoplasmic lipid droplets was not affected statistically significantly by ethanol. Ethanol increased the incorporation of [1-¹⁴C]palmitate into di- and tri-acylglycerol in the membranous fraction. Estimated chemically, the contents of di- and tri-acylglycerol were only slightly affected by ethanol. These results suggest that the effect of ethanol was to increase the turnover of fatty acids in triacylglycerol rather than to increase its accumulation. 5. The results indicate that an increased concentration of fatty acids is more important for the formation of acute fatty liver in fed rats than are the direct effects of ethanol on hepatic fatty acid metabolism.

In the fed unanaesthetized rat a single dose of ethanol increases the concentration of non-esterified fatty acids in the blood 2–3-fold (Brodie & Maickel, 1963; Kondrup & Grunnet, 1973) and increases the hepatic content of triacylglycerol (Brodie & Maickel, 1963). This may be caused mainly by increased peripheral lipolysis mediated via corticosterone and catecholamines (Brodie *et al.*, 1961; Brodie & Maickel, 1963; Maickel & Brodie, 1963). In addition, direct effects of ethanol on the liver probably play a role in the formation of fatty liver, since ethanol decreases the oxidation of radioactively labelled fatty acids in perfused liver from fed rats (Schapiro *et al.*, 1964; Lieber *et al.*, 1967). The effect of ethanol on hepatic metabolism *in vivo* and *in vitro* is dependent on the concentration of ethanol (Grunnet & Thieden, 1972; Guynn & Pielik, 1975; Selmer & Grunnet, 1976) and of fatty acids (Williamson *et al.*, 1969), e.g. the increase in hepatic triacylglycerol *in vivo* depends on the concentration of ethanol in the blood (Johnson, 1974a; Kalant *et al.*, 1975; Abrams & Cooper, 1976).

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Hepatic and extrahepatic effects of ethanol are likely to be involved in the formation of the fatty liver and the relative importance of these effects may depend on the concentrations of ethanol and fatty acids in the blood. In the present study the effect of ethanol on hepatic fatty acid metabolism in perfused liver from fed rats was investigated at high and low concentrations of ethanol and [1-¹⁴C]palmitate. In particular, the effect of ethanol on the deposition of triacylglycerol in the cytoplasmic lipid droplets was investigated by the methods described in the preceding papers (Kondrup, 1979; Kondrup *et al.*, 1979). *In vivo*, ethanol increases the number and size of cytoplasmic lipid droplets (Iseri *et al.*, 1966).

Methods

Most of the methods have been described (Kondrup, 1979). Ethanol at the chosen concentrations was present from the start of the equilibration period.

Samples for measurement of the O₂ saturation were taken from the perfusion medium at the beginning of the equilibration period, at the beginning of the experimental period and from the perfusate twice in each period with 10 min intervals. Samples

for estimation of ethanol were taken simultaneously. The O₂ uptake, as presented below, was calculated from the difference between saturation of haemoglobin in the perfusion medium at the beginning of the experimental period and the mean of saturation of haemoglobin in the two samples from the perfusate in the same period. The ethanol uptake was calculated from the difference between the concentration of ethanol in the perfusion medium at the beginning of the experimental period and the mean of the two samples of perfusate taken in the same period.

Ethanol was determined enzymically by the method of Bonnichsen (1962), and β -hydroxybutyrate by the method of Williamson & Mellanby (1962). β -Hydroxybutyrate dehydrogenase and alcohol dehydrogenase were from Boehringer, Mannheim, Germany.

Results

The uptake of ethanol and O₂ is shown in Table 1. The increase in fatty acid concentration increased the O₂ uptake. Elimination of ethanol was dependent on its concentration, but independent of the fatty acid concentration. At 80 mM-ethanol and 1 mM-palmitate the rate of ethanol removal was significantly higher than the uptake of O₂, suggesting that ethanol was metabolized in part without concomitant O₂ consumption. The elimination of ethanol at 80 mM in the perfusion system, when the liver had been removed, was $0.2 \pm 0.5 \mu\text{mol/min per g wet wt.}$ (mean $\pm$ S.E.M., $n = 5$). The uptake of O₂ and of [1-¹⁴C]-palmitate, the elimination of ethanol and the production of β -hydroxybutyrate were constant with time after 10 min of the equilibration period (results not shown).

Table 1. *Uptake of O₂ and ethanol by perfused rat liver*

The livers were perfused in a single-pass system for 47 min as described in the text. The values are means $\pm$ S.D. The numbers in parentheses refer to the number of experiments.

O ₂ uptake ($\mu\text{mol/min per g wet wt.}$)			
	Without ethanol	10 mM-Ethanol	80 mM-Ethanol
0.2 mM-Palmitate	2.02 ± 0.61 (4)	2.09 ± 0.52 (4)	2.21 ± 0.52 (4)
1 mM-Palmitate	$2.83 \pm 0.20^*$ (6)	$3.05 \pm 0.49^\dagger$ (6)	2.68 ± 0.18 (6)
Ethanol uptake ($\mu\text{mol/min per g wet wt.}$)			
0.2 mM-Palmitate		1.83 ± 0.32 (4)	$2.44\text{--}2.80$ (2)
1 mM-Palmitate		1.93 ± 0.42 (6)	$3.07 \pm 0.36^\ddagger$ (6)

* $P < 0.02$, compared with value for 0.2 mM-palmitate.

† $P < 0.005$, compared with value for 0.2 mM-palmitate.

‡ $P < 0.001$, compared with value for 10 mM-ethanol.

§ $P < 0.05$, compared with the oxygen uptake value for the same group.

Table 2. *Contents of glycerolipids in subcellular fractions*

The experiments are the same as those presented in Table 1. The subcellular fractions were isolated as described (Kondrup, 1979). Results are means $\pm$ S.D., with numbers of experiments shown in parentheses.

		Contents ($\mu\text{mol/g wet wt.}$)					
		Without ethanol		10 mM-Ethanol		80 mM-Ethanol	
		Acylglycerol	Phospholipid	Acylglycerol	Phospholipid	Acylglycerol	Phospholipid
Low-speed pellet	0.2 mM-Palmitate (4)	0.71 ± 0.30	9.04 ± 2.82	0.84 ± 0.39	10.41 ± 4.75	0.87 ± 0.19	10.90 ± 4.20
	1 mM-Palmitate (6)	0.67 ± 0.11	5.88 ± 0.93	0.56 ± 0.26	7.18 ± 1.69	0.73 ± 0.17	7.56 ± 0.93
High-speed pellet	0.2 mM-Palmitate (4)	2.79 ± 0.30	29.26 ± 4.10	2.83 ± 0.58	30.19 ± 2.37	2.67 ± 0.40	33.64 ± 6.45
	1 mM-Palmitate (6)	$4.42 \pm 0.70^*$	31.70 ± 3.50	$4.85 \pm 0.57^\dagger$	30.03 ± 2.96	$3.97 \pm 0.55^*$	32.46 ± 4.11
Diethyl ether fraction	0.2 mM-Palmitate (4)	5.50 ± 2.47	0.29 ± 0.12	3.15 ± 0.68	0.20 ± 0.01	4.73 ± 1.16	0.26 ± 0.05
	1 mM-Palmitate (6)	5.50 ± 1.36	0.22 ± 0.08	$5.56 \pm 1.15^*$	0.24 ± 0.06	5.02 ± 1.19	0.23 ± 0.06

* $P < 0.005$, compared with the value for 0.2 mM-palmitate.

† $P < 0.001$, compared with the value for 0.2 mM-palmitate.

Table 2 shows the contents of glycerolipids in the subcellular fractions. Palmitate (1 mM) caused acylglycerol to accumulate in the high-speed pellet, whereas ethanol itself had no statistically significant

effect. The liver weight after perfusion was slightly higher in the groups perfused with ethanol [control, 6.68 ± 0.64 g (mean $\pm$ S.D., $n = 10$), 10 mM- and 80 mM-ethanol treatment, 7.25 ± 0.73 g (mean $\pm$ S.D.,

Table 3. *Effect of ethanol on the metabolism of exogenous [1- 14 C]palmitate*

The experiments are the same as those presented in Table 1. The radioactive isotope was introduced after an equilibration period of 26 min and the metabolism was followed for 21 min. See the text and Kondrup (1979) for experimental details. The results are means $\pm$ S.D., with $n = 6$ in each group with 1 mM-palmitate, and $n = 4$ in experiments with 0.2 mM-palmitate. Total esterification and total oxidation are defined in the text.

	Concn. of palmitate in perfusion medium (mM)	Rate (μ mol/min per liver)		
		Without ethanol	10 mM-Ethanol	80 mM-Ethanol
Uptake of [1- 14 C]palmitate*	0.2	0.71 ± 0.12	0.70 ± 0.05	0.71 ± 0.08
	1	3.42 ± 0.29	$3.93 \pm 0.34^\dagger$	$3.87 \pm 0.10^\S$
Total esterification*	0.2	0.75 ± 0.06	0.75 ± 0.17	0.79 ± 0.16
	1	2.17 ± 0.26	$3.47 \pm 0.47^\S$	$3.67 \pm 0.34^\parallel$
Total oxidation*	0.2	0.05 ± 0.02	$0.02 \pm 0.01^*$	$0.02 \pm 0.01^*$
	1	0.68 ± 0.12	$0.37 \pm 0.31^\dagger$	$0.27 \pm 0.09^\parallel$
Water-soluble products in the liver	0.2	0.015 ± 0.003	0.010 ± 0.003	$0.008 \pm 0.001^\ddagger$
	1	0.163 ± 0.045	0.094 ± 0.072	$0.082 \pm 0.045^\ddagger$
Water-soluble products in the perfusate	0.2	0.013 ± 0.006	0.008 ± 0.003	0.006 ± 0.002
	1	0.335 ± 0.091	0.209 ± 0.181	$0.137 \pm 0.051^\parallel$
CO ₂ in the perfusate	0.2	0.018 ± 0.008	0.006 ± 0.002	$0.006 \pm 0.001^*$
	1	0.142 ± 0.042	$0.040 \pm 0.010^\parallel$	$0.045 \pm 0.009^\parallel$

* $P < 0.05$, compared with the value for perfusion without ethanol.

† $P < 0.02$, compared with the value for perfusion without ethanol.

‡ $P < 0.01$, compared with the value for perfusion without ethanol.

§ $P < 0.005$, compared with the value for perfusion without ethanol.

|| $P < 0.001$, compared with the value for perfusion without ethanol.

¶ Some of these values have been presented previously (Kondrup *et al.*, 1977). Since then the results have been extended.

Table 4. *Incorporation of palmitate into acylglycerols and phospholipid in subcellular fractions*

The experiments are the same as those presented in Table 1. The results are means $\pm$ S.D. The number of experiments in each group are as shown in Table 3.

Subcellular fraction	Lipid class	Concn. of palmitate (mM)	Esterification (nmol/min per liver)		
			Without ethanol	10 mM-Ethanol	80 mM-Ethanol
High-speed pellet	Phospholipid	0.2	163 ± 38	177 ± 84	165 ± 63
		1	352 ± 82	$481 \pm 123^*$	$526 \pm 87^\dagger$
	Diacylglycerol	0.2	71 ± 31	93 ± 22	108 ± 51
		1	197 ± 50	$341 \pm 76^\dagger$	$575 \pm 171^\ddagger$
	Triacylglycerol	0.2	196 ± 37	179 ± 38	196 ± 26
		1	849 ± 133	1047 ± 334	923 ± 131
Diethyl ether fraction	Phospholipid	0.2	6 ± 4	4 ± 1	2 ± 1
		1	7 ± 2	10 ± 4	9 ± 2
	Diacylglycerol	0.2	11 ± 3	12 ± 5	16 ± 7
		1	34 ± 4	43 ± 13	69 ± 15
	Triacylglycerol	0.2	108 ± 40	77 ± 14	111 ± 10
		1	870 ± 182	980 ± 305	961 ± 207

* $P < 0.05$, compared with the value for perfusion without ethanol.

† $P < 0.005$, compared with the value for perfusion without ethanol.

‡ $P < 0.001$, compared with the value for perfusion without ethanol.

§ $P < 0.02$, compared with the value for perfusion with 10 mM-ethanol.

Table 5. *Contents and relative specific radioactivities of di- and tri-acylglycerol in the high-speed pellet*

The data are from the experiments with 1 mM-palmitate presented in Table 1. The individual acylglycerols were determined as described in Kondrup (1979). The chemical estimations (content) are expressed as μmol of glycerol in acylglycerols/liver and are means $\pm$ s.d. ($n = 6$ in each group). The relative specific radioactivities are relative to exogenous palmitate (= 100).

	Diacylglycerol		Triacylglycerol	
	Content	Relative specific radioactivity	Content	Relative specific radioactivity
Without ethanol	5.07 $\pm$ 1.04	44 $\pm$ 6	19.12 $\pm$ 2.55	32 $\pm$ 6
10mm-Ethanol	9.29 $\pm$ 2.24*	45 $\pm$ 8	18.34 $\pm$ 2.70	38 $\pm$ 4†
80mm-Ethanol	10.43 $\pm$ 1.83‡	56 $\pm$ 8§	16.32 $\pm$ 3.23	40 $\pm$ 5†

* $P < 0.005$, compared with the value for perfusion without ethanol.

† $P < 0.05$, compared with the value for perfusion without ethanol.

‡ $P < 0.001$, compared with the value for perfusion without ethanol.

§ $P < 0.025$, compared with the value for perfusion without ethanol.

$n = 20$; $P < 0.05$]. The weight of the animals was similar in the two groups [control, 166 $\pm$ 6 g (mean $\pm$ s.d., $n = 10$); 10mm- and 80mm-ethanol treatments, 166 $\pm$ 4 g (mean $\pm$ s.d., $n = 20$)].

The recovery of radioactivity taken up by the liver was 102 $\pm$ 9% (mean $\pm$ s.d., $n = 18$) in the experiments with 1mM-palmitate and 113 $\pm$ 23% (mean $\pm$ s.d., $n = 12$) in the experiments with 0.2mM-palmitate. The maximum difference of the mean of recovery between groups perfused with the same palmitate concentration was 5%.

The uptake of [1- ^{14}C]palmitate (Table 3) increased in parallel with the fatty acid concentration. With 1mM-palmitate, ethanol at both concentrations increased the uptake by about 15%. The total esterification is a summation of the incorporation of [1- ^{14}C]palmitate into total lipids in all fractions, 96% of which was esters. The total esterification was not affected by ethanol at the low fatty acid concentration, whereas at 1mM-palmitate the esterification was increased about 30% at both ethanol concentrations. The total oxidation is a summation of the metabolism of [1- ^{14}C]palmitate to radioactively labelled water-soluble products in the liver and in the perfusate and to radioactively labelled CO_2 in the perfusate. The total oxidation was decreased about 50% by ethanol and the decrease was relatively the same at high and low concentrations of ethanol and palmitate.

Table 4 shows the incorporation of [1- ^{14}C]palmitate into various lipid classes in the subcellular fractions. At the low palmitate concentration ethanol had no statistically significant effect. At the high palmitate concentration there was a statistically significant increase in incorporation into diacylglycerol and phospholipid in the high-speed pellet. The increased incorporation into triacylglycerol was not statistically significant in either of the pools. The 5-fold increase in palmitate concentration in-

creased the incorporation into triacylglycerol 8–13-fold in the diethyl ether fraction.

The contents of di- and tri-acylglycerol in the high-speed pellet were determined chemically and the relative specific radioactivity was calculated (Table 5). At both concentrations ethanol increased the content of diacylglycerol. The relative specific radioactivity of diacylglycerol was increased by 80mm-ethanol. The content of triacylglycerol was almost unaffected by ethanol, whereas the relative specific radioactivity was increased. The chemical estimations are expressed per whole liver to allow for the difference in liver weight between the groups. The sum of di- and tri-acylglycerol accounts for 81–93% of the total content of acylglycerol.

The secretion of triacylglycerol was estimated chemically in lipid extracts of the precipitated perfusate. In control livers perfused with 1mM-palmitate, the secretion rate was 43 $\pm$ 17 nmol/min per liver (mean $\pm$ s.d.), with 10mm-ethanol present the rate was 39 $\pm$ 6 nmol/min per liver (mean $\pm$ s.d.) and with 80mm-ethanol it was decreased to 26 $\pm$ 5 nmol/min per liver (mean $\pm$ s.d., $n = 6$, $P < 0.05$ compared with the control value).

Discussion

In previous perfusion studies with liver from fed rats the effect of ethanol on the metabolism of fatty acids was investigated in a recirculating system where acetate would accumulate and possibly contribute to the observed effects of ethanol metabolism (Schapiro *et al.*, 1964; Lieber *et al.*, 1967). The observed effects of ethanol can now more safely be ascribed to the presence of ethanol itself. Also, in the previous studies the perfusion time was 2–4 h without renewal of the fatty acid content of the perfusion medium. By this experimental design the initial effects of ethanol on the metabolism of fatty

acids taken up by the liver is not revealed. This may explain why Schapiro *et al.* (1964) did not observe a decrease in the formation of water-soluble products from labelled palmitate in response to ethanol [this parameter was not measured in the study of Lieber *et al.* (1967)] and why neither of these studies showed an increase in the total esterification of the radioactively labelled fatty acid in response to ethanol. We found ethanol increased the esterification of [$1\text{-}^{14}\text{C}$]palmitate, but the effect was discernible only with 1 mM-palmitate. With 0.2 mM-palmitate 95% of the palmitate taken up was esterified in the absence of ethanol and therefore a statistically significant increase in the esterification, caused by addition of ethanol, could hardly be expected. In this situation the marked decrease in total oxidation was of negligible quantitative importance for the total esterification.

About 50% of the increase in esterification at 1 mM-palmitate could be ascribed to an increased uptake of palmitate. Abrams & Cooper (1976) concluded from studies *in vivo* that ethanol increases the number of open sinusoids in the liver, leading to increased uptake of e.g. fatty acids. The 11% increase in liver weight after perfusion with ethanol (see the Results section) may reflect an increased number of open sinusoids, which could explain the increased uptake of fatty acids at 1 mM-palmitate.

In the preceding paper (Kondrup *et al.*, 1979) it was suggested that, at 1 mM-palmitate, the major part of triacylglycerol newly formed in the endoplasmic reticulum was partially hydrolysed, resynthesized and then deposited in the cytoplasmic lipid droplets. In the presence of ethanol the incorporation of [$1\text{-}^{14}\text{C}$]palmitate into di- and tri-acylglycerol in the high-speed pellet was increased by about $0.4\text{ }\mu\text{mol/min}$ per liver [mean of the two ethanol groups perfused with 1 mM-palmitate (Table 4)]. Provided that this increased esterification occurred during the whole perfusion period it would equal an accumulation of $(0.4 \times 47) = 19\text{ }\mu\text{mol}$ of fatty acids/liver in di- and tri-acylglycerol in the high-speed pellet. After the perfusion the high-speed pellet of the control livers contained $67 \pm 3\text{ }\mu\text{mol}$ of fatty acids/liver in di- and tri-acylglycerol (mean $\pm$ S.E.M., $n = 6$) compared with $72 \pm 3\text{ }\mu\text{mol}$ (mean $\pm$ S.E.M., $n = 12$) in the combined ethanol group. The difference $[(19 - 5) = 14\text{ }\mu\text{mol/liver}]$ by far exceeds the confidence limits of the chemical estimations, which suggests that the majority of the increased esterification in the high-speed pellet occurred by replacement of preexisting fatty acids in that fraction.

The replaced fatty acids could be incorporated into triacylglycerol in the cytoplasmic lipid droplets by the mechanism suggested above. In control livers this fraction contained $113 \pm 16\text{ }\mu\text{mol}$ of fatty acids/liver (mean $\pm$ S.E.M., $n = 6$) compared with a value for the combined ethanol groups of $114 \pm 8\text{ }\mu\text{mol/liver}$ (mean

$\pm$ S.E.M., $n = 12$). The mean values indicate that tri-acylglycerol formation in the cytoplasmic droplets was not increased by ethanol, but the confidence limits of the chemical estimations make such a conclusion speculative. The incorporation of [$1\text{-}^{14}\text{C}$]palmitate into triacylglycerol in the cytoplasmic lipid droplets was increased by ethanol, although the effect was not statistically significant (Table 4). In the preceding paper (Kondrup *et al.*, 1979) it was suggested that diacylglycerol in the high-speed pellet, and acyl-CoA-esters, were precursors for triacylglycerol in the cytoplasmic lipid droplets. Ethanol, at 80 mM, increased the specific radioactivity of diacylglycerol in the high-speed pellet (Table 5) and the increased radioactivity in triacylglycerol in the cytoplasmic lipid droplets in this group may result from this, rather than from increased synthesis. At 10 mM-ethanol, the relative specific radioactivity of diacylglycerol was similar to that in the control group and therefore the increased radioactivity of triacylglycerol in the cytoplasmic lipid droplets in this group may reflect increased synthesis.

As an alternative, the fatty acids replaced in di- and tri-acylglycerol in the high-speed pellet could be β -oxidized. In liver from fed rats, perfused with a high concentration of fatty acids, ethanol decreased ketogenesis negligibly when estimated chemically (Fellenius & Kiessling, 1973). In the present experiments the production of β -hydroxybutyrate was about $2\text{ }\mu\text{mol/min}$ per liver and it was not affected by ethanol (results not shown). β -Oxidation may have been largely unaffected by ethanol in our experiments. The total oxidation of exogenous [$1\text{-}^{14}\text{C}$]palmitate was decreased by $0.36\text{ }\mu\text{mol/min}$ per liver in the presence of ethanol (Table 3). If the replaced fatty acids were oxidized evenly during the experimental period, they would contribute $14:47 = 0.30\text{ }\mu\text{mol/min}$ per liver to β -oxidation, which is very close to the observed decrease in oxidation of exogenous palmitate. It is therefore possible that β -oxidation of endogenous fatty acids was increased in the presence of ethanol at the expense of exogenous palmitate. The observed decrease in [$1\text{-}^{14}\text{C}$]palmitate oxidation could then be due to radioactive isotope dilution with endogenous fatty acids. It follows from this suggestion that the effect of ethanol on palmitate oxidation should be transitory and vanish when replaceable intracellular fatty acids approach the specific radioactivity of infused palmitate. In preliminary experiments it was seen that after a 40 min period of infusion of [$1\text{-}^{14}\text{C}$]palmitate ethanol no longer decreased the appearance of radioactively labelled water-soluble products in the perfusate.

Irrespective of the concentrations of ethanol or palmitate, ethanol decreased the $^{14}\text{CO}_2$ production more than the production of water-soluble products (Table 3). Ethanol apparently had additional effects on the tricarboxylic acid cycle and it is known that

ethanol decreases CO₂ production markedly also when estimated chemically (Lieber *et al.*, 1967). In experiments with isolated hepatocytes from starved rats, Ontko (1973) also found that ethanol decreased the oxidation of radioactively labelled palmitate to CO₂ more than the oxidation to ketone bodies. In addition, he found that ethanol decreased the specific radioactivity of the ketone bodies formed and that this decrease was similar to the decrease in the formation of radioactively labelled ketone bodies. Ontko suggested that the decrease in specific radioactivity was caused by conversion of ethanol to ketone bodies, but his observation is equally compatible with our suggestion that ethanol increases the isotope dilution of the exogenous radioactively labelled fatty acid with endogenous fatty acids.

The secretion of triacylglycerol from the liver was significantly decreased by 80 mM-ethanol, but not by 10 mM-ethanol. This difference may contribute to the explanation of why the accumulation of hepatic triacylglycerol *in vivo* is dependent on the blood ethanol concentration. In other respects the effects on palmitate metabolism of ethanol at the two concentrations were largely similar. *In vivo*, high concentrations of ethanol may have additional indirect effects on the liver. In human studies (Schapiro *et al.*, 1965) ethanol increased the plasma concentration of non-esterified fatty acids and the increase was more pronounced with higher concentrations of ethanol. In the present study it was shown that a 5-fold increase in palmitate concentration increased the incorporation of palmitate into triacylglycerol in cytoplasmic lipid droplets 10-fold, whereas ethanol itself has only small effects. In fed untreated rats plasma concentration of non-esterified fatty acids was found to be about 0.2 mM (Kondrup & Grunnet, 1973; Johnson, 1974b) and at this concentration ethanol has no detectable effect on the hepatic esterification of fatty acids, according to the present study. The formation of fatty liver in fed rats after a single large dose of ethanol therefore most probably is caused by indirect effects of ethanol on the liver cells: increased peripheral lipolysis (Brodie & Maickel, 1963), increased hepatic clearance of fatty acids (Abrams & Cooper, 1976) and a corticosterone-mediated increase in hepatic phosphatidate phosphohydrolase activity, which probably also contributes to the production of fatty liver (Glenny & Brindley, 1978; Brindley *et al.*, 1979).

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