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THE ROLE OF THE HEPATOCELLULAR REDOX STATE IN THE HEPATIC TRIGLYCERIDE ACCUMULATION FOLLOWING ACUTE ETHANOL ADMINISTRATION

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Abstract—The role of the increased hepatocellular redox-state ([NADH]/[NAD$^+$] ratio) as a mechanism underlying hepatic triglyceride deposition after acute ethanol dosing has been investigated in the rat. Following a single dose of ethanol (2 g/kg i.p.) in fasted rats, increases were observed at 1.5 hr in the hepatic [lactate]/[pyruvate] (133%), [3-hydroxybutyrate]/[acetoacetate] (69%) ratios, and the liver triglyceride concentration (129%). At the same time point, ethanol increased radioactivity incorporated into hepatic total lipid and triglyceride, after an injection of [U-^{14}C] palmitic acid, by 76% and 158% respectively.

Treatment of animals with Naloxone hydrochloride (2 mg/kg i.p.) at 1.0 hr and 2.5 hr after ethanol abolished these ethanol-mediated redox-state changes, without inhibiting ethanol oxidation or affecting hepatic acetaldehyde levels. This, however, did not prevent completely the triglyceride accumulation in the liver or reverse the enhanced uptake of radio-labelled palmitate caused by ethanol.

Administration of sorbitol (3.5 g/kg i.p.) caused 199%, 57% and 200% increases in the hepatic [lactate]/[pyruvate], [3-hydroxybutyrate]/[acetoacetate] ratios and glycerol-3-phosphate concentrations respectively. However, the hepatic triglyceride concentration and the incorporation of [U-^{14}C] palmitic acid into hepatic lipids were not influenced by this treatment.

In vitro studies in which rat liver slices were incubated with [1-^{14}C] palmitic acid also indicated that the altered [NADH]/[NAD$^+$] ratio was not responsible for the decreased rate of fatty acid oxidation seen after ethanol administration or after the addition of ethanol to the incubation medium. In conclusion, these experiments indicate that increases in the hepatic [NADH]/[NAD$^+$] ratio resulting from ethanol oxidation may not be directly implicated in the altered hepatic fatty acid utilisation and triglyceride deposition observed after acute ethanol administration in rats.

Acute and chronic administration of ethanol causes accumulation of lipid, chiefly triglyceride, in the livers of experimental animals. In spite of intensive studies over the last twenty-five years, the precise biochemical mechanism underlying alcohol-induced steatosis remains unclear. One of the most commonly quoted mechanisms that could underly fatty liver production is the increase in the hepatocellular redox-state ([NADH]/[NAD$^+$] ratio) that results from ethanol oxidation by alcohol dehydrogenase and aldehyde dehydrogenase in the liver [1]. This altered ratio could influence intermediary metabolism in a number of ways, thereby increasing the hepatic triglyceride content. Firstly, the cytosolic redox-pair [dihydroxyacetone phosphate]/[glycerol-3-phosphate] becomes shifted towards the reduced state, so that availability of glycerol-3-phosphate, for the esterification of fatty acids into triglycerides, is increased [2]. Secondly, transhydrogenation of reducing equivalents from NADH increases hepatic NADPH concentrations, so that fatty acid synthesis may be increased [3]. Thirdly, ethanol inhibits fatty acid oxidation *in vitro* and *in vivo*, possibly as a consequence of the redox-state changes limiting the availability of NAD to the dehydrogenases in the tricarboxylic acid cycle. A combination of these factors might favour esterification of fatty acids into triglycerides, producing the characteristic alcoholic fatty liver.

This "redox-state theory" for ethanol-induced triglyceride accumulation, although plausible, is not supported directly by convincing experimental evidence. Recent studies in this laboratory have shown that supplementation of alcohol-containing liquid diets with the hydrogen acceptor Methylene Blue during chronic ethanol administration in rats largely corrected the ethanol-induced disturbances in the hepatic redox-state, without preventing the production of fatty liver [4]. Furthermore, some compounds, such as antioxidants, will protect against ethanol-induced hepatic triglyceride accumulation without reversing the alcohol-related change of the redox-state [5].

The aim of the present study was to determine whether there is a direct relationship between alterations in the hepatocellular redox-state after a moderate, acute dose of ethanol in the rat, and hepatic fatty acid disposition. Two approaches have been adopted to assess this relationship. Firstly, the effect of correction of ethanol-mediated redox-state

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¶ In this article, the terms ethanol and alcohol are interchangeable.

changes by Naloxone on hepatic triglyceride disposal has been determined [6–8]. Secondly, the effects of sorbitol on hepatic triglyceride and fatty acid metabolism have been compared with the effects of ethanol on the same parameters, since the former produces an increase in the hepatic [NADH]/[NAD^+] ratio comparable to that observed after ethanol administration.

MATERIALS AND METHODS

Chemicals. All reagents were of the highest purity available commercially. Pure Naloxone hydrochloride powder was a gift from Du Pont Ltd. [1-^{14}C] Palmitic acid (sp. act. 55 mCi/mmol), [U-^{14}C] palmitic acid (sp. act. 403 mCi/mmol) and ^{14}C-hexadecane were supplied by Amersham International, Amersham, Bucks., U.K.

Animals and diets. Male Wistar albino rats (University of Surrey strain, 180–230 g) were used throughout. They were housed in pairs in wire-bottomed cages in a room maintained at 22°. Lighting was provided between 0700 hr and 2100 hr daily. They were maintained on Heygates 41B cube diet and water *ad lib*. All animals were fasted for 24 hr prior to ethanol administration.

Dosing. Ethanol (2 g/kg) was administered by the intraperitoneal injection of a 20% (w/v) solution in saline. Control animals received an equivalent volume of saline by the same route. Sorbitol or glucose was given by the same route as 35% (w/v) solutions, at doses isocaloric with the ethanol dose employed (i.e. 3.5 g/kg). Naloxone hydrochloride (2 mg/kg i.p. in saline) was given at 1.0 hr, and where appropriate, 2.5 hr after ethanol dosing as described previously [8].

Fatty acid oxidation studies. Animals were sacrificed 90 min after a dose of ethanol, sorbitol, glucose or saline, and a portion of liver rapidly frozen *in situ* using aluminium tongs which had been precooled in liquid nitrogen. A further portion of liver was removed into ice-cold saline for the preparation of liver slices (0.2–0.4 mm thickness) by using a skin grafting knife. Slices equivalent to about 100 mg wet weight were placed in the outer well of an Ehrlenmeyer flask which contained 3.5 ml Krebs–Henseleit bicarbonate buffer (pH 7.4) supplemented with 1.5 mM lactate and 0.15 mM pyruvate. Glucose, fructose or sorbitol were added at concentrations of 10 mM and ethanol was added at concentrations of either 20 mM or 40 mM. [1-^{14}C] Palmitic acid was bound to fatty acid-free bovine serum albumin and added to each flask (1 μCi/flask; final concentration 60 μM). The flasks were sealed with rubber septum caps, gassed with 100% oxygen for 5 min, and then incubated at 37° for 90 min. The incubations were stopped by injecting 0.5 ml 1 M citric acid into the outer wells of flasks. A 0.6 ml aliquot of hyamine-10X (10% in methanol) was placed in the centre well, and then the flasks further incubated for 45 min to trap the CO_2 released during the incubation. The contents of the centre well were then removed into 10 ml Dimilume-30 liquid scintillation cocktail (Packard Instrument Co. Ltd., Caversham, Bucks, U.K.), the wells washed twice with 1.0 ml portions of scintillant, the washings added to the scintillation vial, and the vials counted. In a preliminary series of experiments, total CO_2 production in some incubations was measured by trapping with 0.3 N barium hydroxide, adding thymolphthalein indicator and titrating with 0.179 N HCl [9]. These studies showed that the specific activity of CO_2 evolved in the incubations was reasonably constant, so that any decreases due to ethanol addition were not due to isotope dilution. In separate experiments, it was established that the rate of $^{14}CO_2$ production from [1-^{14}C] palmitate was linear up to an incubation time of 150 min. The incubation time of 90 min was chosen as it produced conditions in which a reasonable amount of radioactivity was released as $^{14}CO_2$, which could be easily determined in a reproducible manner, giving a coefficient of variation of 5.7%.

^{14}C-*Palmitate uptake* in vivo. Ninety minutes after the administration of either ethanol or sorbitol, [U-^{14}C] palmitic acid, (1μCi/100 g body weight) was injected i.p. in freshly prepared rat serum. Ten minutes later, the animals were sacrificed by cervical dislocation, the liver excised rapidly and homogenised in ice-cold 0.25 M sucrose. Hepatic lipids were extracted and analysed as described below. No radioactivity was recovered in the serum phospholipids, cholesterol esters or triglycerides in these experiments.

Lipid analyses. The incubation medium from the oxidation experiments was homogenized and 1.0 ml aliquots were extracted for total lipids by the method of Folch *et al.* [10]. Liver homogenate from ^{14}C-palmitate uptake studies was treated in the same manner. Individual lipid classes were separated by thin-layer chromatography on Silca Gel 60 TLC plates using hexane:diethyl ether:acetic acid (70:30:1) as the mobile phase. The spots corresponding to various lipid classes were eluted with chloroform:methanol (2:1) and counted for radioactivity incorporated into lipids during the incubations or after injection of ^{14}C-palmitate *in vivo*.

In some experiments, triglycerides were measured in livers homogenised and diluted (1:8) in 0.25 M sucrose by using a colorimetric method [11]. This method has previously been found to give results in excellent agreement with more established and elaborate methods of measuring liver triglycerides [12, 13], and gave coefficient of variation and recovery values of 6.2% and 96.1% respectively.

Metabolite determinations. The redox-state of the liver tissue in the *in vitro* incubations was assessed by measuring the [lactate]/[pyruvate] ratio in a portion of incubation medium (1.0 ml) removed from the flasks 60 min after the start of the initial incubation. The aliquot of medium was mixed with 1.0 ml ice-cold 0.6 N perchloric acid, and lactate and pyruvate determined in the deproteinised supernatant using kits supplied by BCL Ltd., Lewes, Sussex, U.K.

Freeze-clamped liver tissue was extracted with perchloric acid, the extract treated with Florisil, and the [lactate]/[pyruvate] and [3-hydroxybutyrate]/[acetoacetate] ratios determined by standard enzymatic methods as described in detail previously [14]. Glycerol-3-phosphate was determined by the method of Michal and Lang [15] and ATP was assayed using kits supplied by BCL Ltd. Hepatic acetaldehyde was

determined in frozen liver powder which had been extracted with 0.6 N perchloric acid containing 25 mM thiourea to inhibit the non-enzymatic formation of acetaldehyde from ethanol present in the sample [16, 17]. Acetaldehyde was determined by head-space gas chromatography using n-propanol as the internal standard [14].

Ethanol was determined in blood samples taken from the animals at 1.5 or 3.0 hr after ethanol, as described previously [14].

Radioactivity determination and statistics. All liquid scintillation counting was performed using a Nuclear Enterprises NE 8312 radioactivity counter, and counting efficiency was in the range 87–92%. Specific activities of [1-^{14}C] palmitate and [U-^{14}C] palmitate were then used to calculate the nmol of each tracer metabolised under the various experimental conditions.

All results are expressed as mean ± standard deviation. Statistical significance of differences between means for the various treatment groups was assessed using Student's *t*-test for paired data.

RESULTS

Results of the *in vitro* liver slice studies are shown in Table 1. The presence of ethanol in the incubation medium at 20 and 40 mM concentrations, decreased the oxidation of [1-^{14}C] palmitate to $^{14}CO_2$ by 22% and 43% respectively. Addition of sorbitol to the medium caused an increase in the lactate/pyruvate ratio slightly greater than that caused by either 20 mM or 40 mM ethanol, but did not impair the oxidation of [1-^{14}C] palmitate. On the contrary, fructose and sorbitol enhanced this oxidation. Incorporation of palmitate into triglyceride was increased by sorbitol, although the most marked rise was seen after the addition of 40 mM ethanol to the incubation.

In the *in vivo* studies, administration of Naloxone, one hour after ethanol, prevented the disturbances in the hepatic cytosolic and mitochondrial redox-states caused by ethanol, measured as the lactate/pyruvate and 3-hydroxybutyrate/acetoacetate ratios respectively (Table 2). This correction of the ethanol-induced hepatic redox-state changes by Naloxone was not associated with any significant decrease in the hepatic triglyceride accumulation measured either 1.5 hr or 3.0 hr after ethanol. Naloxone treatment did not affect hepatic acetaldehyde concentrations or blood ethanol levels in animals dosed with ethanol (Table 3).

Naloxone alone had an effect on palmitate oxidation when liver slices taken from animals treated with this drug alone were studied *in vitro*. This resulted in an increased esterification of palmitate into triglycerides in these incubations. While liver slices from ethanol-treated rats showed a lowered oxidation of palmitate and increased incorporation of palmitate into triglycerides, these phenomena

Table 1. Effect of sorbitol and ethanol on palmitate oxidation and esterification by rat liver slices

Addition	[1-^{14}C] palmitate oxidised in incubation (nmol/g liver × 10^2)	[1-^{14}C] palmitate incorporated into triglycerides (nmol/g liver × 10^2)	Lactate/pyruvate ratio in incubation medium
Glucose (10 mM)	84.8 ± 4.5 (8)	159.9 ± 4.5 (6)	14.6 ± 0.6 (4)
Fructose (10 mM)	108.9 ± 2.1 (8)*	156.0 ± 20.4 (6)	13.5 ± 2.5 (4)
Sorbitol (10 mM)	118.4 ± 6.1 (8)*	227.1 ± 20.4 (6)*	47.4 ± 5.4 (6)*
Saline	94.8 ± 2.5 (6)	153.2 ± 8.8 (4)	11.7 ± 2.6 (4)
Ethanol (20 mM)	74.1 ± 5.1 (6)†	236.2 ± 53.6 (4)†	37.9 ± 5.9 (4)†
Ethanol (40 mM)	53.8 ± 5.1 (6)†	316.7 ± 42.6 (4)†	39.4 ± 0.4 (4)†

Approximately 100 mg of rat liver slices were incubated for 90 min at 37° in 3.5 ml Krebs–Henseleit buffer (pH = 7.4) containing 1 μCi albumin-bound [1-^{14}C] palmitic acid. 1.0 ml incubation medium was removed after 60 min for determination of lactate and pyruvate concentrations. Results are shown as means ± SD with the number of determinations being given in parentheses.

* $P < 0.001$ vs glucose group.

† $P < 0.001$ vs saline group.

Table 2. Effect of naloxone on the hepatic redox state and triglyceride content after acute ethanol administration

Treatment	Hepatic ATP (nmol/g)	Lactate/pyruvate	3-Hydroxybutyrate/acetoacetate	Liver triglyceride (mg/g) 1.5 hr	3.0 hr
Saline	3726 ± 363	16.5 ± 5.0	2.58 ± 0.30	6.6 ± 1.6	4.9 ± 1.9
Saline + Naloxone	3423 ± 784	15.7 ± 5.1	2.75 ± 0.49	7.0 ± 2.0	5.5 ± 1.0
Ethanol	3973 ± 758	38.5 ± 8.0*	4.35 ± 0.10*	14.5 ± 4.6*	11.9 ± 4.5‡
Ethanol + Naloxone	4070 ± 785	18.2 ± 3.2†	2.54 ± 0.66†	14.2 ± 2.0‖	8.8 ± 2.1§

Determinations, with the exception of triglyceride measurements at 3.0 hr, were made on liver tissue taken by freeze clamping at 90 min after intraperitoneal injection of ethanol (2 g/kg) or an equivalent volume of saline. Naloxone (2 mg/kg i.p.) was given at 1.0 hr after ethanol, and in the case of animals in which triglycerides were measured at 3.0 hr after ethanol, a further Naloxone dose was given at 2.5 hr. Results are shown as mean ± SD (N = 6 rats per group). * $P < 0.001$ vs saline only group. † $P < 0.001$ vs ethanol only group. ‡ $P < 0.01$ vs saline only group. § $P < 0.05$ vs saline groups and N.S. vs ethanol only. ‖ $P < 0.001$ vs saline groups and N.S. vs ethanol only. N.S. = not significant.

Table 3. Effect of Naloxone on hepatic acetaldehyde and blood ethanol concentrations after acute ethanol administration

Treatment	Liver acetaldehyde (nmol/g)	Blood ethanol (mg/100 ml) 1.5 hr	3.0 hr
Ethanol	22.5 ± 4.1	219.8 ± 5.0	139.9 ± 11.3
Ethanol + Naloxone	27.8 ± 6.2	220.5 ± 5.0	135.6 ± 11.1

Acetaldehyde determinations were made 1.5 hr after ethanol (2 g/kg i.p.). Naloxone was given (2 mg/kg i.p.) at 1.0 hr and, where appropriate, 2.5 hr after ethanol. Results are shown as mean ± SD. N = 6 rats per group per time point. None of the differences is statistically significant.

were not reversed by Naloxone pre-treatment (Table 4).

Experiments on the hepatic uptake of palmitate *in vivo* showed that ethanol administration caused increased incorporation of this fatty acid into total hepatic lipids and triglycerides. This effect was not influenced by Naloxone treatment, despite the correction of the redox-state changes by the latter compound.

Sorbitol administration produced a significant increase in the hepatic lactate/pyruvate ratio, similar to that observed after ethanol, whilst also causing a 200% increase in the hepatic glycerol-3-phosphate concentration. Sorbitol also caused an increase in the hepatic 3-hydroxybutyrate/acetoacetate ratio, although this was not statistically significant due to the wide individual variation in the extent of this effect. These changes in the hepatic redox-state observed after sorbitol were not associated with increased incorporation of [U-^{14}C] palmitate into liver lipids or triglycerides, or increased hepatic triglyceride concentrations. The oxidation of palmitate by liver slices from rats dosed with sorbitol was not different from that obtained with control animals which received glucose. Sorbitol dosing did, however, cause cause increased esterification of palmitate into triglycerides in these incubations (Table 5).

DISCUSSION

The results of this study cast some doubts on the role of the altered hepatic redox-state as a mechanism of the acute ethanol-induced hepatic triglyceride accumulation. Data obtained from studies *in vitro* (Table 1) suggest that there is no apparent association between the increased lactate/pyruvate ratio and the decrease in palmitate oxidation in the presence of ethanol. Under these conditions, *in vitro* elevation of the cytosolic [NADH]/[NAD$^+$] ratio by either ethanol or sorbitol occurred in parallel with incorporation of palmitate into triglycerides. However, the fact that the higher concentration of ethanol (40 mM) was much more effective at increasing palmitate esterification, without further raising the [lactate]/[pyruvate] ratio above that found in the presence of 20 mM ethanol, suggested that factors other than the redox-state might be implicated. Experiments *in vivo*, with Naloxone, also support these conclusions drawn from the *in vitro* findings. Correction of the redox-state in ethanol-treated rats by Naloxone did not prevent the hepatic triglyceride accumulation, measured 1.5 hr after acute ethanol administration (Table 2). This may have been the consequence of administration of the drug 1.0 hr after ethanol, so that the redox-state disturbance in ethanol-treated animals prior to Naloxone administration could have contributed to the triglyceride deposition. Prolonging the period of study to 3.0 hr after ethanol, however, with a further Naloxone dose given at 2.5 hr, since the drug has a short half life in the rat [18], still revealed that the drug did not prevent completely the increase in hepatic triglycerides caused by ethanol, although a non-significant, downward trend was observed. This slight apparent trend is probably of little significance with

Table 4. Effect of Naloxone on hepatic palmitic acid disposal after acute ethanol administration

Treatment	[1-^{14}C] Palmitate oxidised to $^{14}CO_2$ by liver slices *in vitro* (nmol/g liver × 10^3)	[1-^{14}C] incorporated into triglyceride in liver slice incubations (nmol × 10^2/g liver)	% Injected [U-^{14}C] palmitate incorporated into liver lipids *in vivo*	[U-^{14}C] Palmitate incorporated into liver triglyceride after *in vivo* injection (nmol/g liver × 10^3)
Saline	29.9 ± 4.6	93.1 ± 28.3	8.2 ± 1.0	9.29 ± 1.14
Saline + Naloxone	19.1 ± 3.2*	166.6 ± 16.4*	8.2 ± 0.7	10.1 ± 2.8
Ethanol	17.7 ± 3.4†	172.3 ± 28.2†	13.9 ± 1.9†	24.0 ± 3.3†
Ethanol + Naloxone	11.9 ± 2.7‡	169.1 ± 51.5†	13.7 ± 4.5§	26.4 ± 5.7§

90 min after administration of ethanol (2 g/kg i.p.) animals were either injected with [U-^{14}C] palmitate (1 μCi/100 mg b.w) and sacrificed 10 min later for determination of radioactivity incorporated into hepatic lipids, or sacrificed, liver slices prepared and incubated for 90 min in Krebs–Henseleit bicarbonate buffer (pH = 7.4) containing 1 μCi albumin-bound [1-^{14}C] palmitic acid. Each value represents the mean ± SD of six determinations. * $P < 0.01$ vs saline only group. † $P < 0.001$ vs saline only group. ‡ $P < 0.001$ vs saline group and N.S. vs ethanol only. § $P < 0.001$ vs both saline groups and N.S. vs ethanol only. N.S. = not significant.

Table 5. Effect of sorbitol on the hepatic redox state and lipid utilisation in the rat

Parameter	Treatment	
	Glucose	Sorbitol
Liver		
Lactate/Pyruvate	20.1 ± 2.2	42.0 ± 10.7*
3-Hydroxybutyrate/Acetoacetate	3.15 ± 0.83	4.97 ± 2.00
Glycerol-3-phosphate (nmol/g liver)	98 ± 11	294 ± 22*
ATP (nmol/g liver)	3986 ± 525	3102 ± 193
% injected [U-^{14}C] palmitate incorporated into liver lipids	8.0 ± 1.4	8.9 ± 0.9
[U-^{14}C] palmitate incorporated into liver triglycerides after *in vivo* injection (nmol/g liver × 10^3)	7.6 ± 1.3	11.7 ± 5.1
[1-^{14}C] palmitate oxidised to $^{14}CO_2$ in liver slice incubations (nmol/g liver × 10^2)	14.7 ± 1.9	15.3 ± 4.6
[1-^{14}C] palmitate incorporated into triglycerides in liver slice incubations (nmol/g liver × 10^2)	42.6 ± 17.3	101.3 ± 15.7*
Liver triglyceride (mg/g)	8.8 ± 1.6	9.7 ± 2.2

90 min after administration of glucose or sorbitol (3.5 g/kg i.p.), animals were sacrificed, freeze clamped liver samples taken for metabolite determinations, and liver slices prepared for incubation with albumin-bound [1-^{14}C] palmitic acid. In experiments where *in vivo* incorporation of [U-^{14}C] palmitate was measured, the label was injected (1 μCi/100 g b.w.) 90 min after glucose or sorbitol dosing and animals sacrificed 10 min later. Values are means ± SD. N = 5 rats per group. * $P < 0.001$ vs glucose group.

regard to a role for the redox-state in causing triglyceride accumulation, since, in separate experiments, sorbitol markedly altered the redox-state without affecting the liver triglyceride concentration (Table 5).

The experiments on the *in vivo* incorporation of radio-labelled palmitate into hepatic lipids also indicate that the redox-state change following ethanol was not responsible for the observed increase in triglyceride synthesis (Tables 2, 4 and 5). This could not be attributed to any difference between the ethanol-treated and control animals in the specific activity of ^{14}C-palmitate, since a dose of ethanol identical to that used here, has been shown to have no influence on the hepatic free fatty acid pool, serum free fatty acid concentration, or the flux of fatty acids from the peripheral tissues [19, 20]. Naloxone treatment failed to reverse the ethanol-mediated increase in the *in vivo* incorporation of palmitate into liver triglycerides, whilst sorbitol only caused a small, non-significant increase. Sorbitol also caused an increase in glycerol-3-phosphate levels *in vivo*, as was expected from the observed effects of this substance on the redox-state. However, this did not have much bearing on triglyceride formation *in vivo* (Table 5).

The data obtained from experiments with liver slices taken from animals dosed with either ethanol or sorbitol, suggest that the redox-state does not influence the hepatic fatty acid oxidising capacity. Sorbitol did not affect the ability of liver tissue to oxidise palmitate to CO_2 when values were compared with those found with control animals which were given glucose. Interpretation of the results from Naloxone-dosed rats, on the other hand, presented a problem, since the drug alone tended to impair palmitate oxidation. This effect of the drug was also associated with increased esterification of this fatty acid into triglyceride by the liver slices. This action of the drug did not appear, however, to influence incorporation of injected palmitate into liver lipids *in vivo* (Table 4). Thus, the question is raised as to whether the decreased fatty acid oxidation rate after ethanol reported here and previously [3, 21, 22], is connected with hepatic triglyceride accumulation in the whole animal. In this respect, other biochemical effects of ethanol that could be relevant include impairment of hepatic lipoprotein secretion [23], increased mobilisation of peripheral fatty acids from adipose tissue [24] or increased hepatic blood flow [19, 20]. However, in a recent study it was reported that administration of 4-pentenoic acid, a specific inhibitor of β-oxidation, to rats, increased hepatic triglyceride concentrations, the increases being quantitatively related to the degree of inhibition of fatty acid oxidation [25].

The mechanism by which Naloxone corrects the ethanol-induced hepatic redox-state changes is not clear from this study, but in view of the lack of effect of the drug on ATP concentrations, non-enzymatic oxidation of NADH or simple uncoupling of oxidative phosphorylation do not seem to be involved. The apparent inhibition of β-oxidation by the drug could be a contributory factor, since this may cause sparing of hepatic NAD. However, no effect of the drug alone on the redox-state was observed in control animals. It is clear from the serum ethanol data that inhibition of ethanol metabolism is not concerned with the action of Naloxone on the redox-state. Regarding the decrease in palmitate oxidation by

liver slices from ethanol-treated animals, it appears that the underlying mechanism may involve mitochondrial injury, rather than direct acute effects of ethanol, including changes in the redox-state, being implicated. Such mitochondrial damage could arise from covalent binding of ethanol-derived acetaldehyde with mitochondrial protein [26], or peroxidative injury to the organelle [27]. Some evidence for the relevance of these mechanisms to disturbed lipid metabolism comes from recent studies in this laboratory, in which antioxidants and thiol compounds were found to prevent many of the altered patterns of fatty acid disposal and increased hepatic triglyceride levels after an acute dose of ethanol [28]. Furthermore, these substances achieved these effects without reversal of the ethanol-induced hepatic redox-state changes, thus further supporting the present proposition that acute ethanol-induced hepatic triglyceride accumulation is not a direct consequence of increases in the hepatocellular [NADH]/[NAD^+] ratio.

In conclusion, the present studies indicate that after a moderate, acute dose of ethanol in fasted rats, triglyceride accumulation occurs in the liver independently of changes in the hepatocellular redox-state ([NADH]/[NAD^+] ratio) or the associated metabolic features, such as raised glycerol-3-phosphate concentrations. Whether this observation also holds true for the hepatic steatosis occurring after larger acute ethanol doses (e.g. 6 g/kg orally), or chronic ethanol intake cannot be discerned from the present findings. However, recent studies using the hydrogen acceptor Methylene Blue also suggest that the ethanol-induced redox-state changes are not primarily responsible for hepatic triglyceride accumulation after long-term alcohol intake in rats [4]. Studies currently in progress in this laboratory [28] suggest that direct toxic effects of acetaldehyde, and/or peroxidative damage may play a part in the altered pattern of hepatic fatty acid utilisation and triglyceride accumulation after acute ethanol doses.

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