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EFFECT OF ETHANOL ON GLYCEROLIPIDS IN RAT LIVER

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## CHAPTER I

## REVIEW OF THE LITERATURE

### Glycerolipid Metabolism

#### Digestion and Absorption

Pancreatic lipase attacks the ester linkage of fatty acids on the α- and α'-positions of glycerides. This intestinal enzyme is specific for long-chain fatty acids and catalyzes formation of α,β-diglycerides and β-monoglycerides. Complete hydrolysis of glycerides probably occurs only after acyl migration of β-monoglycerides to form α-monoglycerides (1-3). Bile salts activate this lipase and act as an anionic detergent in emulsification of lipids to form micellar solutions which contain free fatty acids (FFA), monoglycerides and small amounts of diglycerides and triglycerides. At least 25% of ingested glyceride undergoes complete hydrolysis to glycerol and FFA. The remaining glyceride is absorbed primarily as monoglyceride, with a very small amount of diglyceride and triglyceride.

Absorption of glycerides, glycerol and FFA begins in the distal duodenum and is completed within the first 100 cm of the jejunum. Absorption across the intestinal mucosa involves a passive process and is

therefore concentration but not energy dependent (4-6). Further hydrolysis of monoglyceride is catalyzed by monoglyceride lipase in the luminal cells of the intestine (7). Triglyceride re-synthesis via the α-glycerophosphate pathway (8, 9) and by acylation of monoglycerides and diglycerides (10-13) has been observed in intestinal mucosal cells.

Dietary glycerides containing $C_{10}$ or shorter-chain fatty acids are handled in the intestine somewhat differently than glycerides containing longer-chain fatty acids. Intraluminal hydrolysis of glycerides containing $C_{10}$ or shorter-chain fatty acids is less extensive and re-esterification in the mucosal cell is much slower. These fatty acids are transported in the portal circulation to the liver where a large proportion is oxidized to carbon dioxide (14). $C_{12}$ or longer-chain fatty acids are re-esterified within the cytoplasmic matrix (15-16).

Phospholipids in the intestinal lumen are hydrolyzed by pancreatic phospholipase $A_2$, an enzyme which is specific for fatty acids esterified at the β-position of glycerol (17). Bloom (18) reported that from 60 to 100% of radioactivity administered as phospholipid-fatty acid was absorbed, but only from 15 to 30% remained in the phospholipid fraction. Artom and Swanson (19) concluded that a significant proportion of phospholipid is absorbed intact in micelles. Intestinal mucosal cells contain a lysophosphatide hydrolase which converts lysophosphatide to α-glycerophosphate (20). However, the majority of the lysophosphatide in these cells is re-acylated.

## Transport and Uptake by Tissues

Lipid transport. Glycerides, phospholipids and cholesterol are transported from the intestine as chylomicrons. Chylomicrons are small

light-scattering particles, varying in size from 0.5 to 1.0 mμ. A non-polar triglyceride core constitutes 85 to 90% of these particles and, according to one concept of structure advocated by investigators in this field, is surrounded by a monomolecular film of protein, phospholipid and cholesterol (21-22). Chylomicrons in intestinal mucosal cells are released into the extracellular space, then proceed via the central lymphatic lacteals and the thoracic duct into the systemic circulation (23).

Lipids are also transported in plasma as components of other lipoproteins. Lipoproteins may be separated by ultracentrifugation into three main fractions. The very-low-density lipoprotein fraction, which includes chylomicrons, undergoes flotation at a solvent density of 1.006 g per ml and contains approximately 55% triglyceride, 22% phospholipid, 13% cholesterol and 10% protein. The low-density or β-lipoprotein fraction is isolated in the density range from 1.006 to 1.063 g per ml and contains 11% triglyceride, 22% phospholipid, 46% cholesterol and 21% protein. The high-density or α-lipoprotein fraction is isolated in the density range from 1.063 to 1.210 g per ml and contains 6% triglyceride, 26% phospholipid, 18% cholesterol and 50% protein. Immunochemical studies have demonstrated that the protein moiety of each of these fractions is generally heterogeneous. Plasma FFA are transported as a component of these lipoproteins, although the majority are bound to albumin (24).

Uptake of plasma triglyceride. The liver appears to be the major site of chylomicron uptake from the systemic circulation (25-27). Electron microscopic studies have demonstrated that the endothelium in liver is discontinuous or fenestrated (28). Thus, chylomicrons may pass through the endothelium and enter the sub-endothelial spaces of Disse to

come into direct contact with hepatic parenchymal and reticuloendothelial cells. Chylomicron triglycerides may be hydrolyzed by lipolytic enzymes in or near the outer membrane of these cells (29) or, alternatively, chylomicrons may enter the cells by pinocytosis and then undergo lipolysis (30-31). Borgström and Jordan (32) have reported that chylomicron-triglyceride in which both the glycerol and fatty acid moieties were labeled was taken up initially by the liver as intact triglyceride. After 10 minutes, however, a rapid decrease in the ratio of glycerol to fatty acid radioactivity was observed, indicating that the triglyceride from chylomicrons was being hydrolyzed. Within 4 hours, the radioactivity in fatty acids was equally distributed between triglyceride and phospholipid, principally phosphatidylcholine. In fasted animals, almost all of the radioactivity administered as labeled chylomicrons was recovered in the liver and respiratory carbon dioxide. When a similar experiment was performed in fed animals, however, only one-third of the radioactivity was recovered within the liver, whereas one-third was found in the adipose tissue and the remainder in other organs. The initial disappearance of chylomicrons from the circulation follows a simple exponential function with a half-time in rats of from 4 to 14 minutes (32-36). Liver perfusion studies reported by Morris (37) showed that less than 5% of chylomicron fatty acid was oxidized to carbon dioxide and less than 1% was converted to glycogen and protein. The majority was released into the perfusate as glycerolipids transported as lipoproteins or albumin-bound FFA. The glycerolipid fraction contained approximately twice as much radioactivity as the FFA.

Evidence that liver contains monoglyceride lipase (38) and tri-

glyceride lipase (39-45) has been reported. Triglyceride lipase activity has been observed in liver cell membrane (29, 41) and in microsomes (44) and other subcellular fractions. This lipase may be distinct from lipoprotein lipase, an enzyme which has been demonstrated in plasma after heparin administration and also in adipose tissue, lung, heart and several other tissues (46, 47). Liver from some animal species but not others has been found to contain an enzyme with characteristics similar to those described for lipoprotein lipase. A recent report by Mayes and Felts suggests the possibility that an inactive form of this enzyme is present in liver (48).

Chylomicron uptake by extrahepatic tissue apparently differs from that described for liver. Electron microscopic studies of the capillary endothelium in extrahepatic tissues have failed to demonstrate the fenestrations observed in the endothelium of liver (49). The continuous endothelium found in extrahepatic tissues acts as a barrier for passage of intact chylomicrons. Lipoprotein lipase is bound on or near the capillary endothelium. Robinson (49) has speculated that this enzyme catalyzes an intravascular lipolysis of chylomicrons in extrahepatic tissue and consequently is primarily responsible for providing hydrolytic products of chylomicron triglyceride for utilization by these tissues.

Uptake of plasma phospholipid. Phospholipase $A_1$ (specific for the α-acyl ester) and phospholipase $A_2$ (specific for the β-acyl ester) in liver hydrolyze phosphatidylcholine and phosphatidylethanolamine to form the corresponding β- and α-lysophosphatides (50). Phospholipase $A_1$ is present mainly in the microsomal fraction and $A_2$ in the mitochondrial fraction. Lysophospholipases found primarily in the soluble fraction of

liver catalyze the hydrolysis of these lysophosphatides to form glycerophosphorylcholine and glycerophosphorylethanolamine, respectively (51, 52). Kates (53) has reported that liver also contains phospholipase C, which converts phosphatidylcholine to diglyceride and phosphorylcholine. Phospholipase D, which converts phosphatidylcholine to phosphatidic acid and choline, has not been found in mammalian tissue.

## Fatty Acid Oxidation

Oxidation of fatty acids occurs primarily in the mitochondria of liver, muscle and other tissues. The following series of reactions constitute the β-oxidation pathway by which two-carbon units are removed from the fatty acid:

1. $R\text{-}CH_2\text{-}CH_2\text{-}COOH + CoA\text{-}SH + ATP \underset{Mg^{+2}}{\overset{\text{thiokinase}}{\rightleftharpoons}} R\text{-}CH_2\text{-}CH_2\text{-}\overset{O}{\overset{\|}{C}}\text{-}S\text{-}CoA + AMP + PP$

2. $R\text{-}CH_2\text{-}CH_2\text{-}\overset{O}{\overset{\|}{C}}\text{-}S\text{-}CoA + FAD \xrightarrow{\alpha,\beta\text{-acyl-CoA dehydrogenase}} R\text{-}CH = CH\text{-}\overset{O}{\overset{\|}{C}}\text{-}S\text{-}CoA + FADH_2$

3. $R\text{-}CH = CH\text{-}\overset{O}{\overset{\|}{C}}\text{-}S\text{-}CoA + H_2O \underset{}{\overset{\text{enoyl-CoA hydrase}}{\rightleftharpoons}} R\text{-}CHOH\text{-}CH_2\text{-}\overset{O}{\overset{\|}{C}}\text{-}S\text{-}CoA$

4. $R\text{-}CHOH\text{-}CH_2\text{-}\overset{O}{\overset{\|}{C}}\text{-}S\text{-}CoA + NAD^+ \overset{\beta\text{-hydroxyacyl-CoA dehydrogenase}}{\rightleftharpoons} R\text{-}\overset{O}{\overset{\|}{C}}\text{-}CH_2\text{-}\overset{O}{\overset{\|}{C}}\text{-}S\text{-}CoA + NADH + H^+$

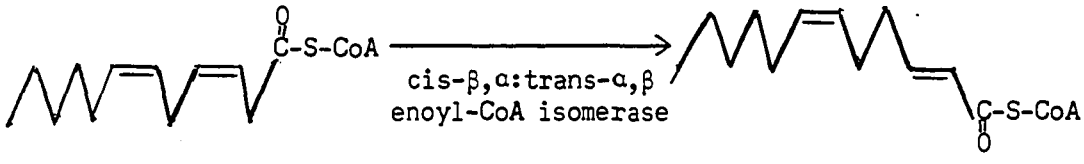
5. $R\text{-}\overset{O}{\overset{\|}{C}}\text{-}CH_2\text{-}\overset{O}{\overset{\|}{C}}\text{-}S\text{-}CoA + CoA\text{-}SH \overset{\beta\text{-ketothiolase}}{\rightleftharpoons} R\text{-}\overset{O}{\overset{\|}{C}}\text{-}S\text{-}CoA + CH_3\text{-}\overset{O}{\overset{\|}{C}}\text{-}S\text{-}CoA$

For palmitic acid, the complete sequence of reactions is outlined by the

following equation:

$$CH_3(CH_2)_{14}\text{-}COOH + ATP + 8\ CoA\text{-}SH + 7\ NAD^+ + 7\ FAD + 7\ H_2O \longrightarrow$$
$$8\ CH_3\overset{O}{\overset{\|}{C}}\text{-}S\text{-}CoA + AMP + PP + 7\ NADH + 7\ H^+ + 7\ FADH_2$$

Complete oxidation of unsaturated fatty acids follows the β-oxidation cycle for the two-carbon units on the carboxylic and terminal methyl ends of the compounds. Enoyl CoA hydrase oxidizes a double bond only when the unsaturation is in the α,β position. Hence an isomerase is frequently required to catalyze the following reaction:




Most naturally occurring polyunsaturated fatty acids have an all-cis configuration with double bonds separated by a methylene group. Hydration of an α,β double bond with cis configuration produces a D-β-hydroxyacyl-CoA. The latter substance must be converted to the L-stereoisomer by an epimerase before further oxidation by β-hydroxyacyl-CoA dehydrogenase, which is specific for the L-stereoisomer. Both these enzymes are present in mitochondria of all organs studied and are non-specific in respect to fatty acid chain length.

## Fatty Acid Biosynthesis

De novo synthesis of fatty acids in parenchymal cells of the liver is catalyzed by a multi-enzyme complex found in the cytoplasm. Saturated fatty acids, principally palmitic acid, are formed by a series of reactions involving acetyl-CoA and malonyl-CoA as substrates and acyl

carrier protein (ACP), fatty acyl transacylases and several other enzymatic activities. This sequence of reactions apparently includes the following:

1. $CH_3\text{-}\overset{O}{\overset{\|}{C}}\text{-S-CoA} + CO_2 + ATP \underset{\text{biotin} + M_n^{++}}{\overset{\text{acetyl-CoA carboxylase}}{\rightleftharpoons}} \overset{COOH}{\overset{|}{CH_2}}\text{-}\overset{O}{\overset{\|}{C}}\text{-S-CoA} + ADP + Pi$

2. $\overset{COOH}{\overset{|}{CH_2}}\text{-}\overset{O}{\overset{\|}{C}}\text{-S-CoA} + \text{ACP-SH} \overset{\text{fatty acyl transacylase}}{\rightleftharpoons} \overset{COOH}{\overset{|}{CH_2}}\text{-}\overset{O}{\overset{\|}{C}}\text{-S-ACP} + \text{CoA-SH}$

3. $CH_3\text{-}\overset{O}{\overset{\|}{C}}\text{-S-CoA} + \text{ACP-SH} \overset{\text{fatty acyl transacylase}}{\rightleftharpoons} CH_3\text{-}\overset{O}{\overset{\|}{C}}\text{-S-ACP} + \text{CoA-SH}$

4. $CH_3\text{-}\overset{O}{\overset{\|}{C}}\text{-S-ACP} + \overset{COOH}{\overset{|}{CH_2}}\text{-}\overset{O}{\overset{\|}{C}}\text{-S-ACP} \rightleftharpoons CH_3\text{-}\overset{O}{\overset{\|}{C}}\text{-}CH_2\text{-}\overset{O}{\overset{\|}{C}}\text{-S-ACP} + CO_2 + \text{ACP-SH}$

5. $CH_3\text{-}\overset{O}{\overset{\|}{C}}\text{-}CH_2\text{-}\overset{O}{\overset{\|}{C}}\text{-S-ACP} + \text{NADPH} + H^+ \rightleftharpoons CH_3\text{-}\overset{OH}{\overset{|}{C}}H\text{-}CH_2\text{-}\overset{O}{\overset{\|}{C}}\text{-S-ACP} + NADP^+$

6. $CH_3\text{-}\overset{OH}{\overset{|}{C}}H\text{-}CH_2\text{-}\overset{O}{\overset{\|}{C}}\text{-S-ACP} \rightleftharpoons CH_3\text{-}CH = CH\text{-}\overset{O}{\overset{\|}{C}}\text{-S-ACP} + H_2O$

7. $CH_3\text{-}CH = CH\text{-}\overset{O}{\overset{\|}{C}}\text{-S-ACP} + \text{NADPH} + H^+ \rightleftharpoons CH_3\text{-}CH_2\text{-}CH_2\text{-}\overset{O}{\overset{\|}{C}}\text{-S-ACP} + NADP^+$

Formation of palmitic acid involves repetition of this cycle seven times and may be expressed by the following over-all equation (54):

$$8\ CH_3\text{-}\overset{O}{\overset{\|}{C}}\text{-S-CoA} + 7\ CO_2 + 14\ NADPH + 14\ H^+ \longrightarrow CH_3\text{-}(CH_2)_{14}\text{-}COOH + 7\ CO_2 + 14\ NADP^+ + 7\ H_2O + CoA\text{-}SH$$

Biosynthesis of monounsaturated fatty acids requires a mixed function oxidase, NADP and oxygen and a stereospecific fatty acid is produced. Although linoleic and linolenic acid cannot be synthesized in mammalian tissue, these fatty acids may be converted into several other polyunsaturated fatty acids, including arachidonic acid, by chain elongation and introduction of additional double bonds. Chain elongation of fatty acids appears to be accomplished primarily in mitochondria by reversal of the β-oxidation cycle. Several of the enzymes which participate in the β-oxidation pathway are apparently involved in this sequence of reactions in which two-carbon units are added to fatty acids.

Ganguly (55), Numa et al. (56) and Martin and Vagelos (57) demonstrated that carboxylation of acetyl-CoA to form malonyl-CoA was rate-limiting in fatty acid synthesis. Langdon (58), Siperstein (59, 60) and others (61, 62) have suggested that in some instances NADPH may also be rate-limiting.

Tricarboxylic acid cycle intermediates, particularly citrate, stimulate fatty acid synthesis. Vagelos and co-workers (63) have suggested that citrate modifies the conformation of acetyl-CoA carboxylase. Citrate may also be converted to acetyl-CoA by the citrate cleavage enzyme, according to the following reaction:

$$\begin{array}{l} CH_2\text{-}COOH \\ HO\text{-}C\text{-}COOH \\ CH_2 \end{array} + ATP + CoA\text{-}SH \xrightarrow[\text{enzyme}]{\text{citrate cleavage}} CH_3\text{-}\overset{O}{\overset{\|}{C}}\text{-S-CoA} + \begin{array}{l} \overset{O}{\overset{\|}{C}}\text{-}COOH \\ CH_2\text{-}COOH \end{array}$$

Inhibition of hepatic acetyl-CoA carboxylase and citrate synthetase, an enzyme involved in citrate formation, has been observed in animals fed a diet rich in fat or when CoA-derivatives of long-chain fatty acids were added to *in vitro* systems containing these enzymes (64, 65).

## Glycerolipid Synthesis

L-α-glycerophosphate may be formed in liver either from glycerol or dihydroxyacetone phosphate. Phosphorylation of the α-hydroxy group of glycerol is catalyzed by glycerokinase in the presence of ATP and magnesium. α-Glycerophosphate may also be formed by hydrogenation of dihydroxyacetone phosphate. The latter pathway produces most of the α-glycerophosphate formed in adipose tissue and intestinal mucosa, since glycerokinase is virtually absent from these tissues. The major pathways involved in synthesis of triglyceride, phosphatidylcholine and phosphatidylethanolamine are shown in Figure 1. Alternate pathways for forming phosphatidylcholine from phosphatidylethanolamine and phosphatidylethanolamine from phosphatidylserine are also presented.

Several radioactive precursors, primarily glycerol and fatty acids, have been utilized for studying glycerolipid metabolism. Gidez and Karnovsky (66) observed that the incorporation of radioactivity into triglycerides following administration of glycerol-$C^{14}$ was considerably greater than in phospholipids. Maximal incorporation into all glycerolipids occurred during the first 30 minutes after isotope administration and was followed by an exponential decline in radioactivity which was most rapid in triglycerides.

Although glycerolipids share a common pathway for the initial steps in their biosynthesis, the fatty acid composition of phospholipids

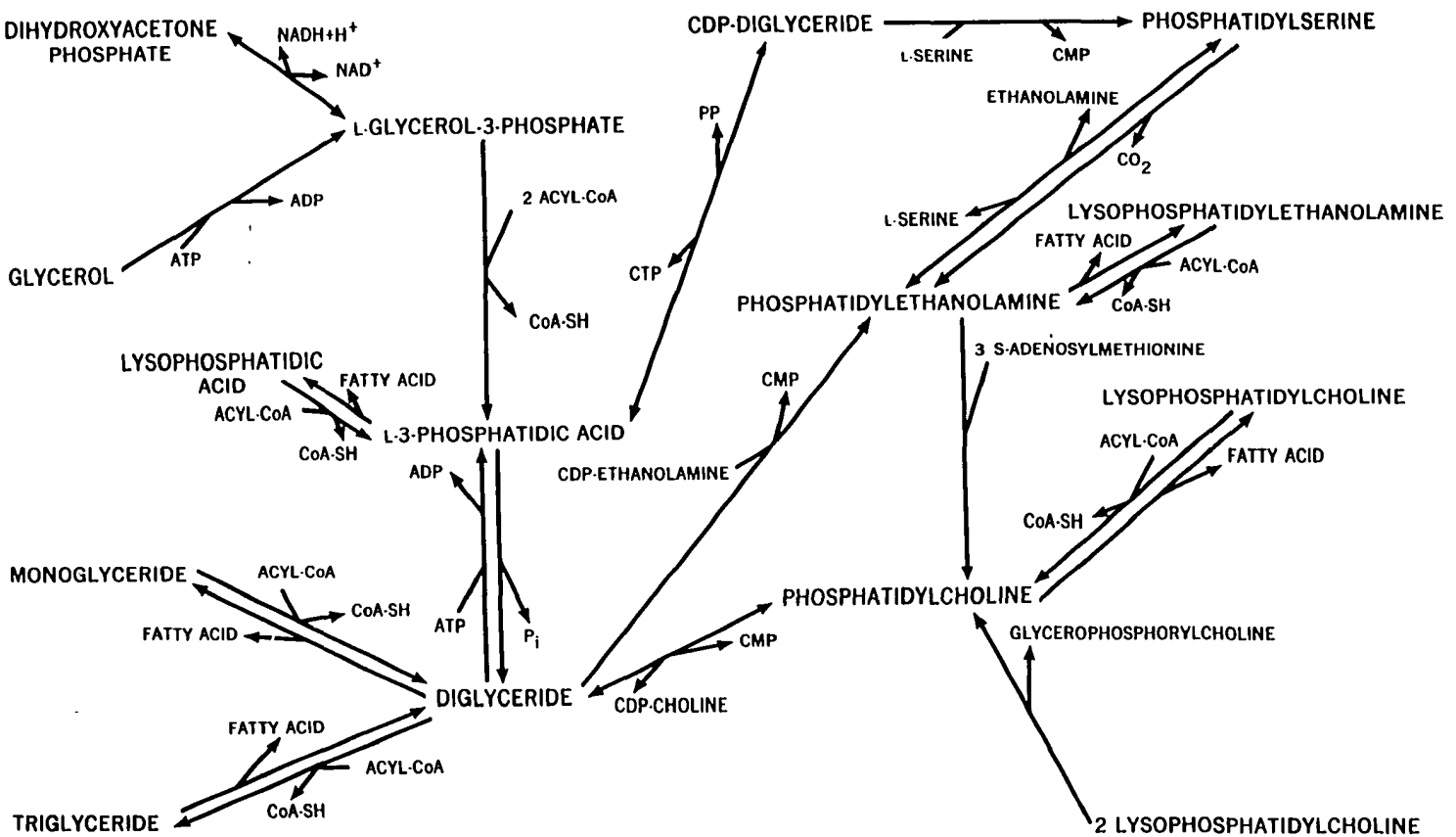



Figure 1. Pathways of glycerolipid metabolism

differs from that of triglycerides. In addition, the fatty acid composition of each lipid species may vary among different tissues. Goldman and Vagelos (67) demonstrated that the rate at which diglycerides were acylated was dependent upon the fatty acid composition of the diglyceride as well as the fatty acyl-CoA derivative utilized. Several investigators (68-70) have observed that the rate at which $C^{14}$-methyl groups were transferred to phosphatidylethanolamine varied with the fatty acid composition of the phosphatidylethanolamine. Recent work reported by Lyman and coworkers (71) indicates that phosphatidylethanolamines containing stearic and arachidonic acid are transmethylated preferentially to form the corresponding phosphatidylcholine.

Lands and associates (72-75) have described a group of fatty acyl CoA:lysophosphatide transferases in liver microsomes. These transferase activities catalyze the transfer of a series of fatty acids from their CoA-derivatives onto α- and β-lysophosphatidylcholine, lysophosphatidylethanolamine and lysophosphatidic acid. Lands has proposed that the fatty acid composition of glycerolipids is determined primarily by the specificity of this group of transferases.

## Triglyceride Metabolism in Adipose Tissue

Triglyceride turnover in adipose tissue is slow when body weight is maintained (76, 77). Studies in human subjects have demonstrated that adipose tissue triglyceride has a half-time greater than one year (78). Some selectivity in the type of fatty acid mobilized has been described (79-82) but in general the amount of each fatty acid released from the tissue parallels the concentration of that fatty acid in the tissue (78, 83).

Hydrolysis of adipose tissue triglycerides is catalyzed primarily by a mitochondrial lipase which is activated by several hormones (hence the designation "hormone-sensitive lipase"). Norepinephrine, which may be released when sympathetic nerves are stimulated, activates this adipose tissue lipase but has little effect on hepatic and muscle phosphorylase. Administration of this catecholamine, therefore, produces a greater increase in plasma FFA than in blood glucose (84, 85). Epinephrine, derived from the adrenal medulla, activates hormone-sensitive lipase of adipose tissue and also hepatic and muscle phosphorylase. Increased levels of plasma FFA as well as blood glucose are elicited by administration of this substance (86). Other hormones, including adrenal corticosteroids, aldosterone, thyroxine, corticotropin, thyroid stimulating hormone, glucagon and growth hormone, influence the lipolytic activity present in adipose tissue. Brodie and co-workers (86) have suggested that adrenal corticosteroids alter the electrolyte environment of this lipase and thereby increase its responsiveness to endogenous catecholamines. Adrenalectomized rats deprived of saline failed to respond to epinephrine. This response could be restored either by prior administration of saline or glucocorticoids. Evidence suggesting that thyroxine is involved in regulating the levels of adipose tissue lipase has been obtained using thyroidectomized rats. Lipase activity in response to epinephrine stimulation was low in these animals and did not return to normal until several days after the administration of thyroxine (87). Corticotropin has some adipokinetic activity, although the dose required to demonstrate this response is large compared with physiological concentrations (87). Thus, corticotropin probably has little direct effect on

lipolysis under normal conditions. Insulin in the presence of glucose accelerates fatty acid esterification in adipose tissue. Wood and co-workers (88) have suggested that this effect is secondary to the presence of increased amounts of α-glycerophosphate formed by insulin-stimulated glycolysis. In a glucose-free media, insulin has been reported to inhibit lipase activity (89).

Activation of lipolytic activity in adipose tissue during starvation appears to be independent of epinephrine and norepinephrine, since neither adrenalectomy nor "chemical sympathectomy" induced by the administration of hexamethonium prevents an increase in plasma FFA levels in fasted animals (90, 91). Inhibition of lipolysis produced by feeding glucose is probably due to an increase in α-glycerophosphate concentration (92), which accelerates the rate of re-esterification of FFA.

## Pathogenesis of Fatty Liver

Lipid accumulation in the liver has been observed following the administration of ethanol and various other substances, including carbon tetrachloride, ethionine, puromycin, white phosphorus, cesium, 4-amino-pyrazolopyrimidine, orotic acid, corticosteroids, corticotropin and fat mobilizing substance from the pituitary. Nutritional deficiencies involving choline, lysine, threonine, tryptophan and prolonged fasting also produce an increased hepatic content of lipids. Changes in liver morphology observed in each of these instances are similar, hence their categorization by the non-specific pathological term "fatty liver". Biochemical mechanisms which may be responsible for accumulation of lipids, particularly triglyceride, in liver include those in which an increased synthesis or a decreased removal of hepatic lipids is involved. Triglyceride

and other lipids are produced by de novo synthesis in liver. In addition, a significant amount of triglyceride can apparently be formed in liver by re-esterification of fatty acids mobilized from adipose tissue and transported to liver in blood. Excessive mobilization of fatty acids from adipose tissue is a significant factor in the fatty liver induced by corticotropin, adrenocorticosteroids (93, 94), fat mobilizing substance (95) and prolonged fasting (96). Carbon tetrachloride (97-102), white phosphorus (103), ethionine (104-107), puromycin (108) and orotic acid (109, 110) impair hepatic protein synthesis. The fatty liver induced by the latter group of substances results from a decreased rate of serum lipoprotein formation and is accompanied by low plasma lipid concentrations. Farber (111, 112) observed low ATP levels in the liver of rats given ethionine and orotic acid and suggested that this deficiency results in disaggregation of polysomes. Adenine administration reverses the fatty liver produced by these substances (113, 114). Puromycin inhibits peptide bond formation during protein synthesis on ribosomes (115).

The fatty liver found in choline deficiency is associated with a decrease in serum lipids (116-118), an increase in hepatic fatty acid oxidation (119) and no change in the rate of hepatic triglyceride biosynthesis (120). Although hepatic phospholipid turnover is increased in this deficiency, the phospholipid content of liver (121) and the rate of conversion of phosphatidylethanolamine to phosphatidylcholine are low (116). Bernhard and associates (122) have postulated that choline deficiency impairs the lipoprotein secretory mechanism. Additional evidence favoring this conclusion was reported by Lombardi (121), who also suggested that this defect is interrelated with an abnormal hepatic phospho-

lipid synthesis.

The association of liver disease and chronic alcoholism was first described by Addison in 1836 (123). One of the more prominent effects of ethanol is the accumulation of triglyceride in the liver (124-127). Hepatic glyceride content is increased within 16 hours after a single large dose and levels up to ten-fold greater than control have been observed (128, 129). Less prominent changes are those associated with increases in hepatic phospholipid, FFA and cholesterol, primarily the esterified fraction. The extent to which the latter group of lipids is increased is dependent on the amount and duration of ethanol consumption (130, 131).

Ethanol administration produces increased serum levels of triglyceride and, when larger doses are given chronically, of total cholesterol and phospholipid (132-136). Plasma FFA levels are increased during chronic ethanol administration but are generally decreased following a single large dose (136-139).

The mechanism whereby ethanol alters hepatic and serum lipids is largely unknown. Lipid accumulations in the liver were initially thought to result from the nutritional deficiencies often found in chronic alcoholics (140, 141). Evidence that ethanol may exert a more direct influence on the pathogenesis of fatty liver is provided by recent studies in which lipid accumulation was observed in liver from human subjects and laboratory animals given moderate amounts of ethanol while receiving a nutritionally-adequate diet (128, 129, 142, 143).

## Metabolism of Ethanol

Only 2 to 10% of ethanol absorbed from the intestine is elimi-

nated through the kidneys and lungs. Dehydrogenases present primarily in the soluble cytoplasmic portion of liver cells oxidize the remaining 90 to 98% sequentially to acetaldehyde and acetate (144). Oxidation of the NADH formed in the conversion of ethanol to acetate involves intermediate carriers, i.e., β-hydroxybutyrate and α-glycerophosphate, to transport hydrogen across the mitochondrial membrane (145). Increased levels of hepatic α-glycerophosphate have been observed following ethanol administration (146-148).

# CHAPTER II

## INTRODUCTION

The present study was undertaken to investigate the effect of chronic ethanol administration on rat liver glycerolipids. Specific objectives include investigating the effect of ethanol on (1) the concentrations of individual glycerolipids present in liver and plasma, (2) the fatty acid composition of triglyceride, phosphatidylcholine and phosphatidylethanolamine in liver from rats fed diets containing corn oil or coconut oil as the sole source of fat, and (3) the fatty acyl-CoA:lysophosphatidylcholine and fatty acyl-CoA:lysophosphatidylethanolamine transferase activities in rat liver microsomes.

# CHAPTER III

## EXPERIMENTAL PROCEDURES

### General Procedure

Male Holtzman rats, 4 to 6 months of age, weighing 350 to 450 g, were divided into two groups. Rats in each group were fed a liquid diet containing the ingredients listed in Table 1, in an amount sufficient to maintain body weight constant. These diets, which are similar to those described by Lieber and associates (128), provided approximately 63% of total calories as carbohydrate (corn dextrose, Clinton Corn Processing Co.), 18% as fat [corn oil (Corn Products Co.) was the sole source of fat calories in all diets except those in which the substitution of coconut oil (Mangus Mabee and Reynard, Inc.) is indicated subsequently] and 19% as protein (vitamin-free casein from Nutritional Biochemical Corp.). Salts and minerals (Nutritional Biochemical Corp.), cod liver oil (E. R. Squibb and Sons) and additional vitamins and co-factors were added and the diet mixture was emulsified with powdered tragacanth gum (S. B. Penick Co.). Animals in the ethanol-treated group were fed a diet identical to that given the control group except that 95% ethanol (U. S. Industrial Chemical Co.) was substituted isocalorically for dextrose to provide approximately 40% of total calories, assuming a caloric equivalent of 7 calories of metabolic energy per g for 100% ethanol. Each rat was main-

TABLE 1

COMPOSITION OF DIET[a]

| | Control | Ethanol |
|---|---|---|
| | g/liter | g/liter |
| Dextrose (corn) | 250.1 | 93.0 |
| Ethanol | 0.0 | 89.8 |
| Corn oil or coconut oil | 30.9 | 30.9 |
| Vitamin-free casein | 75.6 | 75.6 |
| Salts and minerals (Mixture USP XIV) | 7.6 | 7.6 |
| Vitamin and co-factor mixture[b] | 1.7 | 1.7 |
| Cod liver oil | 3.2 | 3.2 |
| Powdered tragacanth gum | 14.0 | 14.0 |
| Water | 697.7 | 697.7 |

[a]Diet contained 1.58 calories per ml. Approximately 50 ml was required daily for maintaining body weight constant.

[b]The vitamin and co-factor mixture contained thiamine hydrochloride 0.25 g, riboflavin 0.50 g, pyridoxine hydrochloride 0.25 g, calcium pantothenate 2.00 g, niacinamide hydrochloride 1.00 g, choline chloride 100.00 g, biotin 0.01 g, Vitamin $B_{12}$ 0.02 g, folic acid 0.10 g, inositol 30.00 g, menadione 0.10 g and vitamin-free casein 258.48 g.

tained on the formula diet for a period of 4 to 6 weeks and then sacrificed by decapitation or exsanguination via the abdominal aorta. Body weight increased by 0.1% of initial value in the control group and decreased by 0.7% in the ethanol-treated group.

## Isolation and Analysis of Hepatic and Serum Lipids

The liver from each rat was perfused immediately at the time of sacrifice with cold, isotonic saline. A portion of each liver was homogenized in a Waring blender with 10 ml of a solvent mixture containing chloroform and methanol (Fisher Scientific Co.) (2:1, v/v) per g of wet liver. Serum samples were extracted using 10 ml of the chloroform-methanol solvent mixture per ml of serum. The chloroform-methanol soluble portion was removed, the residue extracted three additional times with volumes of solvent mixture equal to that used in the initial extraction, and the extracts combined. The final residue was weighed and samples taken for nitrogen and deoxyribonucleic acid estimation. Nitrogen was determined by the Kjeldahl method (149) and deoxyribonucleic acid (DNA) according to the diphenylamine method of Burton (150).

The chloroform-methanol soluble extracts were analyzed for glyceride by the method of Van Handel and Zilversmit (151), lipid phosphorus by the method of Gerlach and Deuticke (152), and total and free cholesterol by the method of Schoenheimer and Sperry (153).

Triglyceride from liver was isolated by thin-layer chromatography on Silica Gel G (E. Merck Co.). Samples of the chloroform-methanol soluble extract were separated in a solvent mixture containing petroleum ether and diethyl ether (Fisher Scientific Co.) (95:5, v/v). The silica

gel containing triglyceride was removed from the chromatography plate and extracted with the chloroform-methanol solvent mixture.

Phospholipids in liver and serum were separated by two-dimensional thin-layer chromatography on Silica Gel G. Samples of the chloroform-methanol soluble extract were separated sequentially in a solvent system containing chloroform, methanol and water (80:35:4, v/v) and a second solvent system containing chloroform, methanol, water and glacial acetic acid (Fisher Scientific Co.) (80:30:2:4, v/v). Good separation of phosphatidylcholine, phosphatidylethanolamine, lysophosphatidylcholine and sphingomyelin was obtained. The silica gel containing individual phospholipid fractions was removed from the chromatography plate and analyzed directly for phosphorus. Results obtained in quantitating individual phospholipids were utilized only from those analyses in which total lipid phosphorus recoveries after separation were at least 95% of the lipid phosphorus value of the sample before separation.

## Lipid Composition of Liver and Serum

Liver and serum from ten control and ten ethanol-treated rats were analyzed for triglyceride, total and free cholesterol and individual phospholipids, following the procedure outlined above.

## Synthesis of Hepatic Triglyceride, Phosphatidylcholine and Phosphatidylethanolamine

Ten microcuries of glycerol-U.L.-$C^{14}$ (New England Nuclear Corp.) was injected into the femoral vein of each of thirty control and thirty ethanol-treated rats under light ether anesthesia. The animals were then divided by a random number technique into six sub-groups and subsequently sacrificed at 15, 30, 45, 70, 90, 120 and 240 minutes after

isotope injection. The liver from each of these animals was frozen immediately in liquid nitrogen to minimize postmortem changes. Lipids were extracted and the triglyceride, phosphatidylcholine and phosphatidylethanolamine fractions isolated by the procedures outlined above. Quantitative chemical analyses were performed on samples from each lipid fraction.

Radioactivity in each fraction was determined using a Nuclear-Chicago Model 701 liquid scintillation counter. Samples of the triglyceride fraction for liquid scintillation counting were dissolved in 10 ml of toluene (Fisher Scientific Co.) containing 2,5-diphenyloxazole (PPO) and 1,4-bis-[2-(5-phenyloxazolyl)]-benzene (POPOP) (Packard Instrument Co.). Samples of the phosphatidylcholine and phosphatidylethanolamine fractions, which have a limited solubility in toluene, were first dissolved in 1.0 ml of methanol and then an aliquot of the toluene solution containing PPO and POPOP was added. Quenching was estimated using both an internal standard and the channels ratio method (154) and appropriate corrections were made to express radioactivity measurements as disintegrations per minute (dpm). Specific and total radioactivities of each lipid fraction were calculated and plotted against time.

## Turnover of Hepatic Triglyceride, Phosphatidylcholine and Phosphatidylethanolamine

Thirty-five control and thirty-five ethanol-treated rats were each given 120 μCi of glycerol-2-$H^3$ (New England Nuclear Co.) by intraperitoneal injection. The animals were divided into six sub-groups using a random number technique and sacrificed at 48, 72, 96, 120 and 144 hours after isotope injection. Triglyceride, phosphatidylcholine and phosphatidylethanolamine fractions were isolated from each liver and quantita-

ted by the chemical and radioactivity analyses described above. Total and specific radioactivities for each glycerolipid fraction were plotted against time and a regression analysis performed to determine the half-time values.

## Fatty Acid Composition of Hepatic Triglyceride, Phosphatidylcholine and Phosphatidyl-ethanolamine

Two groups of twenty control and twenty ethanol-treated rats were each equally divided into four sub-groups. One sub-group from each group was fed a diet for 46 days in which either corn oil or coconut oil was the sole source of fat. The fatty acid composition of corn oil is approximately 60% linoleic acid and less than 2% myristic and shorter-chain length fatty acids, whereas that of coconut oil is approximately 2% linoleic acid and 78% myristic and shorter-chain length fatty acids (Table 2). The dietary fat for the remaining two sub-groups of each group was provided either by coconut oil for 42 days and then corn oil for the final 4 days, or by corn oil for 42 days and then coconut oil for the final 4 days. Triglyceride, phosphatidylcholine and phosphatidyl-ethanolamine fractions were isolated from liver and a specimen of mesenteric adipose tissue obtained from each animal, following the procedures already described. Samples of each fraction were subjected to hydrolytic methanolysis using boron trifluoride in methanol (Applied Science Co.), according to the method of Morrison and Smith (155). The fatty acid methyl esters derived from this reaction were quantitated by gas-liquid chromatography using a Barber-Colman Model 5000 gas-liquid chromatograph, with a 6-foot, U-shaped glass column and a strontium-90 detector. The column was packed with Chromasorb W, 80 to 100 mesh, coated with 15% di-

*TABLE 2*

FATTY ACID COMPOSITION OF CORN OIL AND COCONUT OIL[a]

| Fatty Acid | Corn Oil | Coconut Oil |
|---|---|---|
| $C_{8:0}$ | - | 39.9 |
| $C_{10:0}$ | - | 22.1 |
| $C_{12:0}$ | < 1 | 12.0 |
| $C_{14:0}$ | < 1 | 3.8 |
| $C_{16:0}$ | 10.4 | 10.5 |
| $C_{18:0}$ | 1.2 | 4.0 |
| $C_{18:1}$ | 26.0 | 5.7 |
| $C_{18:2}$ | 60.0 | 2.1 |
| > $C_{18:2}$ | 2.4 | - |

[a]Percent distribution of fatty acids analyzed by gas-liquid chromatography.

ethylene glycol succinate (Applied Science Co.). Separations were performed at a column temperature of 172° C, an injector temperature of 192° C and a detector temperature of 182° C, using argon (64 ml per minute at a pressure of 13.0 psi) as carrier gas. Individual peaks recorded during the separation were identified by comparison with retention times of methyl esters prepared from reference fatty acids. The area under each fatty acid peak was estimated by triangulation and expressed as a percent of the total fatty acid present.

## Assay of Fatty Acyl-Coenzyme A:Lysophosphatide Transferase Activities in Liver Microsomes

### Isolation of Microsomal Fraction from Liver

Liver from five control and five ethanol-treated rats were perfused immediately with a cold, 0.25 M sucrose solution containing 1 mM ethylenediaminetetraacetic acid disodium salt (EDTA), pH 7.4. Each liver was weighed and a portion obtained for lipid analyses. The remainder was homogenized in cold, sucrose-EDTA solution using a glass, Caputto-McCay hand homogenizer. The homogenate was centrifuged at 20,000 x g for 20 minutes in a Servall Model RC-2 centrifuge set at 4° C. The supernatant portion was decanted and centrifuged at 100,000 x g for 1 hour in a Beckman Spinco Model L-2 ultracentrifuge set at 4° C. The latter precipitate, containing the microsomal fraction, was suspended in a volume of sucrose-EDTA solution equal to one-half the wet weight of liver utilized for homogenization and a sample removed for estimation of protein concentration by the Lowry method (156).

## Preparation of Substrates

CoA-derivatives of palmitic, stearic, oleic, linoleic and arachidonic acid (Sigma Chemical Co.) were prepared according to the method of Goldman and Vagelos (67). Gas-liquid chromatographic analysis of each fatty acid demonstrated a single peak with the appropriate retention time. Equal parts of fatty acid and ethyl chloroformate (Eastman Organic Chemical Co.), dissolved in tetrahydrofuran (Eastman Organic Chemical Co.), were reacted at room temperature to form a mixed fatty acyl anhydride in a minimum yield of 95% of theoretical. Four parts of the fatty acyl mixed anhydride, dissolved in tetrahydrofuran, and one part of an aqueous solution of the purified lithium salt of reduced Coenzyme A (Pabst Laboratory) were reacted at 34° C, pH 7.5 to 8.0, under a nitrogen atmosphere. The fatty acyl-CoA derivative was precipitated with cold 10% perchloric acid (Fisher Scientific Co.) and the adenine to fatty acid ratio determined. Adenine was estimated by measuring the absorbance peak at 260 mμ in a Cary Model 14-PM recording spectrophotometer. Fatty acids were quantitated by gas-liquid chromatography, using palmitic or stearic acid as an internal standard. Ratios of adenine to fatty acid for the five fatty acyl-CoA derivatives were from 0.93 to 1.04. Yields of fatty acyl-CoA derivatives were from 8 to 15% of theoretical.

The α-acyl derivatives of L-α-glycerophosphorylcholine and L-α-glycerophosphorylethanolamine, hereinafter designated as α-lysophosphatidlycholine and α-lysophosphatidylethanolamine, respectively, were obtained from Pierce Laboratories. Information provided by the vendor indicates that these compounds were prepared by enzymatic deacylation of purified egg phosphatidylcholine and phosphatidylethanolamine. Thin-

layer chromatography of the sample of α-lysophosphatidylcholine demonstrated a single area which reacted with iodine and Dragendorff reagent for choline (157). Similar analysis of the sample of α-lysophosphatidylethanolamine revealed a major area and a faint minor area, with a slightly lower $R_f$ value than the major area. Both reacted with ninhydrin and iodine.

The β-acyl derivatives of L-α-glycerophosphorylcholine and L-α-glycerophosphorylethanolamine, hereinafter designated as β-lysophosphatidylcholine and β-lysophosphatidylethanolamine, respectively, were prepared from the corresponding plasmalogens. Phosphatidalcholine was isolated from beef heart and phosphatidalethanolamine from beef brain. Approximately 1.5 kg of beef heart and 1.0 kg of beef brain were each extracted with 20 volumes of a solvent mixture containing chloroform and methanol (2:1, v/v). The chloroform-methanol soluble portion was evaporated to dryness in vacuo at room temperature using a rotating evaporator and the residue dissolved in diethyl ether. Two volumes of this ether solution was mixed with ten volumes of acetone (Fisher Scientific Co.) and one volume of a solution containing 50% magnesium chloride in ethanol. The precipitate which formed was washed several times with a mixture containing acetone and 50% magnesium chloride in ethanol (10:1, v/v). This phospholipid-containing precipitate was dissolved in chloroform and separated using silicic acid column chromatography. Silicic acid (Mallinckrodt Chemical Co.), 100 to 200 mesh, was activated at 120° C for 3 hours. A slurry of activated silicic acid in chloroform was placed in a 2.5 x 45 cm glass column. The amount of the phospholipid fraction applied to each column was adjusted to provide approximately 1 mg of phos-

phorus per g of silicic acid. Flow was regulated at 1.0 to 1.25 ml per minute and the eluate was collected using a Buchler Model 3C-4004 automatic fraction collector. Sequential elution was performed in a 4° C cold room, using chloroform followed by mixtures containing 5, 10, 20 and 30% methanol in chloroform. Samples of each eluate fraction were analyzed by thin-layer chromatography and those fractions which demonstrated a single area with the appropriate mobility for phosphatidylcholine and phosphatidylethanolamine were combined individually. Neither silicic acid nor thin-layer chromatography separates phosphatidalcholine from phosphatidylcholine or phosphatidalethanolamine from phosphatidylethanolamine. Quantitation of plasmalogen performed by potentiometric titration using the method described by Norton (158) indicated that phosphatidalcholine contained approximately 60% of the phosphorus in the phosphatidylcholine fraction and phosphatidalethanolamine contained approximately 30% of the phosphorus in the phosphatidylethanolamine fraction. Phosphatidalcholine and phosphatidalethanolamine were converted to β-lysophosphatidylcholine and β-lysophosphatidylethanolamine, respectively, just prior to use by treating the plasmalogen with iodine according to the procedure described by Lands and Merkl (72). Further purification of the β-lyso derivatives was not attempted since Lands and Merkl (72) reported that extensive acyl migration, resulting in a mixture of α- and β-lyso derivatives, had been obtained when the β-lyso derivatives were separated by silicic acid chromatography. These investigators demonstrated however that the addition of phosphatidylcholine did not affect the rate at which lysophosphatidylcholine was acylated.

Procedure for Assay of Transferase Activity

Transferase activity was assayed by a modification of the procedure described by Lands and co-workers (75). Five milligrams of the α- or β-lyso derivative of phosphatidylcholine or phosphatidylethanolamine was dissolved in a small volume of chloroform and mixed with 10 ml of a 3% aqueous solution of gum arabic. The chloroform was evaporated in vacuo at room temperature. The assay mixture contained, in a final volume of 1.0 ml, 30 mμmoles of the lysophosphatide suspended in 3% gum arabic, 30 mμmoles of the fatty acyl-CoA derivative, 0.2 ml of microsomal fraction containing 0.10 to 0.25 mg of protein, 0.1 ml of a 0.01 M solution (pH 7.0) of the sodium salt of 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTN) (Sigma Chemical Co.) and 70 μmoles of Tris buffer, pH 7.4. DTN reacts with reduced thiol groups to form a product which absorbs at 414 mμ. Transferase activity in the assay mixture was measured by monitoring the appearance of reduced Coenzyme A, a product of the transferase-catalyzed acylation reaction. Optical density at 414 mμ was recorded continuously during the first several minutes of reaction using a Beckman Model DU spectrophotometer equipped with a Sargent multiple sample recorder. A linear increase in optical density was observed during the initial period of reaction, as demonstrated by the representative example shown in Figure 2. The linear portion of each curve was evaluated to determine maximal velocity (maximal change in optical density during a 1-minute period). The maximal velocity values from duplicate assays were averaged and the latter values used in calculating rates. These results are presented as mμmoles of Coenzyme A released per minute per mg of microsomal protein. Microsomal acyl-CoA hydrolase activity for each

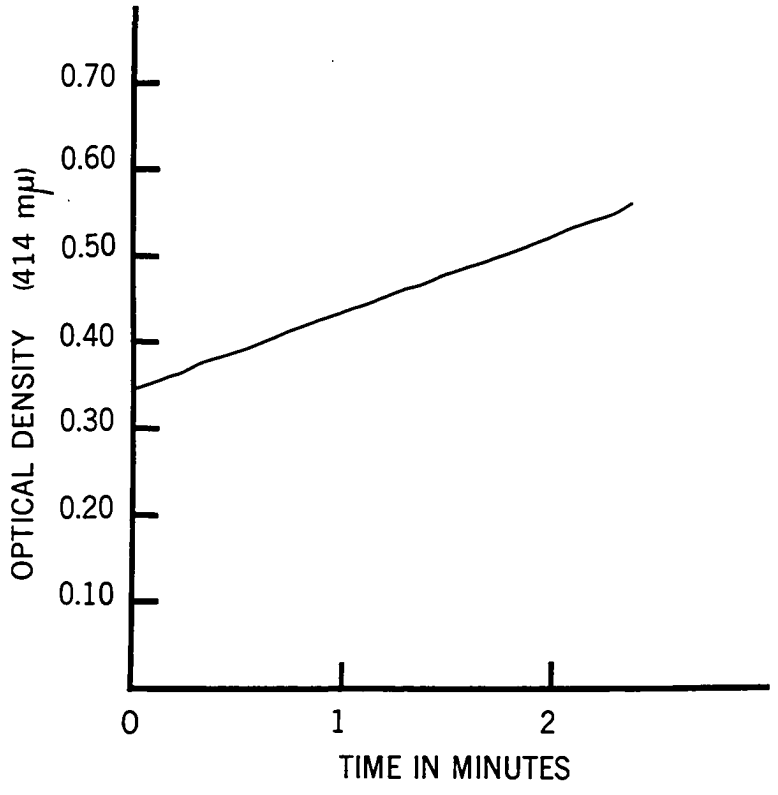



Figure 2. Time course of a representative assay of fatty acyl-Coenzyme A:lysophosphatide transferase activity in rat liver microsomes.

This assay mixture contained, in a final volume of 1.0 ml, 30 mμmoles of β-lysophosphatidylcholine suspended in 3% gum arabic, 30 mμmoles of oleyl-CoA, 0.2 ml of microsomal fraction (containing 0.116 mg of protein) from the liver of a control, 0.1 ml of 0.01 M DTN solution and 70 μmoles of Tris buffer, pH 7.4.

fatty acyl-CoA derivative was measured using an assay mixture from which the lysophosphatide was omitted. Transferase activity observed for each fatty acyl-CoA derivative was corrected for acyl-CoA hydrolase activity by subtracting the value obtained in the assay mixture containing no lysophosphatide from that obtained in the complete assay mixture.

# CHAPTER IV

# RESULTS

## Effect of Ethanol on Hepatic and Serum Glycerolipids

Chronic administration of ethanol to rats produced an increase in hepatic triglyceride, cholesterol and phospholipid (Table 3). The most pronounced change was observed in the hepatic content of triglyceride, which increased by 383% of control ($p < 0.001$), and esterified cholesterol, which increased by 964% ($p < 0.001$). A significant increase was also observed in the total content of hepatic phospholipid (32% of control, $p < 0.001$), as well as in phosphatidylcholine (38%, $p < 0.001$), phosphatidylethanolamine (26%, $p < 0.001$) and sphingomyelin (79%, $p < 0.001$). The magnitude of the increment in phospholipids, however, was much less than that observed in triglyceride and esterified cholesterol. Changes produced by ethanol in the concentration of individual hepatic lipid fractions, calculated relative to wet weight of liver, were similar qualitatively to those observed in the total content of these lipids in liver. The wet weight of liver from ethanol-treated rats increased by 14% of control ($p < 0.01$) and the protein content by 66% ($p < 0.01$) (Table 4). Hepatic DNA content was similar in control and ethanol-treated rats.

Increased serum concentrations of triglyceride (107% of control,

TABLE 3

EFFECT OF ETHANOL ON HEPATIC LIPID COMPOSITION[a]

| | Total Content (mg) | | | Percent of Total | | Concentration (mg/g wet wt) | | |
|---|---|---|---|---|---|---|---|---|
| | Control | Ethanol | p[b] | Control | Ethanol | Control | Ethanol | p[b] |
| Triglyceride | 71.5±4.20 | 345.4±52.50 | 0.001 | | | 7.2±0.55 | 30.7±4.44 | 0.001 |
| Cholesterol | | | | | | | | |
| Total | 17.6±0.98 | 53.3± 4.96 | 0.001 | | | 1.7±0.15 | 4.0±0.65 | 0.01 |
| Free | 14.1±1.07 | 21.3± 2.66 | 0.02 | 82.0 | 39.5 | 1.4±0.10 | 1.6±0.18 | ns |
| Ester | 3.0±0.46 | 32.0± 5.91 | 0.001 | 18.0 | 60.5 | 0.3±0.05 | 2.4±0.47 | 0.001 |
| Phospholipid | | | | | | | | |
| Total | 191.4±4.47 | 252.4± 5.33 | 0.001 | | | 16.7±1.03 | 21.0±2.30 | ns |
| Phosphatidyl-choline | 100.1±2.30 | 138.3± 3.66 | 0.001 | 52.3 | 54.8 | 8.7±0.54 | 11.7±0.50 | 0.001 |
| Phosphatidyl-ethanolamine | 49.1±1.17 | 61.9± 0.99 | 0.001 | 25.7 | 24.9 | 4.3±0.26 | 5.2±0.18 | 0.01 |
| Sphingomyelin | 8.9±0.65 | 15.9± 0.95 | 0.001 | 3.0 | 4.0 | 0.8±0.09 | 1.4±0.15 | 0.01 |

[a]Values are presented as the mean, ± standard error. Samples from 10 rats in each group were analyzed for triglyceride and phospholipids; samples from 7 rats in each group were analyzed for cholesterol.

[b]Statistical significance of difference between mean values obtained in ethanol-treated and control groups; ns indicates difference is not significant at 0.05 level.

TABLE 4

EFFECT OF ETHANOL ON HEPATIC WEIGHT AND PROTEIN AND DNA CONTENT[a]

| | Control | Ethanol | p[b] |
|---|---|---|---|
| Liver Weight (g) | 11.3±0.53 | 14.1±0.66 | 0.01 |
| Protein (g) | 1.4±0.12 | 2.3±0.22 | 0.01 |
| DNA (mg) | 16.9±1.41 | 17.2±3.55 | ns |

[a]Values are presented as the mean, ± standard error. Liver weights were obtained for all 10 rats in each group. Samples from 7 rats in each group were analyzed for protein; samples from 5 rats in each group were analyzed for DNA.

[b]Statistical significance of difference between mean values obtained in ethanol-treated and control groups; ns indicates difference is not significant at 0.05 level.

$p < 0.01$), cholesterol (110%, $p < 0.001$) and phospholipid (42%, $p < 0.001$) were found in the rats given ethanol (Table 5). The proportion of serum cholesterol present in the ester fraction was lower in the ethanol-treated rats than in the control group. The proportions of serum phospholipid present as phosphatidylcholine and phosphatidylethanolamine were increased in ethanol-treated rats, whereas the relative amount of sphingomyelin was unchanged and lysophosphatidylcholine was decreased.

## Effect of Ethanol on Glycerolipid Synthesis

The incorporation of radioactivity from glycerol-U.L.-$C^{14}$ into hepatic glycerolipids is presented in Figures 3 and 4. Specific radioactivities of hepatic triglyceride (Fig. 3) were greater, usually by a substantial amount, than those of hepatic phosphatidylcholine and phosphatidylethanolamine (Fig. 4). Maximal incorporation into triglyceride occurred within 30 minutes after glycerol administration. During the first 2-hour period after isotope administration, the total radioactivity incorporated into hepatic triglyceride was 56% greater in ethanol-treated rats than controls (Table 6).

The total radioactivity incorporated into phosphatidylcholine in control and ethanol-treated rats increased rapidly and reached a peak at 30 minutes after isotope administration (Fig. 4). A second peak in these incorporation curves was observed at 90 minutes. Approximately 35% more radioactivity was incorporated into phosphatidylcholine in the ethanol-treated rats than in controls during the first 2-hour period after isotope injection (Table 6).

The incorporation curves for hepatic phosphatidylethanolamine from control and ethanol-treated rats were relatively flat, although a

TABLE 5

EFFECT OF ETHANOL ON SERUM LIPIDS[a]

| | Concentration (mg/100 ml) | | | Percent of Total | |
|---|---|---|---|---|---|
| | Control | Ethanol | p[b] | Control | Ethanol |
| Triglyceride | 24.1±2.07 | 49.9± 7.21 | 0.01 | | |
| Cholesterol | | | | | |
| Total | 47.0±2.45 | 98.5± 8.65 | 0.001 | | |
| Free | 9.5±1.29 | 41.4± 6.40 | 0.01 | 20.4 | 43.6 |
| Ester | 37.5±2.87 | 57.1±11.84 | ns | 79.6 | 56.4 |
| Phospholipid | | | | | |
| Total | 113.3±4.64 | 160.6± 5.01 | 0.001 | | |
| Phosphatidylcholine | 57.4±2.35 | 94.4± 2.94 | 0.001 | 50.7 | 58.8 |
| Phosphatidylethanolamine | 2.0±0.08 | 4.3± 0.13 | 0.001 | 1.8 | 2.7 |
| Sphingomyelin | 10.0±0.41 | 13.7± 0.43 | 0.001 | 8.8 | 8.5 |
| Lysophosphatidylcholine | 38.2±1.56 | 37.9± 1.18 | ns | 33.7 | 23.6 |

[a]Values are presented as the mean, ± standard error. Samples from 18 rats in each group were analyzed for triglyceride and phospholipids; samples from 5 rats in each group were analyzed for cholesterol.

[b]Statistical significance of difference between values obtained for ethanol-treated and control groups; ns indicates difference is not significant at 0.05 level.

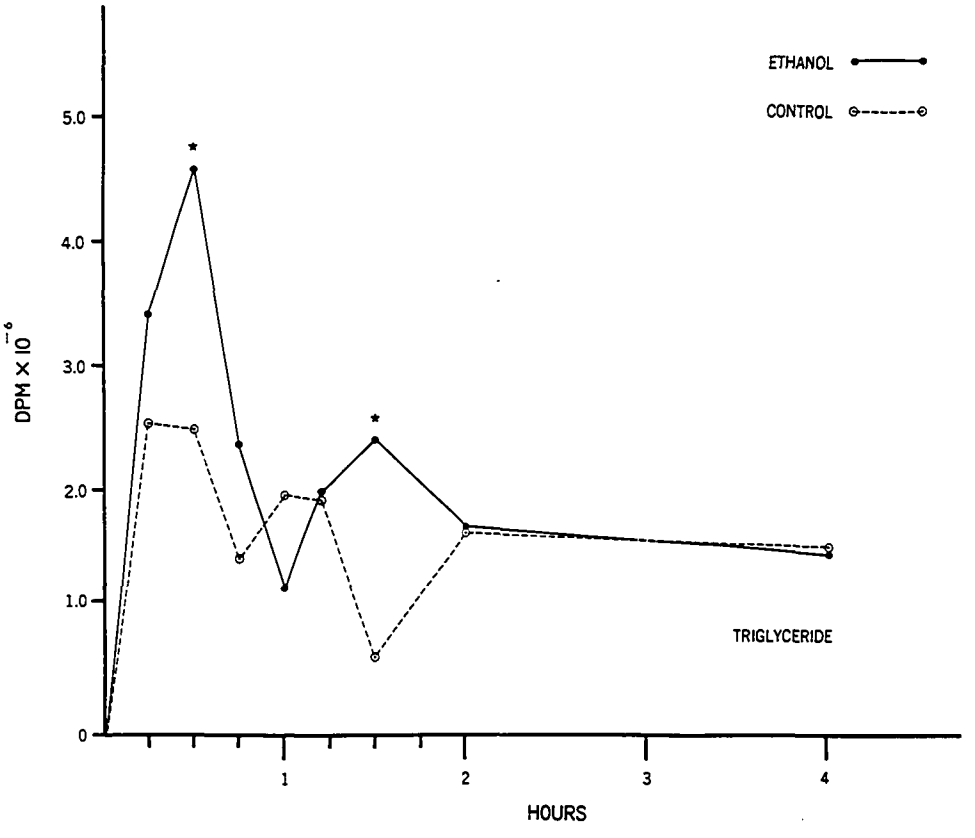



Figure 3. Effect of ethanol on incorporation of radioactivity into hepatic triglyceride in rats given glycerol-U.L.-$C^{14}$. Each point represents the mean of results obtained in five animals.

* - Indicates that the difference between control and ethanol-treated groups was statistically significant ($p < 0.05$).

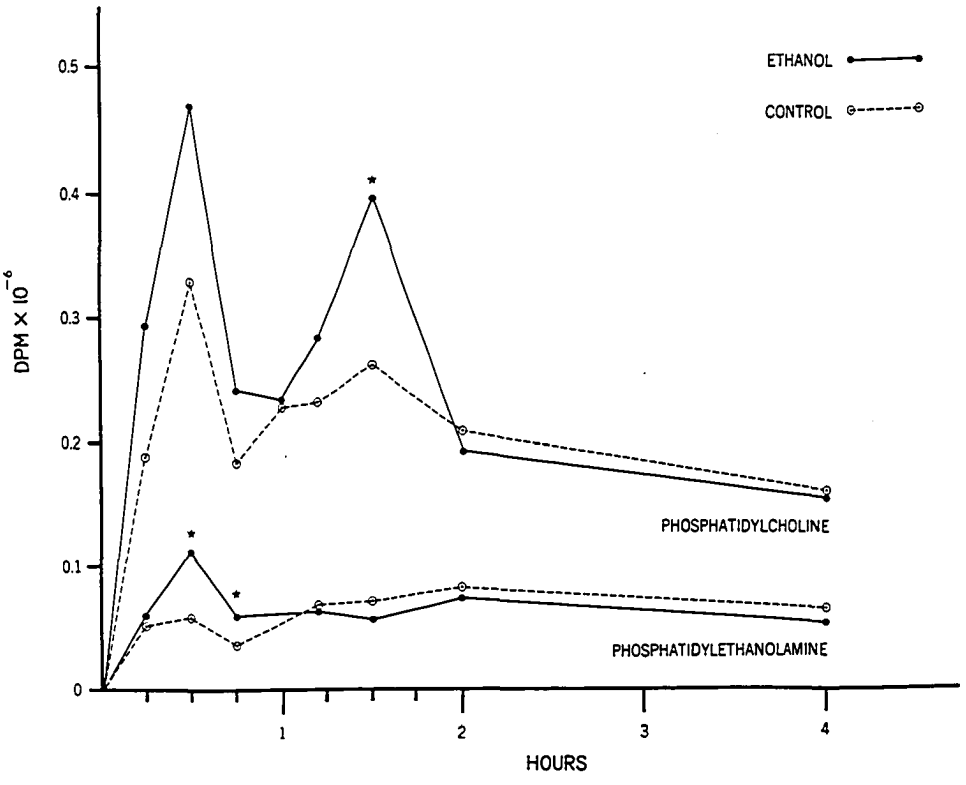



Figure 4. Effect of ethanol on incorporation of radioactivity into hepatic phosphatidylcholine and phosphatidylethanolamine in rats given glycerol-U.L.-$C^{14}$. Each point represents the mean of results obtained in five animals.

* - Indicates that the difference between control and ethanol-treated groups was statistically significant ($p < 0.02$).

TABLE 6

EFFECT OF ETHANOL ON INCORPORATION OF RADIOACTIVITY INTO HEPATIC GLYCEROLIPIDS IN RATS GIVEN GLYCEROL-U.L.-$C^{14}$ [a]

| | Duration Following Isotope Administration | | | | | |
|---|---|---|---|---|---|---|
| | 0 to 45 min. | | 45 to 120 min. | | Total (0 to 120 min.) | |
| | Control | Ethanol | Control | Ethanol | Control | Ethanol |
| Triglyceride | 57.28 | 90.16 | 67.76 | 105.52 | 125.04 | 195.68 |
| Phosphatidylcholine | 5.85 | 8.34 | 11.34 | 14.82 | 17.20 | 23.16 |
| Phosphatidylethanolamine | 1.26 | 2.00 | 3.28 | 3.07 | 4.54 | 5.07 |

[a]Values, in square inches, were obtained by planimetric analysis of the area beneath the radioactivity incorporation curves (Figures 3 and 4) in which total radioactivity (dpm X $10^{-6}$) in each glycerolipid was plotted versus time.

small peak was observed at 30 minutes in the incorporation curve for the ethanol-treated group (Fig. 4). Specific and total radioactivity values for this phospholipid were much lower in both groups during the 4-hour period of study than those observed for phosphatidylcholine. The total radioactivity incorporated into hepatic phosphatidylethanolamine during the first 2-hour period after isotope injection was slightly greater (9%) in the ethanol-treated rats than in controls (Table 6).

## Effect of Ethanol on Glycerolipid Turnover

Half-time values and turnover rates were calculated from radioactivity incorporation curves for hepatic glycerolipids obtained following the administration of glycerol-3-$H^3$ to control and ethanol-treated rats (Table 7). The half-time for hepatic triglyceride in the control group (30 hours) was less than that for phosphatidylcholine (34 hours) and phosphatidylethanolamine (49 hours). Half-time values in the rats given ethanol were higher for hepatic triglyceride, slightly higher for phosphatidylcholine and slightly lower for phosphatidylethanolamine than were comparable values observed in controls. Ethanol increased the absolute turnover rate (mg per day) of each glycerolipid (triglyceride by 22% of control; phosphatidylcholine, 16%; and phosphatidylethanolamine, 10%) and the fractional turnover rate (percent per day) of phosphatidylethanolamine. Fractional turnover rates for triglyceride and phosphatidylcholine in ethanol-treated rats, however, were lower than in controls.

## Effect of Ethanol and Type of Dietary Fat on the Fatty Acid Composition of Hepatic and Adipose Tissue Glycerolipids

Hepatic triglyceride from control rats fed a diet containing

TABLE 7

EFFECT OF ETHANOL ON THE HALF-TIME AND TURNOVER RATES OF HEPATIC GLYCEROLIPIDS

| | Total Content (mg) | | Half-time (day) | | Turnover Rate (mg/day) | | Turnover Rate (%/day) | |
|---|---|---|---|---|---|---|---|---|
| | Control | Ethanol | Control | Ethanol | Control | Ethanol | Control | Ethanol |
| Triglyceride | 84.5±6.44 | 149.0±21.53 | 1.25 | 1.79 | 49.8 | 61.0 | 58.9 | 40.9 |
| Phosphatidylcholine | 110.4±6.84 | 134.9± 5.77 | 1.43 | 1.51 | 53.6 | 62.0 | 48.5 | 45.9 |
| Phosphatidylethanolamine | 57.7±3.51 | 56.6± 1.95 | 2.04 | 1.83 | 19.6 | 21.5 | 34.0 | 38.0 |

corn oil as the sole source of fat calories contained primarily palmitic, oleic and linoleic acid (Table 8). These three fatty acids comprise in excess of 95% of the fatty acids present in corn oil (Table 2). Hepatic triglyceride from ethanol-treated rats fed corn oil contained a greater proportion of linoleic acid ($p < 0.02$) and a smaller proportion of myristic, palmitic ($p < 0.01$), palmitoleic, stearic and oleic acid than controls.

Substitution of coconut oil for corn oil produced a relatively large increase in the proportion of capric, lauric and myristic acid and a marked decrease in the proportion of linoleic acid in hepatic triglyceride from controls (Table 8). Coconut oil contains more than 75% $C_8$-$C_{14}$ fatty acids and less than 3% linoleic acid (Table 2). Less pronounced increases were observed in the two principal fatty acids (palmitic and oleic acid) which are derived from endogenous synthesis. Hepatic triglyceride from ethanol-treated rats fed coconut oil contained a greater proportion of capric, lauric ($p < 0.05$), myristic ($p < 0.001$) and palmitoleic acid and a smaller proportion of palmitic, stearic ($p < 0.01$), oleic ($p < 0.02$) and linoleic acid than controls.

Fatty acid analysis of hepatic phosphatidylcholine from control rats fed a diet containing corn oil demonstrated primarily palmitic, stearic, linoleic and arachidonic acid (Tables 9-10). Hepatic phosphatidylcholine from ethanol-treated rats fed corn oil contained a greater proportion of stearic, oleic and arachidonic acid ($p < 0.001$) and a smaller proportion of palmitic ($p < 0.001$), palmitoleic and linoleic acid than controls. The hepatic content of only one of the phosphatidylcholine fatty acids, i.e., palmitoleic acid, was less in the ethanol-treated rats

TABLE 8

EFFECT OF ETHANOL AND TYPE OF DIETARY FAT ON PERCENT COMPOSITION OF INDIVIDUAL TRIGLYCERIDE-FATTY ACIDS IN LIVER[a]

| Fatty Acid | Corn Oil[b] | | | Coconut Oil[b] | | |
|---|---|---|---|---|---|---|
| | Control | Ethanol | p[e] | Control | Ethanol | p |
| $C_{10:0}$ | 0.0 | 0.0 | | 0.5±0.44 | 0.8±0.34 | ns |
| $C_{12:0}$ | 0.1±0.60 | 0.0 | ns | 4.8±1.27 | 11.4±2.37 | 0.05 |
| $C_{14:0}$ | 0.7±0.12 | 0.4±0.03 | ns | 7.1±0.74 | 16.4±1.37 | 0.001 |
| $C_{16:0}$ | 40.8±3.03 | 30.6±1.14 | 0.01 | 43.0±5.38 | 40.1±5.00 | ns |
| $C_{16:1}$ | 3.6±0.74 | 1.9±1.11 | ns | 4.7±0.89 | 5.4±1.09 | ns |
| $C_{18:0}$ | 2.7±0.57 | 1.2±0.56 | ns | 3.0±0.42 | 1.4±0.11 | 0.01 |
| $C_{18:1}$ | 23.9±2.41 | 23.3±1.61 | ns | 30.5±4.65 | 20.5±1.06 | 0.02 |
| $C_{18:2}$ | 28.3±4.10 | 42.8±1.65 | 0.02 | 6.1±1.19 | 3.8±0.48 | ns |

[a]Values are presented as percent of total, ± standard error of the mean.

[b]Diets containing either corn oil or coconut oil were fed for 46 days.

[c]Diet containing coconut oil was fed for 42 days, followed by one containing corn oil for 4 days.

[d]Diet containing corn oil was fed for 42 days, followed by one containing coconut oil for 4 days.

[e]Statistical significance of difference between mean values obtained in ethanol-treated and control groups; ns indicates difference is not significant at 0.05 level.

TABLE 8--Continued

| Coconut Oil, then Corn Oil[c] | | | Corn Oil, then Coconut Oil[d] | | |
|---|---|---|---|---|---|
| Control | Ethanol | p | Control | Ethanol | p |
| 0.0 | 0.0 | | 0.0 | 0.0 | |
| 0.7±0.40 | 0.8±0.26 | ns | 2.3±0.93 | 5.2±2.20 | ns |
| 2.9±0.67 | 2.2±0.13 | ns | 4.9±0.72 | 8.9±2.91 | ns |
| 41.3±4.34 | 37.8±3.75 | ns | 40.2±2.63 | 37.5±5.45 | ns |
| 4.0±0.38 | 3.7±0.67 | ns | 3.5±0.22 | 6.7±1.00 | 0.02 |
| 2.8±0.34 | 1.4±0.53 | ns | 11.6±2.64 | 4.3±0.72 | 0.05 |
| 30.4±1.92 | 28.3±1.42 | ns | 19.7±0.62 | 28.3±5.62 | ns |
| 18.0±1.11 | 25.6±2.84 | 0.05 | 17.9±1.18 | 9.2±1.56 | 0.01 |

TABLE 9

EFFECT OF ETHANOL AND TYPE OF DIETARY FAT ON PERCENT COMPOSITION OF INDIVIDUAL PHOSPHATIDYLCHOLINE-FATTY ACIDS IN LIVER[a]

| Fatty Acid | Corn Oil[b] | | | Coconut Oil[b] | | |
|---|---|---|---|---|---|---|
| | Control | Ethanol | p[e] | Control | Ethanol | p |
| $C_{14:0}$ | 0.1±0.09 | 0.0 | | 1.0±0.24 | 1.6±0.22 | ns |
| $C_{16:0}$ | 34.4±0.17 | 25.6±1.41 | 0.001 | 49.9±4.12 | 50.3±2.64 | ns |
| $C_{16:1}$ | 2.3±0.99 | 0.9±0.33 | ns | 2.0±0.45 | 2.9±0.68 | ns |
| $C_{18:0}$ | 26.9±2.20 | 30.2±2.16 | ns | 25.3±2.28 | 24.0±2.57 | ns |
| $C_{18:1}$ | 6.3±0.48 | 7.1±0.86 | ns | 10.5±1.97 | 12.2±1.85 | ns |
| $C_{18:2}$ | 16.0±1.55 | 13.5±1.98 | ns | 5.9±0.73 | 3.5±0.65 | 0.05 |
| $C_{20:4}$ | 13.8±0.11 | 22.7±0.72 | 0.001 | 5.4±1.41 | 5.4±1.28 | ns |

[a]Values are presented as percent of total, ± standard error of the mean.

[b]Diets containing either corn oil or coconut oil were fed for 46 days.

[c]Diet containing coconut oil was fed for 42 days, followed by one containing corn oil for 4 days.

[d]Diet containing corn oil was fed for 42 days, followed by one containing coconut oil for 4 days.

[e]Statistical significance of difference between mean values obtained in ethanol-treated and control groups; ns indicates difference is not significant at 0.05 level.

TABLE 9--Continued

| Coconut Oil, then Corn Oil[c] | | | Corn Oil, then Coconut Oil[d] | | |
|---|---|---|---|---|---|
| Control | Ethanol | p | Control | Ethanol | p |
| 0.8±0.29 | 0.2±0.11 | ns | 1.4±0.19 | 1.5±0.18 | ns |
| 38.4±2.55 | 35.7±2.00 | ns | 33.3±3.06 | 28.2±0.75 | ns |
| 1.4±0.20 | 1.0±0.19 | ns | 2.1±0.51 | 2.3±0.42 | ns |
| 23.6±2.03 | 32.0±2.90 | 0.05 | 34.4±0.32 | 35.0±2.34 | ns |
| 7.8±0.53 | 6.4±0.46 | ns | 11.2±1.53 | 13.6±1.14 | ns |
| 19.1±1.93 | 15.7±1.99 | ns | 7.9±1.05 | 8.4±1.50 | ns |
| 8.7±1.09 | 9.1±1.18 | ns | 9.4±2.35 | 10.9±2.20 | ns |

TABLE 10

EFFECT OF ETHANOL AND TYPE OF DIETARY FAT ON THE CONTENT OF INDIVIDUAL PHOSPHATIDYLCHOLINE-FATTY ACIDS IN LIVER[a]

| Fatty Acid | Corn Oil[b] | | Coconut Oil[b] | |
|---|---|---|---|---|
| | Control | Ethanol | Control | Ethanol |
| $C_{14:0}$ | 0.2±0.23 | 0.0 | 2.6± 0.60 | 5.6±0.90 |
| $C_{16:0}$ | 86.2±0.42 | 92.1±4.84 | 124.8±10.31 | 174.0±9.11 |
| $C_{16:1}$ | 5.8±2.50 | 3.1±1.14 | 5.0± 1.15 | 10.0±2.35 |
| $C_{18:0}$ | 67.3±5.51 | 104.4±7.47 | 63.2± 5.71 | 83.0±8.89 |
| $C_{18:1}$ | 15.9±1.20 | 24.6±2.97 | 26.2± 4.93 | 42.0±6.40 |
| $C_{18:2}$ | 40.0±3.88 | 46.7±7.40 | 14.8± 1.83 | 13.7±2.25 |
| $C_{20:4}$ | 34.6±2.83 | 78.4±2.49 | 13.4± 3.53 | 18.6±4.39 |

[a]Values are presented as μmoles, ± standard error of the mean. Results were calculated by multiplying the percent of individual fatty acid (Table 9) by the total μmoles of fatty acid in phosphatidylcholine.

[b]Diet containing either corn oil or coconut oil was fed for 46 days.

[c]Diet containing coconut oil was fed for 42 days, followed by one containing corn oil for 4 days.

[d]Diet containing corn oil was fed for 42 days, followed by one containing coconut oil for 4 days.

TABLE 10--Continued

| Coconut Oil, then Corn Oil[c] | | Corn Oil, then Coconut Oil[d] | |
|---|---|---|---|
| Control | Ethanol | Control | Ethanol |
| 1.9±0.74 | 5.9± 0.62 | 3.6±0.48 | 5.2±0.62 |
| 96.1±6.38 | 123.5± 6.92 | 83.4±7.66 | 97.4±2.59 |
| 3.6±0.50 | 3.5± 0.66 | 5.3±1.28 | 8.1±1.45 |
| 59.1±5.08 | 110.5±10.03 | 86.2±0.80 | 121.0±8.09 |
| 19.5±1.33 | 22.1± 1.59 | 28.1±3.83 | 47.1±3.94 |
| 47.8±4.83 | 54.3± 6.88 | 19.9±2.63 | 29.2±5.19 |
| 21.8±2.73 | 31.4± 4.08 | 23.5±5.88 | 37.8±7.61 |

given a diet containing corn oil than in controls (Table 10).

Hepatic phosphatidylcholine from rats fed coconut oil, compared with those fed corn oil, contained greater amounts of myristic, palmitic and oleic acid and smaller amounts of palmitoleic, stearic, linoleic and arachidonic acid. The magnitude of changes produced by ethanol in the fatty acid composition of hepatic phosphatidylcholine of coconut oil-fed rats was small. However, a slight increase in the mean values of myristic, palmitic, palmitoleic and oleic acid and a slight decrease in stearic and linoleic ($p < 0.05$) acid was observed in ethanol-treated rats. The hepatic content of each phosphatidylcholine-fatty acid except linoleic acid was greater in the latter group of rats than in controls (Table 10).

The principal fatty acids found in hepatic phosphatidylethanolamine and phosphatidylcholine of control rats fed corn oil were similar (Tables 11-12). Although ethanol produced no statistically significant change in the fatty acid composition of phosphatidylethanolamine in rats fed corn oil, a small decrease in the mean percentage of linoleic acid and a small increase in arachidonic acid were found. The hepatic content of each phosphatidylethanolamine-fatty acid was increased in ethanol-treated rats, except for palmitoleic acid which was unchanged and myristic and linoleic acid which were decreased (Table 12). Hepatic phosphatidylethanolamine from coconut oil-fed rats contained a greater proportion of myristic, palmitic, palmitoleic and oleic acid and less stearic, linoleic and arachidonic acid than that from corn oil-fed rats (Table 11). Ethanol produced an increase in the relative amount of stearic acid and a small decrease in oleic and arachidonic acid, although none of these differences was statistically significant. The hepatic contents of pal-

TABLE 11

EFFECT OF ETHANOL AND TYPE OF DIETARY FAT ON PERCENT COMPOSITION OF INDIVIDUAL PHOSPHATIDYLETHANOLAMINE-FATTY ACIDS IN LIVER[a]

| Fatty Acid | Corn Oil[b] | | | Coconut Oil[b] | | |
|---|---|---|---|---|---|---|
| | Control | Ethanol | p[e] | Control | Ethanol | p |
| $C_{14:0}$ | 0.5±0.34 | 0.0 | ns | 1.1±0.63 | 0.2±0.18 | ns |
| $C_{16:0}$ | 31.4±1.59 | 31.7±1.75 | ns | 44.8±1.90 | 43.4±1.41 | ns |
| $C_{16:1}$ | 1.0±0.21 | 0.8±0.34 | ns | 1.8±0.44 | 2.1±0.45 | ns |
| $C_{18:0}$ | 34.8±1.84 | 35.2±1.89 | ns | 30.3±3.78 | 37.7±0.61 | ns |
| $C_{18:1}$ | 7.1±0.39 | 7.4±0.73 | ns | 8.7±2.80 | 6.5±0.81 | ns |
| $C_{18:2}$ | 12.8±2.37 | 9.7±1.45 | ns | 3.8±1.49 | 3.4±0.78 | ns |
| $C_{20:4}$ | 12.4±1.28 | 15.3±2.27 | ns | 9.3±2.48 | 6.5±1.38 | ns |

[a]Values are presented as percent of total, ± standard error of the mean.

[b]Diets containing either corn oil or coconut oil were fed for 46 days.

[c]Diet containing coconut oil was fed for 42 days, followed by one containing corn oil for 4 days.

[d]Diet containing corn oil was fed for 42 days, followed by one containing coconut oil for 4 days.

[e]Statistical significance of difference between mean values obtained in ethanol-treated and control groups; ns indicates difference is not significant at 0.05 level.

TABLE 11--Continued

| Coconut Oil, then Corn Oil[c] | | | Corn Oil, then Coconut Oil[d] | | |
|---|---|---|---|---|---|
| Control | Ethanol | p | Control | Ethanol | p |
| 0.7±0.29 | 0.6±0.23 | ns | 1.4±0.29 | 1.1±0.25 | ns |
| 37.0±1.88 | 34.5±2.20 | ns | 33.4±0.83 | 35.1±1.28 | ns |
| 2.0±0.25 | 0.9±0.40 | 0.05 | 2.2±0.43 | 1.8±0.31 | ns |
| 29.9±2.32 | 40.0±1.67 | 0.01 | 31.2±0.52 | 33.1±1.46 | ns |
| 7.7±1.45 | 4.4±0.66 | ns | 14.1±1.70 | 11.2±2.26 | ns |
| 11.8±1.75 | 5.5±1.21 | 0.02 | 6.1±0.59 | 5.9±1.03 | ns |
| 10.8±0.88 | 14.3±1.96 | ns | 11.4±0.89 | 11.5±2.60 | ns |

TABLE 12

EFFECT OF ETHANOL AND TYPE OF DIETARY FAT ON THE CONTENT OF INDIVIDUAL PHOSPHATIDYLETHANOLAMINE-FATTY ACIDS IN LIVER[a]

| Fatty Acid | Corn Oil[b] | | Coconut Oil[b] | |
|---|---|---|---|---|
| | Control | Ethanol | Control | Ethanol |
| $C_{14:0}$ | 0.6±0.36 | 0.0 | 1.3±0.78 | 0.4±0.36 |
| $C_{16:0}$ | 37.3±1.95 | 49.0±2.71 | 54.9±2.33 | 67.1±2.17 |
| $C_{16:1}$ | 1.2±0.26 | 1.2±0.52 | 2.2±0.54 | 3.3±0.69 |
| $C_{18:0}$ | 42.6±2.26 | 54.4±2.93 | 37.1±4.64 | 58.4±0.94 |
| $C_{18:1}$ | 8.7±0.49 | 11.5±1.13 | 10.6±3.44 | 10.1±1.25 |
| $C_{18:2}$ | 15.8±2.91 | 15.0±2.25 | 4.7±1.83 | 5.3±1.20 |
| $C_{20:4}$ | 15.2±1.57 | 23.7±3.50 | 11.5±3.04 | 10.1±2.13 |

[a]Values are presented as μmoles, ± standard error of the mean. Results were calculated by multiplying the percent of individual fatty acids (Table 11) by the total μmoles of fatty acid in phosphatidylethanolamine.

[b]Diet containing either corn oil or coconut oil was fed for 46 days.

[c]Diet containing coconut oil was fed for 42 days, followed by one containing corn oil for 4 days.

[d]Diet containing corn oil was fed for 42 days, followed by one containing coconut oil for 4 days.

*TABLE 12--Continued*

| Coconut Oil, then Corn Oil[c] | | Corn Oil, then Coconut Oil[d] | |
|---|---|---|---|
| Control | Ethanol | Control | Ethanol |
| 0.9±0.36 | 0.9±0.36 | 1.8±0.36 | 1.8±0.39 |
| 45.5±2.31 | 53.3±3.40 | 41.0±1.02 | 54.4±1.98 |
| 2.5±0.31 | 1.4±0.62 | 2.7±0.53 | 2.8±0.48 |
| 36.8±2.85 | 61.8±2.58 | 38.3±0.64 | 51.2±2.26 |
| 9.5±1.78 | 6.9±1.02 | 17.3±2.09 | 17.4±3.50 |
| 14.4±2.15 | 8.4±1.86 | 7.5±0.72 | 9.1±1.59 |
| 13.3±1.08 | 22.2±3.03 | 14.0±1.09 | 17.8±4.02 |

mitic, palmitoleic, stearic and linoleic acid in phosphatidylethanolamine were increased in ethanol-treated rats fed coconut oil, whereas total amounts of myristic, oleic and arachidonic acid were decreased (Table 12).

Adipose tissue triglyceride from corn oil-fed rats contained primarily palmitic, oleic and linoleic acid (Table 13). Ethanol produced a decrease in the proportion of lauric ($p < 0.001$), myristic ($p < 0.05$), palmitic ($p < 0.01$) and palmitoleic acid ($p < 0.05$) and an increase in the relative amount of stearic, oleic and linoleic acid ($p < 0.05$). When coconut oil was the sole source of dietary triglyceride, lauric, myristic, palmitic and oleic acid were the principal fatty acids present in adipose tissue triglyceride. Substitution of coconut oil for corn oil increased the proportion of capric, lauric, myristic and palmitoleic acid and decreased the relative amount of palmitic, stearic, oleic and linoleic acid. Administration of ethanol to coconut oil-fed rats produced an increase in the proportion of capric and lauric acid ($p < 0.01$) in adipose tissue triglyceride and a decrease in myristic, palmitic, palmitoleic, stearic, oleic ($p < 0.05$) and linoleic acid.

Hepatic and adipose tissue glycerolipids were studied also in rats fed corn oil as the sole source of fat calories for 42 days, followed for the last 4 days prior to sacrifice by a diet containing coconut oil, or vice versa (Tables 8-13). Response of hepatic triglyceride, phosphatidylcholine and phosphatidylethanolamine to change in the fatty acid composition of the dietary triglyceride was clearly evident by the end of the 4-day period. A substantial decrease in the proportion of lauric acid and increase in linoleic acid were observed in hepatic triglyceride from controls in which corn oil was substituted for coconut

TABLE 13

EFFECT OF ETHANOL AND TYPE OF DIETARY FAT ON PERCENT COMPOSITION OF INDIVIDUAL TRIGLYCERIDE-FATTY ACIDS IN MESENTERIC ADIPOSE TISSUE[a]

| Fatty Acid | Corn Oil[b] | | | Coconut Oil[b] | | |
|---|---|---|---|---|---|---|
| | Control | Ethanol | p[e] | Control | Ethanol | p |
| $C_{10:0}$ | 0.0 | 0.0 | | 0.5±0.03 | 0.7±0.05 | ns |
| $C_{12:0}$ | 0.2±0.01 | 0.1±0.01 | 0.001 | 28.5±2.16 | 51.5±3.62 | 0.01 |
| $C_{14:0}$ | 2.0±0.14 | 1.5±0.08 | 0.05 | 14.8±1.12 | 13.3±0.88 | ns |
| $C_{16:0}$ | 33.2±1.21 | 25.5±1.04 | 0.01 | 22.7±1.88 | 16.0±0.94 | ns |
| $C_{16:1}$ | 6.4±0.47 | 4.1±0.67 | 0.05 | 6.9±0.37 | 2.5±2.19 | ns |
| $C_{18:0}$ | 2.6±0.43 | 3.2±0.56 | ns | 2.0±0.30 | 1.3±0.58 | ns |
| $C_{18:1}$ | 27.8±0.59 | 32.4±1.89 | ns | 21.7±1.28 | 12.9±2.14 | 0.05 |
| $C_{18:2}$ | 27.7±1.63 | 32.9±1.16 | 0.05 | 2.8±0.37 | 1.5±0.92 | ns |

[a]Values are presented as percent of total, ± standard error of the mean.

[b]Diets containing either corn oil or coconut oil were fed for 46 days.

[c]Diet containing coconut oil was fed for 42 days, followed by one containing corn oil for 4 days.

[d]Diet containing corn oil was fed for 42 days, followed by one containing coconut oil for 4 days.

[e]Statistical significance of difference between mean values obtained in ethanol-treated and control groups; ns indicates difference is not significant at 0.05 level.

TABLE 13--Continued

| Coconut Oil, then Corn Oil[c] | | | Corn Oil, then Coconut Oil[d] | | |
|---|---|---|---|---|---|
| Control | Ethanol | p | Control | Ethanol | p |
| 0.0 | 7.1±0.17 | | 0.0 | 0.0 | |
| 31.1±5.64 | 37.0±0.87 | ns | 14.2±1.39 | 11.7±1.47 | ns |
| 10.7±1.95 | 10.8±0.25 | ns | 6.7±0.65 | 5.5±0.69 | ns |
| 22.8±2.19 | 15.6±1.63 | 0.05 | 24.0±1.08 | 20.2±1.17 | 0.05 |
| 4.9±0.67 | 2.8±0.35 | 0.05 | 3.7±0.50 | 3.3±0.38 | ns |
| 2.2±0.28 | 1.6±0.27 | ns | 2.3±0.18 | 1.9±0.22 | ns |
| 20.9±3.26 | 17.8±0.90 | ns | 24.0±0.86 | 25.2±0.74 | ns |
| 7.4±2.56 | 6.5±1.53 | ns | 25.1±2.38 | 32.6±1.51 | 0.05 |

oil, when compared with results obtained in controls given coconut oil throughout the study. An opposite pattern of change in the fatty acid composition of hepatic triglyceride, characterized by a marked decrease in the proportion of linoleic acid and increase in lauric acid, was observed when the results obtained in controls in which coconut oil was substituted for corn oil were compared with those obtained in controls given corn oil throughout the study. Similar comparisons of the proportions of linoleic and arachidonic acid, individually or as the sum of the percentages of these fatty acids, in hepatic phosphatidylcholine and phosphatidylethanolamine demonstrated that these values were increased in controls in which corn oil was substituted for coconut oil and decreased when corn oil was replaced by coconut oil. The proportions of lauric and linoleic acid in hepatic triglyceride from ethanol-treated rats resembled the fatty acid composition of the diet to a greater degree than those from controls. Ethanol failed to produce a similar consistent effect, in response to the fatty acid composition of diet, on the proportions of linoleic and arachidonic acid in hepatic phosphatidylcholine and phosphatidylethanolamine.

The lauric and linoleic acid composition of adipose tissue triglyceride also was altered within a 4-day period after the fatty acid composition of the diet was modified, but the magnitude of the response was generally much less than that observed in hepatic triglyceride. The proportions of these two fatty acids in adipose tissue triglyceride from controls resembled the fatty acid composition of the diet to a greater degree than those in adipose tissue triglyceride from ethanol-treated rats.

## Effect of Ethanol and Type of Dietary Fat on Fatty Acyl-Coenzyme A:Lysophosphatide Transferase Activities in Rat Liver Microsomes

Transferase activities in liver microsomes from rats fed a diet containing corn oil were assayed using the CoA-derivative of palmitic, stearic, oleic, linoleic or arachidonic acid as fatty acid donor and α- or β-lysophosphatidylcholine as fatty acid acceptor. When α-lysophosphatidylcholine served as acceptor, much higher transferase activities were observed with oleic, linoleic and arachidonic acid than with palmitic and stearic acid (Table 14). The transferase activities observed when β-lysophosphatidylcholine was used as acceptor were highest with palmitic acid, intermediate with stearic and oleic acid and lowest with linoleic and arachidonic acid. These results are consistent with observations reported by others (159-162), indicating that saturated fatty acids are esterified preferentially at the α-position of phosphatidylcholine and phosphatidylethanolamine, and unsaturated fatty acids at the β-position. Ethanol produced a substantial increase in activities for transfer of oleic, linoleic and arachidonic acid onto α-lysophosphatidylcholine, but had little effect on the transfer of stearic and palmitic acid onto this substrate. The sum of the activities for transfer of unsaturated fatty acids onto α-lysophosphatidylcholine was much greater in ethanol-treated rats, whereas this value for saturated fatty acids was essentially unchanged. Ethanol decreased the transferase activities for palmitic and stearic acid onto β-lysophosphatidylcholine, but produced only a small and inconstant effect on the activities for the unsaturated fatty acids studied. A pronounced decrease was observed in the sum of the activities for transfer of saturated fatty acids onto β-lysophosphatidylcholine in

TABLE 14

EFFECT OF ETHANOL AND TYPE OF DIETARY FAT ON FATTY ACYL-COENZYME A:LYSOPHOSPHATIDYLCHOLINE TRANSFERASE ACTIVITY IN RAT LIVER MICROSOMES[a]

| Diet | Fatty Acid | α-Lysophosphatidylcholine[b] | | β-Lysophosphatidylcholine[b] | | Total (α + β) | |
|---|---|---|---|---|---|---|---|
| | | Control | Ethanol | Control | Ethanol | Control | Ethanol |
| Corn Oil | $C_{16:0}$ | 17.7± 3.69 | 16.3± 1.11 | 66.5± 7.30 | 36.9± 9.99 | 84.2 | 53.2 |
| | $C_{18:0}$ | 5.5± 1.26 | 8.7± 3.57 | 33.3±10.20 | 21.6± 4.09 | 38.8 | 30.3 |
| | $C_{18:1}$ | 61.5±14.31 | 108.3±14.17 | 26.1± 4.36 | 21.5± 5.62 | 87.6 | 129.8 |
| | $C_{18:2}$ | 55.5± 4.72 | 95.3±23.51 | 5.5± 1.75 | 10.5± 3.12 | 61.0 | 105.8 |
| | $C_{20:4}$ | 50.2± 7.37 | 87.7±17.28 | 8.5± 3.80 | 4.2± 1.07 | 58.7 | 91.9 |
| Coconut Oil | $C_{16.0}$ | 17.1± 1.33 | 9.1± 1.35 | 56.0± 8.71 | 55.6± 7.41 | 73.1 | 64.7 |
| | $C_{18:0}$ | 4.2± 1.04 | 0.8± 0.04 | 34.8± 3.07 | 46.0±10.91 | 39.0 | 46.8 |
| | $C_{18:1}$ | 64.9± 6.81 | 76.5± 7.39 | 27.1± 5.85 | 26.5± 4.15 | 92.0 | 103.0 |
| | $C_{18:2}$ | 56.6± 8.29 | 49.9± 1.94 | 10.9± 2.18 | 20.4± 3.99 | 67.5 | 70.3 |
| | $C_{20:4}$ | 27.0± 6.05 | 24.6± 6.02 | 4.1± 1.19 | 11.3± 2.18 | 31.1 | 35.9 |

[a]Values are expressed as mμmoles of CoA released per minute per mg of microsomal protein, ± standard error of the mean.

[b]Substrate used as fatty acid acceptor.

ethanol-treated rats fed corn oil, while little change was observed in this value for unsaturated fatty acids. The sum of activities for transfer of all fatty acids onto α-lysophosphatidylcholine was increased in ethanol-treated rats fed corn oil, whereas this value for β-lysophosphatidylcholine was decreased. Total transferase activities (the sum of the activities for transfer of individual fatty acids onto α- and β-lysophosphatidylcholine) in ethanol-treated rats were decreased for saturated fatty acids (palmitic and stearic acid) and increased for unsaturated fatty acids (oleic, linoleic and arachidonic acid). The sum of total transferase activities for saturated fatty acids was less and that for unsaturated fatty acids was much greater in these ethanol-treated rats. Ethanol produced a moderate increase in the sum of the total transferase activities for all fatty acids in rats fed corn oil.

Transferase activities using α- and β-lysophosphatidylcholine as acceptors were also assayed in liver microsomes obtained from rats fed coconut oil (Table 14). Results for control rats fed coconut oil were similar to those for controls fed corn oil, with the exceptions that the activities for transfer of arachidonic acid onto α-lysophosphatidylcholine was much lower in the coconut oil-fed rats. Ethanol produced much less effect on the activities for transfer of fatty acids onto α- or β-lysophosphatidylcholine in liver microsomes from rats fed coconut oil than from those fed corn oil. However, small decreases in activities, relative to control values, were observed for the transfer of palmitic, stearic, linoleic and arachidonic acid onto α-lysophosphatidylcholine and for the transfer of stearic, linoleic and arachidonic acid onto β-lysophosphatidylcholine in ethanol-treated rats fed coconut oil. The sum of the

activities for transfer of saturated fatty acids onto α-lysophosphatidylcholine was decreased slightly in ethanol-treated rats, while this value for unsaturated fatty acids was unchanged. Ethanol produced a small increase in the sum of the activities for transfer of saturated and unsaturated fatty acids onto β-lysophosphatidylcholine. The sum of the activities for transfer of all fatty acids onto α-lysophatidylcholine was essentially unchanged in ethanol-treated rats fed coconut oil, whereas this value for β-lysophosphatidylcholine was moderately increased. The sum of the total transferase activities for unsaturated fatty acids was increased slightly in ethanol-treated rats, whereas no difference was observed for saturated fatty acids. When the sum of total transferase activities for all fatty acids was calculated, the value obtained in ethanol-treated rats fed coconut oil was only slightly greater than the control value. Thus, ethanol had much less effect on this value in rats fed coconut oil than in those fed corn oil.

Transferase activities in liver microsomes from rats fed a diet containing corn oil were assayed using α- and β-lysophosphatidylethanolamine as acceptors (Table 15). In controls, activities for transfer of linoleic and arachidonic acid onto α-lysophosphatidylethanolamine were higher than those for the other three fatty acids studied. The highest activity for fatty acid transfer onto β-lysophosphatidylethanolamine in these rats was observed with palmitic acid. Intermediate values were obtained with linoleic and arachidonic acid and lowest values with stearic and oleic acid. The activities for transfer of each fatty acid onto α-lysophosphatidylethanolamine were higher in ethanol-treated rats than in controls, although the magnitude of this increase was small. When com-

TABLE 15

EFFECT OF ETHANOL ON FATTY ACYL-COENZYME A:LYSOPHOSPHATIDYLETHANOLAMINE TRANSFERASE ACTIVITY IN RAT LIVER MICROSOMES[a]

| Fatty Acid | α-Lysophosphatidylethanolamine[b] | | β-Lysophosphatidylethanolamine[b] | | Total (α + β) | |
|---|---|---|---|---|---|---|
| | Control | Ethanol | Control | Ethanol | Control | Ethanol |
| $C_{16:0}$ | 4.0±1.24 | 7.4±1.37 | 21.3±3.65 | 15.0±3.74 | 25.3 | 22.4 |
| $C_{18:0}$ | 1.6±0.41 | 8.2±1.26 | 4.2±0.85 | 14.8±3.55 | 5.8 | 23.0 |
| $C_{18:1}$ | 6.0±1.99 | 12.8±1.56 | 4.4±1.50 | 6.0±2.61 | 10.4 | 18.8 |
| $C_{18:2}$ | 10.8±3.13 | 15.2±1.91 | 10.1±3.22 | 11.6±3.80 | 20.9 | 26.8 |
| $C_{20:4}$ | 13.0±3.19 | 14.6±2.64 | 7.7±2.45 | 2.6±0.67 | 20.7 | 17.2 |

[a]Fatty acyl-CoA:lysophosphatidylethanolamine transferase activity was measured only in rats fed corn oil. Values are expressed as mμmoles of CoA released per minute per mg of microsomal protein, ± standard error of the mean.

[b]Substrate used as fatty acid acceptor.

pared with values obtained in controls, the sum of the activities for transfer of fatty acids onto α-lysophosphatidylethanolamine in ethanol-treated rats was slightly increased for saturated and unsaturated fatty acids. Activities for transfer of stearic, oleic and linoleic acid onto β-lysophosphatidylethanolamine in ethanol-treated rats were slightly greater than in controls, whereas slightly lower activities were observed for palmitic and arachidonic acid. Ethanol treatment had little effect on the sum of activities for transfer of either saturated or unsaturated fatty acids onto β-lysophosphatidylethanolamine. The sum of the activities for the transfer of all fatty acids onto α-lysophosphatidylethanolamine was somewhat greater in ethanol-treated rats than in controls, whereas little change was observed in this value when β-lysophosphatidylethanolamine served as acceptor. In ethanol-treated rats, total transferase activities for palmitic and arachidonic acid were decreased and those for stearic, oleic and linoleic acid were increased. Ethanol increased the sum of total transferase activities for saturated and for unsaturated fatty acids. The sum of total transferase activities for all fatty acids was higher in ethanol-treated rats than controls.

## Effect of Ethanol on Fatty Acyl-Coenzyme A Hydrolase Activities in Rat Liver Microsomes

Hydrolase activities in liver microsomes from rats fed corn oil were higher with palmitic, linoleic and arachidonic acid than with oleic acid and stearic acid (Table 16). Ethanol administration produced a small increase in the hydrolase activity for each of the fatty acids studied.

TABLE 16

EFFECT OF ETHANOL ON FATTY ACYL-COENZYME A HYDROLASE ACTIVITY IN RAT LIVER MICROSOMES[a]

| Fatty Acid | Control | Ethanol |
|---|---|---|
| $C_{16:0}$ | 9.5±1.50 | 10.2±1.60 |
| $C_{18:0}$ | 3.9±0.67 | 7.8±1.14 |
| $C_{18:1}$ | 6.3±1.80 | 13.8±1.30 |
| $C_{18:2}$ | 10.1±1.34 | 17.1±0.98 |
| $C_{20:4}$ | 12.5±1.65 | 17.3±1.42 |

[a]Values are expressed as mμmoles of CoA released per minute per mg of microsomal protein, ± standard error of the mean.

## Comparison of Fatty Acid Composition of Hepatic Phosphatides with Hepatic Microsomal Fatty Acyl-Coenzyme A:Lysophosphatide Transferase Activities

Relative microsomal activities for the transfer of palmitic, stearic, oleic, linoleic and arachidonic acid onto lysophosphatidylcholine were calculated for comparison with the fatty acid distribution in phosphatidylcholine (Table 17). In liver from rats fed the diet containing corn oil, the relative transferase activity values were lower for saturated fatty acids (palmitic and stearic acid) and higher for unsaturated fatty acids (oleic, linoleic and arachidonic acid) than the percentages of these fatty acids in phosphatidylcholine. Ethanol accentuated the disparity between relative transferase activities and the fatty acid composition of phosphatidylcholine for all fatty acids except arachidonic acid. In ethanol-treated rats, values obtained for relative transferase activity with arachidonic acid and for the percentage of this fatty acid in phosphatidylcholine were similar.

The relationship between relative transferase activities with lysophosphatidylethanolamine and the fatty acid distribution of phosphatidylethanolamine was similar generally to that between relative transferase activities with lysophosphatidylcholine and the fatty acid distribution of phosphatidylcholine (Table 18). Relative activities for the transfer of the series of fatty acids onto lysophosphatidylethanolamine were lower for saturated fatty acids and higher for unsaturated fatty acids than the percentages of these fatty acids in phosphatidylethanolamine. Ethanol produced an increase in the difference between these two values for palmitic, oleic and linoleic acid and a decrease in that for stearic and arachidonic acid.

TABLE 17

COMPARISON OF THE FATTY ACID COMPOSITION OF HEPATIC PHOSPHATIDYLCHOLINE WITH HEPATIC MICROSOMAL FATTY ACYL-COENZYME A:LYSOPHOSPHATIDYLCHOLINE TRANSFERASE ACTIVITY[a]

| | Control | | Ethanol | |
|---|---|---|---|---|
| | Fatty Acid Distribution[b] | Relative Transferase Activity[c] | Fatty Acid Distribution[b] | Relative Transferase Activity[c] |
| $C_{16:0}$ | 34.4 | 25.5 | 25.6 | 12.9 |
| $C_{16:1}$ | 2.3 | [d] | 0.9 | [d] |
| $C_{18:0}$ | 26.9 | 11.7 | 30.2 | 7.4 |
| $C_{18:1}$ | 6.3 | 26.5 | 7.1 | 31.6 |
| $C_{18:2}$ | 16.0 | 18.5 | 13.5 | 25.7 |
| $C_{20:4}$ | 13.8 | 17.8 | 22.7 | 22.4 |

[a]Results were obtained from rats fed a diet containing corn oil.

[b]Values are expressed as percent of the total fatty acid composition of phosphatidylcholine, determined by gas-liquid chromatography.

[c]Values are expressed as percent of total transferase activity, calculated for each fatty acid as the sum of the activities observed when α- and β-lysophosphatidylcholines were used as substrates.

[d]Assays of transferase activity were not performed with palmitoleic acid.

TABLE 18

COMPARISON OF THE FATTY ACID COMPOSITION OF HEPATIC PHOSPHATIDYLETHANOLAMINE WITH HEPATIC MICROSOMAL FATTY ACYL-COENZYME A: LYSOPHOSPHATIDYLETHANOLAMINE TRANSFERASE ACTIVITY[a]

| | Control | | Ethanol | |
|---|---|---|---|---|
| | Fatty Acid Distribution[b] | Relative Transferase Activity[c] | Fatty Acid Distribution[b] | Relative Transferase Activity[c] |
| $C_{16:0}$ | 31.4 | 30.4 | 31.7 | 20.7 |
| $C_{16:1}$ | 1.0 | [d] | 0.8 | [d] |
| $C_{18:0}$ | 34.8 | 7.0 | 35.2 | 21.3 |
| $C_{18:1}$ | 7.1 | 12.5 | 7.4 | 17.3 |
| $C_{18:2}$ | 12.8 | 25.2 | 9.7 | 24.8 |
| $C_{20:4}$ | 12.4 | 24.9 | 15.3 | 15.9 |

[a]Results were obtained from rats fed a diet containing corn oil.

[b]Values are expressed as percent of total fatty acid composition of phosphatidylethanolamine, determined by gas-liquid chromatography.

[c]Values are expressed as percent of total transferase activity, calculated for each fatty acid as the sum of the activities observed when α- and β-lysophosphatidylethanolamines were used as substrates.

[d]Assays of transferase activity were not performed with palmitoleic acid.

# CHAPTER V

# DISCUSSION

Results obtained in the present study demonstrate that chronic administration of ethanol to rats produces significant changes in liver lipids, including marked increases in triglyceride and esterified cholesterol and moderate increases in free cholesterol, phosphatidylcholine, phosphatidylethanolamine and sphingomyelin. Other investigators have reported that increased amounts primarily of triglyceride and esterified cholesterol may accumulate in the fatty liver produced by ethanol (128, 129), although the magnitude of change appears to be influenced by the type and amount of fat in the diet (163-166) and by the amount of ethanol given and the duration of its administration (128, 142, 143).

## Mechanisms Responsible for Lipid Accumulation in Liver

Mechanisms which may produce lipid accumulation in the liver include increased hepatic lipogenesis, decreased lipoprotein formation or release by liver and decreased hepatic oxidation of lipid. Results obtained in the present study and those reported by other investigators permit some evaluation of the relative importance of these mechanisms in the pathogenesis of the fatty liver produced by ethanol. Emphasis in the discussion which follows is focused on the effects of ethanol on the me-

tabolism of hepatic triglyceride, phosphatidylcholine and phosphatidylethanolamine.

## Effect of Ethanol on Hepatic Lipogenesis

The fatty acids incorporated into hepatic glycerolipids may be derived either from de novo fatty acid synthesis in liver or from dietary and adipose tissue triglyceride.

Evidence favoring the conclusion that ethanol may increase the rate of de novo synthesis of fatty acids in liver has been obtained from studies of precursor incorporation into liver lipids. Ethanol stimulates the incorporation of radioactivity from acetate-$C^{14}$ into liver lipids in vivo (167) and into fatty acids of liver slices (168). Scheig and Isselbacher (169) also found that the in vitro addition of ethanol to rat liver slices enhanced free fatty acid, triglyceride and phospholipid synthesis from acetate-$C^{14}$ and pyruvate-$C^{14}$, although they observed only a moderate increase in the radioactivity incorporated from these precursors into triglyceride and a decrease in that incorporated into phospholipid in liver slices from rats given a single dose of ethanol in vivo 16 hours prior to sacrifice.

The rate of fatty acid synthesis in liver may be accelerated following ethanol treatment as a result of an increase in available acetate (170) and NADH (167, 168, 171-174). The influence of increased levels of NADH on fatty acid synthesis might be expected to involve primarily elongation of fatty acid chain length (175) and formation of unsaturated fatty acids (176, 177), rather than de novo synthesis. The latter appears to require NADPH in preference to NADH as the hydrogen donor (173). It should be noted, however, that an increase in hepatic

NADH content similar to that observed when ethanol is given has been described in animals following the administration of sorbitol, xylitol, glucose, or fructose (134), although none of these sugars produces an accumulation of lipids in the liver.

Howard and Lowenstein (178) have shown that the availability of α-glycerophosphate influences the rate of fatty acid synthesis, and presumably that of glycerolipid formation, in microsomal preparations from rat liver. Nikkila and Ojala (146, 148) observed that the hepatic α-glycerophosphate content was increased in ethanol-treated rats. These investigators and others (169) demonstrated an increased incorporation of palmitate-$C^{14}$ into hepatic triglyceride and phospholipid in ethanol-treated rats and concluded that this increase in palmitate-$C^{14}$ incorporation, as well as that of acetate-$C^{14}$ and pyruvate-$C^{14}$, may result from increased hepatic α-glycerophosphate levels in these animals (146).

Fatty acids mobilized from adipose tissue and transported in the plasma provide a second major source of fatty acids available to the liver for esterification into glycerolipids. Increasing the rate of fatty acid mobilization from peripheral sites by administration of norepinephrine to rats has been shown to lead to development of fatty liver (179). Evidence has been presented suggesting that adipose tissue triglycerides may be the principal source of fatty acids accumulating in the fatty liver produced by a single large dose of ethanol (163, 169, 180, 181). Under these conditions, the fatty acid composition of hepatic triglyceride resembles that of adipose tissue rather than that expected from endogenous synthesis (181). Several investigators have reported that plasma FFA levels were elevated following large amounts of ethanol given

as a single dose or within a period of several hours, suggesting that ethanol increased fatty acid mobilization from adipose tissue (125, 138, 139, 182). Additional evidence favoring this conclusion is the observation that fat accumulation in liver following alcohol administration may be prevented by hypophysectomy (131, 167), adrenalectomy (125, 131), cordotomy (167), or administration of ganglionic-blocking agents (182). Other investigators, however, have found either no change (180, 183) or a decrease in plasma FFA levels, a decrease in plasma glycerol concentration, a reduction in plasma FFA turnover (137, 184-186) and no evidence of enhanced mobilization of fatty acids from labeled epididymal fat pads (180), following an acute episode of alcohol ingestion. In subjects given ethanol over short periods of time and studied by hepatic vein catheterization, net hepatic uptake was reduced (187). The apparent inconsistencies in these results may reflect a dose response of FFA metabolism to the amount of ethanol administered. In addition, the possibility cannot be excluded that ethanol, under these conditions, may alter hepatic metabolism of FFA, thereby increasing their utilization for triglyceride formation.

Results obtained in the present study and those reported by others (188-190) favor the conclusion that fatty acids derived from diet provide the majority of the triglyceride-fatty acids present in the fatty liver produced by chronic ethanol administration. Results presented here indicate that the oleic and linoleic acid composition of hepatic triglyceride and, to a lesser extent, that of hepatic phosphatidylcholine and phosphatidylethanolamine resembled the fatty acid composition of the diet to a greater extent than the fatty acid composition of mesenteric adipose

tissue. Other investigators have reported that the fatty acid composition of hepatic triglyceride in the fatty liver produced by prolonged ethanol ingestion resembled that found in dietary triglyceride but not that of depot fat when a diet containing fat was fed (163, 164, 188-190). The fatty liver produced in ethanol-treated animals fed a fat-free diet or one containing triglycerides of medium-chain length fatty acids (predominantly $C_8$ and $C_{10}$) as the sole source of fat contained much less lipid than was observed when a more nearly normal amount and type of dietary fat was fed (129, 163, 191). The hepatic triglyceride-fatty acids found in the ethanol-treated rats fed a low-fat or fat-free diet were primarily those which may be synthesized endogenously rather than those present in the adipose tissue (163, 191).

The mechanism whereby dietary triglyceride-fatty acids are utilized for glycerolipid synthesis in preference to fatty acids derived from adipose tissue in animals administered ethanol chronically has not been defined. The possibility that ethanol might alter gastrointestinal absorption or hepatic uptake of triglyceride has been investigated. Ethanol had no effect on absorption of $C^{14}$-palmitate (163), but produced a decrease in xylose absorption (192, 193). Uptake of $C^{14}$-labeled chylomicrons by the liver was not altered by ethanol (124, 163).

The majority of studies of the effect of ethanol on hepatic lipids have been concerned with changes produced in triglyceride, rather than those in phospholipid. The present study and those reported by others (128, 129) have demonstrated that the magnitude of increase in hepatic phospholipid produced by ethanol is much less impressive than that found in triglyceride. Maling, Wakabayashi and Horning (194) and

Scheig and Isselbacher (195) have reported that the incorporation of palmitate-$C^{14}$ and acetate-$C^{14}$ into phospholipid is decreased, whereas that into triglyceride is increased, in liver slices and homogenates from rats given a single dose of alcohol. However, studies involving intact animals have shown that incorporation of radioactivity from palmitate-$C^{14}$ into both hepatic triglyceride and phospholipid is increased in ethanol-treated rats, although much greater amounts of radioactivity were generally incorporated into triglyceride than phospholipid (146, 148). In these studies, the radioactivity incorporated into hepatic triglyceride in ethanol-treated rats was increased severalfold, when compared with controls, whereas only a slight increase was found in the radioactivity present in hepatic phospholipid.

Glycerol-$C^{14}$ and glycerol-$H^3$ were selected for the present study in preference to labeled fatty acids and related precursors since other investigators have shown that less evidence of re-cycling is observed with glycerol (196). Results obtained in the present study indicate that the total radioactivity from glycerol-$C^{14}$ incorporated into hepatic triglyceride in the control group of rats was approximately seven-fold greater than that incorporated into phosphatidylcholine and approximately 25-fold greater than into phosphatidylethanolamine during the first two hours following isotope injection. Ethanol produced an increase, relative to control values, in the total radioactivity incorporated into each of these glycerolipids. The greatest ethanol-induced increase was found in triglyceride and the least in phosphatidylethanolamine.

Results from the present study also indicate that the absolute turnover rates (mg per day) of hepatic phosphatidylcholine in control and

ethanol-treated rats are slightly higher than those of hepatic triglyceride. Each of these values for triglyceride and phosphatidylcholine in control and ethanol-treated rats is much higher than those observed for hepatic phosphatidylethanolamine. Nevertheless, during the 4-hour interval following glycerol-$C^{14}$ administration the specific and total radioactivities of hepatic triglyceride were much greater than those of phosphatidylcholine, which has relatively similar turnover rates. One explanation of these observations is that a significant portion of the hepatic triglyceride formed during the 4-hour period of this experiment may be synthesized using glycerol-$C^{14}$ derived from a pool distinct from that utilized for phosphatidylcholine synthesis.

Results obtained using labeled glycerol may infer erroneously low rates of glycerolipid synthesis, since an increased hepatic concentration of α-glycerophosphate has been found in ethanol-treated rats (146) and the glycerol uptake by splanchnic organs has been found to be reduced following ethanol administration (197).

## Effect of Ethanol on Release of Lipoproteins by Liver

Low plasma lipid concentrations have been observed in association with the fatty liver produced by several agents which impair hepatic protein synthesis, including carbon tetrachloride (97-102), ethionine (104-107), puromycin (108) and orotic acid (109, 110). Evidence of impaired lipoprotein release has been obtained from liver perfusion studies in which palmitate-$C^{14}$ was used, although the physiological significance of this study is questionable due to the high concentrations of ethanol to which the organ was subjected (198). Other investigators have con-

cluded that ethanol administered *in vivo* has no effect on lipoprotein synthesis in liver (103, 138). Results obtained in the present study favor the latter conclusion, since the concentration of each of the serum lipids was increased in ethanol-treated rats. A similar response in serum lipid concentrations to ethanol has been observed by other investigators in studies involving laboratory animals (136) and human subjects (133, 184).

The possibility exists that ethanol-induced elevations in serum lipid levels may reflect impaired removal of triglyceride and other lipids from blood. Measurements of plasma post-heparin lipoprotein lipase activities have generally demonstrated normal levels following ethanol administration (199, 200), although low values of this lipolytic activity have been described in six of a group of eight chronic alcoholics studied following a drinking bout (133). In addition, plasma obtained from a chronic alcoholic in whom lipemia and pancreatitis were observed following an episode of acute alcoholism, was found to inhibit plasma post-heparin lipoprotein lipase activity from a normal subject (201). Studies performed in human subjects fed a fat load have demonstrated that ethanol increases the lipemia (199). The possibility that ethanol alters lipolytic enzymes other than those appearing in plasma after heparin administration has not been excluded by these studies.

## Effect of Ethanol on Fatty Acid Oxidation by Liver

Wooles (202) studied fatty acid metabolism following the administration of a single large dose of ethanol to rats and concluded from indirect *in vivo* measurements that oxidation of hepatic fatty acids was depressed. Addition of ethanol to rat liver homogenates decreased the

rate of palmitate-$C^{14}$ oxidation to radioactive carbon dioxide (167). Ethanol also depressed the oxidation of octanoate-$C^{14}$ to carbon dioxide in liver slices and liver perfused *in vitro*, although oxidation of this medium chain-length fatty acid was less inhibited than was palmitate (191).

Lieber and Schmid (168) have proposed that the high intracellular concentration of NADH in liver may decrease the rate of substrate oxidation via the citric acid cycle and thereby limit the conversion of fatty acid to carbon dioxide. The significance of any impairment in fatty acid oxidation produced by ethanol in relation to the development of fatty liver remains to be defined, since inhibition of fatty acid oxidation similar to that produced by ethanol has been observed following the administration of several other substances, including sorbitol, xylitol, nicotinamide and isocaloric amounts of glucose, without evidence in any of the latter instances of fat accumulation in the liver (167).

## Effect of Ethanol on Fatty Acid Composition of Hepatic Glycerolipid

Results obtained in the present study demonstrate that the fatty acid composition of hepatic triglyceride, phosphatidylcholine and phosphatidylethanolamine may be modified by altering the fatty acids present in dietary triglyceride from a saturated (approximately 92% of the fatty acids in coconut oil are saturated) to an unsaturated type (approximately 88% of the fatty acids in corn oil are unsaturated). A larger proportion of saturated fatty acids in each of these glycerolipids was found in liver from rats fed coconut oil, when compared with results obtained in rats fed corn oil.

Triglyceride-fatty acids from ethanol-treated rats fed corn oil were relatively less saturated (decrease in saturated fatty acids from 44.3 to 32.2% of total) than controls, whereas triglyceride-fatty acids from ethanol-treated rats fed coconut oil were relatively more saturated (increase from 58.4 to 70.1%). These results and those reported by other investigators (163) demonstrate that the fatty acid composition of the triglyceride which accumulates in the fatty liver produced by prolonged ethanol administration resembles, in part, dietary triglyceride. A similar response to ethanol was observed in hepatic phosphatidylcholine from rats fed corn oil. Although the magnitude of the response to ethanol was less, statistically significant changes (decrease in the proportion of palmitic acid and increase in arachidonic acid) were observed in phosphatidylcholine-fatty acids from these animals. Ethanol had little effect either on the proportions of individual fatty acids or on the total proportion of saturated fatty acids in phosphatidylcholine from rats fed coconut oil. A similar lack of response was observed in phosphatidylethanolamine from ethanol-treated rats fed either corn oil or coconut oil.

The changes in fatty acid composition of hepatic glycerolipids, particularly triglyceride and phosphatidylcholine, observed in ethanol-treated rats might possibly reflect ethanol-induced alterations in the fatty acid composition of the diglyceride which is considered to be a common precursor in the synthesis of triglyceride, phosphatidylcholine and phosphatidylethanolamine. Zakim (203) has reported that ethanol lowers the hepatic concentration of long-chain acyl-CoA derivatives. If the concentration of acyl-CoA were limiting in liver of ethanol-treated rats,

it is conceivable that a greater proportion of the fatty acids utilized for synthesis of the diglyceride intermediate, and other glycerolipids formed therefrom, might be derived from the fatty acids provided in dietary triglyceride. Results reported by Lands and Hart (74), however, demonstrated that stearic and linoleic acid were esterified at both the α- and β-position of glycerophosphate. This observation suggests that the acyltransferases involved in the formation of phosphatidic acid and, subsequently, the diglyceride intermediate, are not sufficiently specific to account for the relative distribution of saturated and unsaturated fatty acids found in glycerolipids. Additional evidence favoring the conclusion that other factors are involved in the ethanol-induced effects on glycerolipid metabolism is the observation, obtained in the present study, that ethanol altered the fatty acid composition of hepatic triglyceride and phosphatidylcholine to a much greater extent than that of phosphatidylethanolamine.

The possibility exists that ethanol might alter the activity of the transferases involved in the reaction of diglycerides with acyl-CoA, CDP-choline or CDP-ethanolamine. Goldman and Vagelos (67) have observed evidence of fatty acid specificity, in respect to both the diglyceride and acyl-CoA substrates, in the transferase activity which catalyzes the acylation of diglyceride to form triglyceride. The effect of ethanol, if any, on this group of transferases has not been evaluated.

Lands and co-workers (72, 74, 75) have presented evidence favoring the conclusion that the characteristic fatty acid compositions of phosphatidylcholine and phosphatidylethanolamine are determined in a significant degree by a re-distribution of fatty acids after the nitrogenous

base has been attached to the molecule. This re-structuring of phospholipids is catalyzed apparently by a group of fatty acyl-CoA:lysophosphatide transferases found in liver microsomes. This group of investigators (204) has also shown that acyltransferase activities measured in vitro correlate favorably with the distribution of fatty acids, particularly the relative positional specificity of saturated and unsaturated fatty acids between the α- and β-positions of glycerol, in glycerolipids in vivo.

Results obtained in the present study indicate that the activities of those acyltransferases for which α-lysophosphatidylcholine and α-lysophosphatidylethanolamine serve as acyl acceptors were increased in ethanol-treated rats fed corn oil. The fatty acyl specificities of the acyltransferase activities for α-lysophosphatidylcholine, however, differed from those for α-lysophosphatidylethanolamine. Ethanol produced a marked increase in the rate at which unsaturated fatty acids were transferred onto α-lysophosphatidylcholine, while the rate of transfer of saturated fatty acids was unchanged. When α-lysophosphatidylethanolamine served as acceptor, the rate of transfer of both saturated and unsaturated fatty acids was moderately increased. Ethanol had less effect on the acyltransferase activities for which β-lysophosphatidylcholine and β-lysophosphatidylethanolamine served as acceptors. The rate of transfer of saturated fatty acids onto β-lysophosphatidylcholine was decreased, however, in ethanol-treated rats. The effect of ethanol on acyltransferase activities for saturated and unsaturated fatty acids measured in vitro correlates favorably with the changes observed in the saturated and unsaturated fatty acid composition of phosphatidylcholine, as

well as the lack of change of phosphatidylethanolamine, in ethanol-treated rats fed corn oil. These observations suggest that the administration of ethanol to these rats produced specific changes in the acyltransferases involved in phosphatidylcholine metabolism.

Results obtained in the present study also suggest that nutritional factors may influence these acyltransferases. Ethanol had little effect on the activities which transfer unsaturated fatty acids onto α-lysophosphatidylcholine in rats fed coconut oil, whereas a marked increase in these activities was observed when ethanol was given to rats fed corn oil. This lack of response to ethanol in respect to the acyltransferases in rats fed coconut oil is consistent with the observation that similar proportions of saturated and unsaturated fatty acids were found in controls and ethanol-treated rats fed coconut oil.

Fallon and co-workers (205, 206) have reported that ethanol increased the rate of conversion of phosphatidylethanolamine to phosphatidylcholine, based on the incorporation into the latter of radioactivity from methionine labeled in the methyl group. Additional studies are required to evaluate whether the enzyme system catalyzing this transmethylation reaction demonstrates a fatty acid specificity.

## CHAPTER VI

## SUMMARY

1. Chronic administration of ethanol to rats produced an increase in hepatic contents of triglyceride, cholesterol, phosphatidylcholine and phosphatidylethanolamine, when compared with values obtained from controls fed an isocaloric liquid diet. Serum concentrations of these lipids were also increased in ethanol-treated rats.

2. Ethanol increased the incorporation of glycerol-$C^{14}$ into hepatic triglyceride (increased by 56% of control value), phosphatidylcholine (35%) and phosphatidylethanolamine (9%) during the first two hours after isotope administration.

Measurements of half-time values for hepatic glycerolipids, obtained using glycerol-$H^3$, demonstrated that ethanol produced a moderate increase in the absolute turnover rate of triglyceride and phosphatidylcholine, as well as a small increase in the absolute and fractional turnover rates of phosphatidylethanolamine. The fractional turnover rates of triglyceride and phosphatidylcholine in ethanol-treated rats were lower, however, than in controls.

These observations suggest that the accumulation of triglyceride, phosphatidylcholine and, to a lesser extent, phosphatidylethanolamine in the liver of ethanol-treated rats is due primarily to an increase

in the rates of synthesis of these glycerolipids, rather than an impairment in removal mechanisms.

3. The total proportions of unsaturated fatty acids present in hepatic triglyceride, phosphatidylcholine and phosphatidylethanolamine were higher in controls fed corn oil as the sole source of dietary triglyceride than in those fed coconut oil. Ethanol increased the total proportion of unsaturated fatty acids in hepatic triglyceride and phosphatidylcholine (compared with controls fed corn oil), as well as the total proportion of saturated fatty acids in hepatic triglyceride from rats fed coconut oil (compared with controls fed coconut oil). The total proportion of saturated fatty acids in phosphatidylcholine was similar in ethanol-treated and control rats fed coconut oil. In addition, ethanol produced no statistically significant change in the fatty acid composition of hepatic phosphatidylethanolamine in rats fed either corn oil or coconut oil.

These results are consistent with the conclusion that a significant proportion of the triglyceride which accumulates in the liver during prolonged ethanol administration contains fatty acids derived from dietary triglyceride.

4. Transferase activity in rat liver microsomes was assayed using CoA-derivatives of palmitic, stearic, oleic, linoleic and arachidonic acid as fatty acyl donors and α- and β-lysophosphatidylcholine and α- and β-lysophosphatidylethanolamine as fatty acyl acceptors. A marked increase in rates of transfer of unsaturated fatty acids onto α-lysophosphatidylcholine and a decrease in the rates of transfer of saturated fatty acids onto β-lysophosphatidylcholine were observed in ethanol-

treated rats fed corn oil. Ethanol failed to produce a similar response in rats fed coconut oil, however, suggesting that the fatty acid composition of dietary triglyceride may modify the ethanol-induced changes in these acyltransferases. The rates of transfer of both saturated and unsaturated fatty acids onto α-lysophosphatidylethanolamine were increased slightly in ethanol-treated rats, but little change was evident in the values obtained for the transfer of fatty acids onto β-lysophosphatidylethanolamine.

The effect of ethanol on acyltransferase activities for saturated and unsaturated fatty acids measured in vitro correlates favorably with the changes in the saturated and unsaturated fatty acid composition of phosphatidylcholine, as well as the lack of change of phosphatidylethanolamine, in ethanol-treated rats.

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