Metabolic Effects of Ethanol on the Rabbit Heart

By T. Kikuchi, M.D., and K. J. Kako, M.D.

ABSTRACT
Ethanol in saline solution (15%, v/v) was infused into anesthetized rabbits at a rate of 0.494 ml/min for the first 12 minutes and then at 0.247 ml/min for 108 minutes. Three hours after the infusion, heart triglyceride and lipoprotein lipase were assayed. Oxidation and esterification of fatty acids (palmitate-14C as an indicator) were assayed by using either tissue homogenates or perfused hearts taken from the rabbits. Oxidation-reduction states of the perfused hearts were examined by measuring the tissue levels of dehydrogenase-linked substrates. The infusion of ethanol resulted in 180% increase in heart triglyceride content, but the infusion of norepinephrine (3 μg/kg/min) did not change the content. No change in plasma free fatty acids and triglyceride or heart lipoprotein lipase activity was detected. Addition of ethanol had little effect on the distribution of palmitate-14C in the lipids of tissue slices and homogenates. On the other hand, prior infusion of ethanol resulted in depression of 14CO2 production (70 and 50%) and enhanced fatty acid esterification into triglyceride (270 and 170%) both in homogenates and perfused hearts. Mitochondrial and cytoplasmic redox states were shifted to more oxidized states by ethanol infusion. It is postulated from these results that an accumulation of triglyceride in the rabbit heart in response to ethanol administration is a result of decreased fatty acid oxidation rather than of increased triglyceride uptake or increased fatty acid synthesis.

ADDITIONAL KEY WORDS palmitate oxidation lipoprotein lipase fatty acid esterification heart triglyceride rabbit heart perfusion mitochondrial redox state cytoplasmic redox state plasma lipids

Chronic ingestion of alcohol affects cardiac function and metabolism causing cardiomyopathy (1, 2). Histological and histochemical changes observed in this syndrome include large amounts of neutral lipid deposit (1). However, accumulation of triglyceride in the heart was found even after an acute, moderate intoxication of ethanol in experimental animals (3, 4). Furthermore, previous reports from other laboratories describe a decreased uptake of free fatty acids (FFA) and a release of zinc potassium, phosphate and enzymes from the heart (5-6). These results together with the morphological findings, suggest that ethanol may alter intermediary metabolism in myocardial cells, resulting in an accumulation of triglyceride.

The present study was made to observe the biochemical changes in rabbit hearts under the influence of a moderate dose of ethanol. The esterification of labeled palmitate was quantified by using tissue slices, homogenates and perfused hearts. Three hours after a 2-hour infusion of ethanol, a decreased oxidation and an increased esterification of exogenous fatty acid was recorded, but there was no change in lipoprotein lipase activity. Indirect evidence further indicates that triglyceride accumulation is due neither to enhanced fatty acid synthesis nor to an increased triglyceride uptake.

Materials and Methods

Albino male rabbits weighing 1.9 kg to 2.7 kg (average 2.5 kg) were anesthetized with 2.5 ml/kg of 2% chloral hydrate in 20% urethane. The jugular vein was cannulated, and 19% (v/v) ethanol in physiological saline solution was infused at a rate of 0.496 ml/min for the first 15 minutes and then at a rate of 0.247 ml/min for 108 minutes (total 1.05 g/kg). This infusion plus (3) was adapted to produce moderate intoxication (i.e., blood alcohol level of approximately 200 mg/dl ref. 7, 8). Three hours after stopping the infusion, the heart was excised and used for the following experiments: (1) triglyceride determination, (2) lipoprotein lipase assay, (3) tissue slice experiments, (4) tissue homogenate experiments, and (5) heart perfusion experiments.

Triglyceride Determination.—Tissue lipids were extracted with chloroform-methanol (2:1, v/v) according to the method of Folch et al. (9). The chloroform extract was washed twice with a mixture of chloroform-methanol-salt solution (3:47:48, v/v/v, "pure solvent upper phase"). Silicic acid was then added to the extract to remove phospholipids. Triglyceride was determined by a modification of the method based on chromotropic acid formation (10). The extraction procedure resulted in recovery of 83% of the triglycerine, 89% of the phospholipid phosphorus (11) and 73% of the palmitic acid (12). Tissue lipids were also measured in the heart of rabbits which received, instead of ethanol, norepinephrine infusion (14, 15) during ethanol infusion. Three hours after completion of the infusion the chest was opened and the heart lipid was analyzed.

Lipoprotein Lipase Activity.—This activity was assayed by a method similar to that described by Alousi and Mallov (16). Each incubating flask contained 0.5 ml of 1.5 (w/v) homogenates of the left ventricle in Krebs' phosphate buffer (pH 7.4), followed by homogenizing with a Potter-type glass and Teflon homogenizer (21). Each incubation employed 1.5 ml of this homogenate per flask. Before tissue addition, Edman many flasks with attached center wells (22), each containing 0.06 ml of potassium palmitate-4°C (specific activity; 7 to 8 X 10⁶ cpm/μg, i.e. 0.34 μEq palmitate per flask) in 0.2 ml of water were flushed with a mixture of 85% oxygen and 15% CO₂ for approximately 5 minutes. Without this aeration, a high blank value of 3°C0 was invariably observed. The tissue homogenate (about 150 mg) in buffer solution was added, and the incubation at 37°C was started in a Dubnoff metabolic shaker. In some experiments, ethanol was added to a final concentration of 12 to 15% or compound F (20 mg hydrocortisone succinate in toluene containing 10% alcohol) was added to a final concentration of 0.3 mg/kg/min for 2 hours. After 3 hours incubation at 37°C, free fatty acids (FFA) were extracted with petroleum ether-ether-acetic acid (80:20:2, v/v/v, "pure solvent upper phase"). The spots were identified by iodine vapor, then scraped off and collected in toluene containing 4% Cab-O-Sil (w/v).4 FFA in the medium were quantified (12) and their specific activity calculated.

Tissue Homogenate Experiments.—These experiments were carried out by using 700 mg of the left ventricle in 7 ml of Krebs' phosphate buffer (pH 7.4), followed by homogenizing with a Potter-type glass and Teflon homogenizer (21). Each incubation employed 1.5 ml of this homogenate per flask. Before tissue addition, Edman many flasks with attached center wells (22), each containing 0.08 ml of potassium palmitate-4°C (specific activity; 7 to 8 X 10⁶ cpm/μg, i.e. 0.34 μEq palmitate per flask) in 0.2 ml of water were flushed with a mixture of 85% oxygen and 15% CO₂ for approximately 5 minutes. Without this aeration, a high blank value of 3°C0 was invariably observed. The tissue homogenate (about 150 mg) in buffer solution was added, and the incubation at 37°C was started in a Dubnoff metabolic shaker. In some experiments, ethanol was added to a final concentration of 12 to 15% or compound F (20 mg hydrocortisone succinate in toluene containing 10% alcohol) was added to a final concentration of 0.3 mg/kg/min for 2 hours. After 3 hours incubation at 37°C, free fatty acids (FFA) were extracted with petroleum ether-ether-acetic acid (80:20:2, v/v/v, "pure solvent upper phase"). The spots were identified by iodine vapor, then scraped off and collected in toluene containing 4% Cab-O-Sil (w/v).4 FFA in the medium were quantified (12) and their specific activity calculated.

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1Ediol: 50% emulsion of coconut oil containing 12.5% sucrose, 1.5% glycerylmonostearate, and 2% polyoxyethylene sorbitan monostearate, Calbiochem.

2The tissue slices were purchased from Harvard Apparatus Co.

3Omnifluor: 98% PPO and 2% bis-MSB, New England Nuclear Corp.

4Cab-O-Sil thixotropic gel suspension powder, New England Nuclear Corp.

had been added. The reaction mixture was then brought to pH 4.5 by adding 1 ml of 1M potassium phosphate buffer (22). CO₂ was liberated by shaking the flask at 37°C for 30 minutes. Radioactivity was counted by adding the filter paper to 0.4% Omnifluor in a toluene-ethanol (2:1, v/v) mixture. In this experiment, distilled water was boiled to expel CO₂. An average of 0.5% of the label was recovered from NaH¹⁴CO₃ after completion of the entire procedure. The rate of ¹⁴CO₂ production was constant between 10 and 40 minutes of incubation under these conditions. Incorporation of palmitate-¹⁴C into tissue triglyceride and phospholipid was quantified by applying the techniques of thin-layer chromatography and liquid scintillation counting as previously described above. Protein was measured by the method of Lowry et al. (23). The specific activity of the precursor was calculated in individual experiments by using measured values (dpm and μEq) of FFA in the incubation mixture containing tissue. Quantitative distribution of fatty acids was expressed in μEq/g protein per 30 minutes by assuming that the specific activity remained constant. Initially, other incubation media containing ATP, CoA, α-glycerophosphate, reducing agents, etc. (24, 25) were studied and found not to influence the result greatly.

Perfusion Experiments.—The rabbit heart perfusion technique was previously described (26). In this experiment, 20 ml of blood was taken from a rabbit just before perfusion and mixed with the same volume of Krebs' bicarbonate buffer (pH 7.4) to which albumin-bound palmitate-¹⁴C (2 μC/S.A. = 28 μCi/μmole) was added. The perfusing apparatus, which is similar to that used by Morgan et al. (27), was filled with this perfusing solution. The heart was excised from the animal, which also served as blood donor, and attached to the perfusing system. Coronary perfusing pressure was 60 to 80 mm Hg. Perfusate samples were taken at 0 minutes and after 30 minutes of perfusion, at which time the heart was cooled and used for analysis. The perfusate was analyzed for FFA (12) and ¹⁴C, and the analytical methods for heart lipids were as described above. Incorporation of FFA was expressed in μEq/g wet weight by using the specific activity of FFA found in the perfusing solution, although there might be some objection in using palmitate alone to represent fatty acids of various chain lengths and unsaturation since the myocardium may preferentially utilize some fatty acids (28). For assessment of redox states, the heart was frozen with tongs cooled in liquid nitrogen (26). The frozen pellet was pulverized by a percussion and a porcelain mortar, then weighed in 6% HClO₄ and mixed. (VirTis homogenizer). The extract was neutralized (pH 7.4) with K₂CO₃. Dihydroxyacetone phosphate, α-glycerophosphate, malate, pyruvate, glucose-6-phosphate and α-ketoglutarate were assayed by enzymatic, fluorometric methods (29) using a metabolite fluorimeter. Glutamate and lactate were similarly assayed, using an Eppendorf photometer (29). NH₃ was determined colorimetrically by applying the indophenol reaction of amino acids (30). In a few experiments, ¹⁴CO₂ was collected as follows: The recirculating perfusion system, which was constantly oxygenated by a CO₂-O₂ mixture, was closed during the last 10 minutes of a 30-minute perfusion period. At the end of this period, 4N H₂SO₄ was injected into the system to release CO₂ into the gaseous phase. The accumulated CO₂ was flushed through the system by nitrogen gas into 15 ml of 10% KOH. The washing with

Metabolite fluorimeter was made in a workshop of the Johnson Foundation, University of Pennsylvania.
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Effect of Ethanol Infusion on the Plasma Free Fatty Acid and Triglyceride Levels

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<td>Free fatty acids</td>
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The concentration is expressed as \(\mu\)Eq/ml of plasma (means \(\pm\) SE). The number in parentheses is the number of determinations.

nitrogen was continued for 1 hour. An aliquot of KOH solution was counted in Bray's solution (31) with about 55% efficiency.

Miscellaneous.—In some experiments, plasma levels of triglyceride and FFA were measured 3 hours after the end of ethanol infusion. Plasma was extracted by using chloroform containing Florasil (activated magnesium silicate) for the triglyceride determinations (10) or by chloroform-heptane-methanol (200:150:7, v/v/v) containing silicic acid for the FFA determinations (15). All lipid solvents (ethanol, methanol, chloroform, heptane) were redistilled. This was found to be particularly important for sensitive colorimetry of the FFA. Bovine serum albumin, fraction V (Sigma) contained approximately 0.01 \(\mu\)Eq FFA/mg. Enzymes were purchased from Boehringer Corp., New York. The efficiency of counting of the radioactivity was calculated by the channels ratio method.

Results

Triglyceride content in the heart under the influence of various interventions in our experiment is shown in Figure 1. There was a significant increase in triglyceride 3 hours after the infusion of ethanol. In contrast, the infusion of norepinephrine at a rate of 2 to 3 \(\mu\)g/kg/min or that of 20 mg of compound F did not influence the myocardial triglyceride level (Fig. 1). None of these interventions influenced myocardial FFA and phospholipid levels. The prior administration of reserpine (10 or 20 mg, 20 hours before) did not prevent triglyceride accumulation by ethanol administration (32.7 \(\mu\)Eq/g, \(n = 2\)). Since FFA uptake by the heart depends on the plasma FFA concentration (28), it could have been possible that FFA or triglyceride concentrations were increased by the infusion of ethanol, resulting in an increase of the uptake. However, Table 1 shows that this is not the case. The plasma level of these lipids was not significantly changed. These values were measured at the time of heart extirpation, 3 hours after the end of infusion. However, in two cases the FFA and triglyceride levels in plasma were determined 30 and 120 minutes after start of the infusion and were found to be unchanged. This finding differs again from that reported as the effect of catecholamines (3, 6, 32, 33, 34).

Although the question regarding the penetration of triglyceride molecules across the myocardial cell membrane is still open, the most likely mode of triglyceride transport is through prior hydrolysis by lipoprotein lipase (34). To test this, the enzyme activity was measured. The results indicated that an increase in activity under the influence of ethanol infusion is unlikely, namely, the activity O.I.

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**Table 1**

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**Figure 1**

Effects of adding various concentrations of ethanol on palmitate distribution in fats of tissue slices of normal rabbit hearts. Tissue slices were incubated in the presence of ethanol (0-72 \(\mu\)M), sodium palmitate-\(1^1\)C, albumin and Krebs' phosphate buffer for 3 hours at 37°C. Incorporation in \(\mu\)Eq/g wet weight into triglyceride (TG), phospholipid (PhL) and free fatty acids (FFA) is shown. The number of experiments was 3 in each case.
Approximately 150 mg of 1:10 homogenates of rabbit left ventricle were incubated at 37°C for 30 minutes in 1.5 ml of the medium containing 0.14 μEq (0.06 μM) of potassium palmitate and Krebs' phosphate buffer (pH 7.4). The concentration of ethanol added was 24 μM. The infusion rate was 74 mg/min for the first 12 minutes, and then 37 mg/min for 102 minutes. The rabbit was killed 3 hours after cessation of the infusion. "CO₂ was collected by absorbing it with a filter paper soaked in 0.1 ml of 10% KOH after the medium pH was brought down to 4.5. The homogenates were treated with chloroform-methanol and the lipid extract was separated by thin-layer chromatography. Specific activities of the precursor in individual experiments were calculated by using the results of chemical and radiochemical determinations of fatty acids in the incubation mixture. All the above results are means of six experiments expressed as μEq/g protein ± SE.

was 90.5 ± 21.9% (mean ± SE) of the control in seven paired cases (140 ± 24.4 μEq/hour/g of the control, vs. 103.3 ± 15.2 μEq/hour/g of the ethanol-treated).

The effect of ethanol on the distribution of labeled fatty acids in the tissue slice obtained from the normal rabbit is shown in Figure 2. The addition of increasing concentrations of ethanol from 0 to 72 mM resulted in a decreasing esterification; concomitantly, an increasing proportion of the tissue label was found in the fatty acid fraction as the concentration of ethanol was raised. Since the tissue slice is a broken cell preparation and since the cellular transport of FFA is governed by the concentration, chain length and unsaturation of the fatty acids and FFA-albumin ratio (28, 34), the fatty acid uptake was presumably uninfluenced by the above experimental conditions. Therefore, these results suggest less esterification and probably less beta oxidation of palmitate as the medium ethanol concentration becomes higher. In another experiment in which 144 mM ethanol was added, 92% of the total tissue label was found in the FFA fraction as compared to 62.7 ± 2.8% at 72 mM and 62.5 ± 2.9% at 0 mM (data shown in Fig. 1). Addition of ethanol did not change the chemically determined content of heart lipids.

More isotope was incorporated into tissue lipids when homogenates were used for the experiment than when slices were used (Table 2), probably because of different methods of calculation of specific activity of the precursor and because of the presence of albumin in the slice experiment. Although the addition of ethanol, 12 to 95 mM (24 mM in Table 2), did not influence the oxidation and esterification of FFA significantly, homogenates prepared from the animal that had received the ethanol infusion incorporated more (270%) fatty acids into the triglyceride fraction than did homogenates from the normal rabbit heart. The "CO₂ production from fatty acids, on the other hand, was depressed to approximately 70% of the control level. Interestingly, the incorporation into phospholipid was uninfluenced by prior treatment with ethanol (Table 2).

In the perfused heart, the incorporation of labeled FA was even greater than that of the homogenates (Table 3). In particular, incorporation into triglyceride was more than 50-fold greater (1.96 μEq/g vs. 0.027 μEq/g). Although the medium fatty acid concentration was also greater by 13-fold (1.02 vs. 0.08 μEq/ml), the incorporation into phospholipid in the homogenate and perfusion experiments was of a similar magnitude. The labeling found in FFA and phospholipid of the heart was again not influenced by the addition of 2 mg/ml (44.5 mM) of ethanol to the perfusing fluid or by prior infusion of ethanol into the...
TABLE 3

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 8)</th>
<th>Ethanol addition (x in 3)</th>
<th>Ethanol infusion (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incorporation into triglyceride (μEq/g)</td>
<td>1.96 ± 0.18</td>
<td>2.36 ± 0.46</td>
<td>3.42 ± 0.34 (P &lt; 0.01)</td>
</tr>
<tr>
<td>Incorporation into phospholipid (μEq/g)</td>
<td>0.37 ± 0.07</td>
<td>0.45 ± 0.11</td>
<td>0.36 ± 0.08</td>
</tr>
<tr>
<td>Incorporation into free fatty acid (FFA) (μEq/g)</td>
<td>0.16 ± 0.04</td>
<td>0.21 ± 0.03</td>
<td>0.10 ± 0.02</td>
</tr>
<tr>
<td>Concentration of FFA in perfusate (μEq/ml)</td>
<td>10.2 ± 0.06</td>
<td>1.28 ± 0.20</td>
<td>1.28 ± 0.20</td>
</tr>
<tr>
<td>Specific activity of FFA in perfusate (cpm/μEq)</td>
<td>21,000</td>
<td>34,000</td>
<td></td>
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</table>

The perfusion was performed by using the heart and blood of the same rabbit. The blood was diluted 1:1 with Krebs' bicarbonate buffer. The perfusion pressure was 60 to 80 mm Hg and the duration 30 minutes. Heart lipids were extracted by chloroform-methanol and analyzed by thin-layer chromatography. Free fatty acids in the medium were determined according to Laurell and Tibbling (12). The concentration of ethanol added was 44.5%. Ethanol was infused as described in the legend of Table 2. g indicates gram of wet weight. Values are means ± SE.

rabbit from which the blood and heart were obtained for perfusion (Table 3).

In contrast, esterification to triglyceride was significantly increased by prior infusion of ethanol to 174% of the value found in the control heart (Table 3). This relative increase was comparable in magnitude to that observed in in-vivo experiments in which triglyceride concentration was determined (Fig. 1). Incorporation into triglyceride was somewhat increased in the heart perfused in the presence of ethanol, although the change was statistically insignificant (Table 3). The production of 14CO2 was semiquantitatively compared, since the work level and the heart rate were not rigidly controlled in the perfusion experiments. Nevertheless, a 10-minute CO2 collection indicated that ethanol suppressed fatty acid oxidation (from 0.122 ± 0.010, n = 3, to 0.058 ± 0.005, μEq/g/10 min, n = 2).

The ratios of redox pairs of substrates, α-glycerophosphate/dihydroxyacetone phosphate, lactate/pyruvate and glutamate/(α-ketoglutarate) × (NH4) were measured in hearts perfused under conditions similar to the above study (Fig. 3). All of the ratios tended to decrease, indicating that both cytoplasmic and mitochondrial redox states shifted to a more oxidized state (35). In other words, free pyridine nucleotides of the two compartments became more oxidized by the action of ethanol. These results again suggest that the mitochondrial fatty acid oxidation decreased, together with a decreased glycolytic flux in the cytoplasm. Simultaneously, an increase in the lactate level (93 ± 13, n = 9, to 128 ± 14, n = 5, μmole/g wet weight) and in the glucose-6-phosphate level (108 ± 18 to 200...
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± 33 μmoles/g wet weight) were observed in the hearts of alcohol-infused rabbits.

Discussion

This study demonstrates that a moderate dose of ethanol infused into rabbits induces concurrently a decrease in fatty acid oxidation and an increase in fatty acid esterification in the myocardium. These findings were observed both by using heart homogenates and hearts perfused with diluted autologous blood. The results showing a decrease in CO₂ production from fatty acids were supported by the finding that the mitochondrial redox state shifted to a more oxidized state under the influence of ethanol. Esterification data was in agreement with the increase in triglyceride content of the heart in vivo caused by the infusion of ethanol.

Although it was shown that oxidation in the tricarboxylic acid cycle in the liver was inhibited by ethanol (36, 37), such an inhibition in heart mitochondria seems unlikely. This is because (1) the inhibition of the Krebs cycle in the liver is related to the production of reducing equivalents during ethanol metabolism (37), but in the heart, the redox state was not reduced (our results), and (2) the ventricles demand a continuous supply of energy for contraction and the oxygen consumption does not decrease (3, 38). Furthermore, it was recently reported that the glucose, lactate, and pyruvate metabolism of the perfused rat heart was unchanged in the presence of a toxic concentration of ethanol (38). Thus, the myocardial usage of lactate, acetate and acetoacetate may even be increased as a consequence of their increased plasma concentration (3, 6, 39-41). This alone could cause the suppression of oxidation of palmitate-¹⁴C and enhance esterification, as shown in the isolated rat heart (42). This cannot, however, be the sole mechanism, since our experiment with tissue homogenates, in which the medium contained exogenous and perhaps endogenous fatty acids as the sole substrate, produced a change in esterification, which suggested the existence of some control mechanisms other than that mediated by acetate. Thus a mode of action of ethanol in directing the flow of fatty acid synthesis from oxidation to esterification in the heart cell remains unsolved.

Under the experimental conditions adopted in this study, the myocardial level of α-glycerophosphate tended to decrease (206 ± 37 to 146 ± 20 μmoles/g). This is in agreement with the finding of a shift to oxidation of cytoplasmic pyridine nucleotides (Fig. 3), which suggests that glycolytic flux is decreasing (42), and hence the supply of α-glycerophosphate should also be limited. The results thus indicate that the level of α-glycerophosphate does not primarily control fatty acid esterification in the heart. An increased glucose-6-phosphate level implies that the tissue citrate may be increased, as was observed when acetate availability was raised in the isolated perfused rat heart (43).

The measured dehydrogenase system indicated that the mitochondrial state was reduced. This is in contrast to the findings obtained from the hepatic actions of ethanol (36, 37). In the latter case, presumably because of the presence of powerful alcohol dehydrogenase and aldehyde dehydrogenase, both mitochondrial and cytoplasmic redox states changed dramatically to a more reduced state (37), with its numerous biochemical consequences (36, 37, 40). A portion of pyridine nucleotides is bound to cellular proteins and does not necessarily participate in the oxidation-reduction reactions (35, 43). It would be feasible, then, to observe a reduction of the total NAD/NADH of the heart by large doses of ethanol (44), whereas the determination of dehydrogenase-linked substrates, as carried out in this study, shows the oxidation of free nucleotides in the cell compartment. Since reduced states in cellular compartments are prerequisite for fatty acid synthesis to take place (34, 36), these results suggest that the increased synthesis by the action of ethanol is unlikely. Nevertheless, the possibility exists that an additional mechanism is responsible for the change in heart lipid metabolism caused by ethanol, particularly in fed rabbits, since the change in the triglyc-
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Our study provides only an indirect answer to the question whether an increase in myocardial triglyceride is the result of an increase in uptake of FFA or triglyceride. The uptake of FFA depends on their arterial level at a given concentration of albumin, a carrier of FFA (28). Since plasma FFA levels remained unchanged (Table 1), the FFA uptake presumably did not change. Rather, FFA uptake by hearts in vivo was decreased in previous studies (3, 6, 39).

Factors governing myocardial triglyceride uptake, on the other hand, have not been studied in much detail. Most studies favor hydrolysis of triglyceride prior to its entry into the myocardial cell (34). If this assumption is valid, increased uptake of triglyceride-fatty acids is the result of an increased plasma triglyceride level, increased heart lipoprotein lipase activity, or both. Neither of these predictions was substantiated in the experiments reported here, although higher doses of ethanol may cause such changes (47, 48).

Regan et al. (3, 6) suggested an increased permeability of myocardial membrane to triglyceride as a possible mechanism. However, our work provides two pieces of circumstantial evidence against the hypothesis that an increase in triglyceride penetration may be a cause of ethanol-induced lipid accumulation: namely, (1) an increase in fatty acid esterification was observed in the experiment in which homogenates were used in the absence of exogenous triglyceride, and (2) an even greater degree of esterification was observed by using the perfused heart instead of the homogenate. This would not have been the case if an appreciable amount of triglyceride uptake had occurred, since the triglyceride-fatty acids would then have diluted the palmitate-14C, resulting in an apparently low incorporation.

The heart does not appear to metabolize ethanol (38), and its addition to the incubating medium or to the perfusing fluid has been shown by our studies to be without effect. Therefore, the changes in intermediary metabolism reported here are probably not a result of direct action of ethanol but more likely mediated by the action of acetaldehyde, an alteration in membranous components of the cell (1, 3, 6), or a change in palmitoyl-CoA acyltransferase (49), for example. The influence of secondarily released catecholamines is negligible (3, 6, 32, 33, 50, and our results). Further studies are required to elucidate the functional significance of the fatty infiltration and depression of fatty acid oxidation which follow a moderate dose of alcohol.

Acknowledgment

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References

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