Effects of ethanol on lipid metabolism

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INTRODUCTION

Accumulation of fat in the liver is the most common disturbance of lipid metabolism produced by alcohol. Since steatosis constitutes the most striking histologic feature of the early stages of alcoholic liver injury, this initial phase is generally defined as alcoholic fatty liver. It must be pointed out, however, that less conspicuous but not less important alterations affecting protein metabolism and subcellular organelles occur concomitantly. These associated changes may play a key role in perpetuating disturbances in hepatic lipid metabolism beyond the immediate effects of the oxidation of ethanol on intermediary metabolism.

Alcoholic fatty liver is often associated with hyperlipemia. Both the accumulation of lipids in the liver and in the blood are multifactorial phenomena. Though a great deal of evidence has been gathered to establish an etiologic role of ethanol in the pathogenesis of these syndromes, other factors associated with alcohol abuse also play important roles.

Accumulation of fat in the liver is also prominent in alcoholic hepatitis (defined by necrosis and inflammation) and in alcoholic cirrhosis (characterized by fibrosis and distortion of the normal architecture of the liver). Recently developed animal models indicate that these varieties of alcoholic liver disease probably represent steps of a process that should be considered as a continuous spectrum. With progressive liver damage, the predominant mechanism leading to steatosis and hyperlipemia changes: some lipid alterations appear to represent the initial interactions between ethanol and lipid metabolism; others appear to represent compensatory efforts to alleviate consequences of the altered lipid metabolism; and, finally, others reflect the liver failure during progression of alcoholic liver injury. A challenging but still unresolved question is to what extent the serum lipid changes may serve as sensitive indicators of this progression. The alterations in hepatic and serum lipids may also bear important consequences on other tissues: possible relationships between the alcohol-induced lipid changes and the pathogenesis of some hemolytic anemias, pancreatitis, and, particularly, atherosclerosis have been emphasized.

PATHOGENESIS OF ALCOHOLIC FATTY LIVER

Etiologic role of ethanol

Ethanol administration is associated with a variety of secondary effects, which by themselves can alter lipid metabolism. These include local toxic effects at the site of administration, particularly in the gastrointestinal tract (1, 2) and the peritoneum (3), and systemic stressful effects secondary to alcohol intoxication. Chronic ethanol administration, on the other hand, is complicated by interference with nutrition. Therefore, considerable efforts have been spent to differentiate the effects due to ethanol itself from those of associated complications. This is of more than academic interest since the treatment of the medical complications of alcoholism directly depends upon the recognition of the etiologic factor.

Acute effects of ethanol on liver lipids

Mallov and Bloch (4) first reported that a single large dose of ethanol (4.2–6.2 g/kg body weight) given to rats by stomach tube or by intraperitoneal injection, provoked significant accumulation of triglycerides in the liver within 12–16 hr. Probably because of its apparent simplicity, this "acute alcoholic fatty liver" has been employed as a model by numerous investigators and continues to be used, despite the original recognition of the fact that most of this acute effect may be nonspecifically due to the stress generated by

Abbreviations: FFA, fatty free acids; ADH, alcohol dehydrogenase; MEOS, microsomal ethanol-oxidizing system; PHLA, postheparin lipoprotein lipase activity; LCAT, lecithin:cholesterol acyltransferase.
alcoholic administration. Although the changes in liver lipids can be related in part to the oxidation of ethanol, the degree of fat accumulation varies over a dosage range far above that required to saturate ethanol-oxidizing capacity (5) and the fatty liver is markedly diminished if the ethanol dose is fractionated and given at intervals in order to achieve smaller blood ethanol concentrations (6). Like other stressful situations, alcohol intoxication stimulates both the hypothalamus–pituitary–adrenocortical (7–10) and sympatho–adrenomedullar (11, 12) systems. The release of catecholamines has been particularly implicated because of their recognized ability to promote free fatty acid (FFA) release from the adipose tissue and triglyceride accumulation in the liver (13). Moreover, the acute alcoholic fatty liver is prevented by the administration of β-adrenergic blocking agents, hypophysectomy, adrenalectomy, and spinal cord transection. It is likely that any stress that depletes catecholamine stores prior to alcohol administration will also prevent acute alcoholic fatty liver (6). These acute effects are not only nonspecific but they may not pertain to the fatty liver observed after chronic ethanol ingestion.

**Chronic effects of ethanol on liver lipids**

The concept that malnutrition is primarily responsible for the development of chronic alcoholic fatty liver was based on experimental evidence in growing rats, in which deficiencies in dietary protein and lipotropic factors (choline and methionine) produced fatty liver, whereas ethanol was devoid of apparent toxicity (14). Moreover, alcohol administration to patients recovering from fatty liver failed to prevent the disappearance of liver fat or to elicit any deleterious effect (15, 16). In these studies, however, the amounts of ethanol given were considerably less than the usual intake of alcohols, which not uncommonly amounts to 50% or more of the caloric requirement. When amounts of ethanol similar to those usually consumed by alcoholics were given to patients with fatty liver, the clearance of fat from the liver was indeed prevented (17). In the rat experiments in which ethanol had no effect, the alcohol was administered in the drinking water. This technique of alcohol administration results in ethanol intakes equivalent to only 10–25% of the caloric requirement of the animals. A comparable amount, when given in liquid diets, results in negligible blood ethanol concentrations and there is no development of fatty liver (18). By incorporating ethanol in a liquid diet containing all required nutrients and offered as the only source of fluid and food, the consumption of ethanol by the rat was increased to 36% of the caloric requirement, a proportion comparable to moderate alcohol consumption in man. Isocaloric replacement of some of the carbohydrate of the diet by ethanol consistently produced a 5- to 10-fold increase in hepatic triglyceride concentrations (18–20). The accumulation of lipids developed gradually during the first month of ethanol feeding and persisted thereafter for at least 1 yr in the rat (21) and 3 yr in the baboon (22). The nutritional adequacy of the diets was manifested by their ability to support growth and maintain health and normal liver function and structure in the pair-fed controls. Animals fed alcohol also gained weight, though the growth-promoting ability of ethanol calories was smaller than that of carbohydrate (23). Isocaloric replacement of carbohydrate by fat or administration of carbohydrate-deficient diets did not reproduce the effect of ethanol (Fig. 1). This indicates that the fatty liver is due to ethanol and not to the manipulation of the other sources of calories.

In man with morphologically normal liver (with and without a history of alcoholism), fatty liver develops when ethanol is given as a supplement to normal diet or in isocaloric substitution for carbohydrate (18, 24–26). These fatty livers are evident both morphologically and by the lipid content of liver biopsy samples which reveal up to a 25-fold rise in triglyceride concentration. The lipid accumulation is apparent already after a few days (26) or even after one day (27) of ethanol administration. Though the greatest increase affects liver triglycerides, other lipid classes such as phospholipid (18) and cholesterol (19) also accumulate in the liver of rats after chronic ethanol feeding. The increase in hepatic cholesterol occurs mainly in the esterified fraction, with little (if any) increase in free cholesterol (19).

Though the ingestion of the alcohol-containing diets provided all nutrients in the required amounts, the possibility of malabsorption needed to be considered. Indeed, the ingestion of ethanol in concentrations commonly found in alcoholic beverages produces intraluminal concentrations in the upper gastrointestinal tract (including proximal jejunum) considerably higher than those found in blood, even during extreme alcohol intoxication (28). These high concentrations of ethanol have been shown to produce damage and functional impairment of the intestinal epithelium (2). High ethanol concentrations also interfere with active transport of glucose, amino acids, and vitamins both in vitro and in vivo (29–32). Moreover, malabsorption has been found in malnourished alcoholics (33, 34). However, chronic administration of ethanol along with nutritionally adequate diets to both man and animals failed to produce abnormal fecal losses of calories, nitrogen, or fat (23, 35, 36), suggesting that the malabsorption observed in some alcoholics
is due in part to the associated malnutrition. Furthermore, it is unlikely that a deficiency state can develop in the short time required for the development of alcoholic fatty liver in volunteers (26, 27).

In nonhuman primates (baboons), the intake of ethanol was increased up to 50% of the caloric requirement in the absence of malabsorption or malnutrition. Under these conditions, the entire spectrum of alcoholic liver disease (from fatty liver to cirrhosis) was reproduced (37).

Influence of other factors on ethanol-induced fatty liver

The evidence that ethanol produces fatty liver (which actually progresses to the stage of cirrhosis) in the absence of malnutrition does not exclude a possible secondary role of nutritional factors in the liver damage of alcoholics.

a) Role of dietary lipids. The degree of steatosis produced by alcohol depends on the lipid content of the diet. Reduction of dietary fat to a level of 25% or less of total calories was accompanied by a significant reduction of the lipid accumulation in the liver of ethanol-fed rats (21) (Fig. 2). However, even when the dietary fat was decreased to only the necessary supply of linoleate (to avoid essential fatty acid deficiency), the fatty liver was not fully prevented (21). The importance of dietary fat was confirmed in volunteers: for a given amount of alcohol intake, much more steatosis developed with a diet of normal fat content than with low-fat diet (38). In addition to the amount, the chain length of the dietary fatty acids is also important in determining the degree of fat deposition. Replacement of dietary fat containing triglycerides of long chain fatty acids by triglycerides containing medium chain fatty acids reduced the amount of lipid accumulated in the liver of ethanol-fed rats (39). This effect was probably due to the propensity of medium chain fatty acids to undergo oxidation rather than esterification (40).

b) Role of protein and lipotropic factors. As mentioned before, the deficiency in protein and lipotropic factors can produce fatty liver in growing rats, but primates are far less susceptible to these deficiencies than rodents (41). The effects of protein deficiency on the liver have not been clearly delineated in human adults. In children, protein deficiency leads to steatosis, one of the manifestations of kwashiorkor. In rats, ethanol-induced fatty liver was potentiated when dietary protein was reduced to 4% of total calories and the diet was made deficient in choline (42). However, such potentiation failed to occur in baboons given 7% of total calories as protein (43). Moreover, an excess of protein (25% of total calories or twice the recommended amount) and massive supplementation with choline failed to prevent the fatty liver produced by alcohol in volunteers (26). This is not surprising since there is no evidence that a diet deficient in choline is deleterious to man. Unlike rodent livers, human liver contains very little choline oxidase activity, a fact which may explain the species differences with regard to choline deficiency. Hepatic injury produced by lipotropic deficiency appears to be primarily an experimental disease of the rat with little (if any) relevance.
TABLE 1. Differences between the fatty liver induced by ethanol and by choline deficiency

<table>
<thead>
<tr>
<th>Species susceptibility</th>
<th>Ethanol</th>
<th>Choline Deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of liver lipids</td>
<td>Both rodents and primates are susceptible (18, 37)</td>
<td>Rodents are particularly susceptible, (14) whereas primates are resistant (41)</td>
</tr>
<tr>
<td>Influence of dietary fat</td>
<td>Hepatic lipid accumulation is enhanced by high fat diets (21)</td>
<td>Hepatic lipid accumulation is unaffected by dietary fat (47)</td>
</tr>
<tr>
<td>Serum lipids</td>
<td>Associated with increased production of serum lipoproteins and hyperlipemia (48)</td>
<td>Associated with decreased production of serum lipoproteins and hypolipemia (49)</td>
</tr>
<tr>
<td>Ultrastructural changes</td>
<td>Marked alterations of the mitochondria (50)</td>
<td>Slight alterations of the mitochondria (51)</td>
</tr>
<tr>
<td>Effect of orotic acid</td>
<td>None (52)</td>
<td>Reduces fatty liver (53, 54)</td>
</tr>
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To human alcoholic liver injury. Even in rats massive choline supplementation failed to prevent the ethanol-induced lesion (39). Furthermore, the fatty liver induced in the rat by choline deficiency differs from that induced by ethanol. Some of these differences are illustrated in Table 1. Among the similarities, we should mention that both types of fatty liver are associated with delayed export of plasma proteins, such as albumin (55, 56) and both lead to the development of cirrhosis.

**Mechanisms of the ethanol-induced accumulation of fat in the liver**

Fatty liver involves not only marked alterations in hepatic lipid metabolism, but also profound alterations in protein metabolism, which may have important implications in the ability of the liver to handle lipids.

The two most conspicuous features of alcoholic fatty liver are the deposition of fat and the enlargement of the organ. This hepatomegaly was traditionally attributed to fat accumulation, but it has been recently shown in ethanol-fed rats that fat accounts for only half of the increase in liver dry weight. The other half is almost totally accounted for by an increase in protein (57) (Fig. 3). This protein increase is not associated with changes in total liver protein concentration or wet/dry weight ratios, indicating that the accumulation of protein is accompanied by a proportional retention of water. These increases in lipid, protein, and water are associated with an increase in size of the hepatocytes (57). The protein accumulation is due, at least in part, to retention of secretory proteins in the liver (56).

There is no convincing evidence that ethanol by itself, rather than its oxidation, causes these changes. The effects of alcohol dehydrogenase (ADH) inhibitors (such as pyrazole) on acute alcoholic fatty liver have been inconclusive, prevention being found by some (58, 59), no effect at all by others (60, 61), or effects depending on the experimental conditions such as dose of the drug (62) or sex of the animal (63). This variability is not unexpected from the conspicuous role of stress on the pathogenesis of acute alcoholic fatty liver. Chronic administration of inhibitors together with ethanol have been reported to increase the effects of ethanol on the liver with appearance of inflammation and cell necrosis (64, 65). The interpretation of the latter results have been complicated by the inherent toxicity of the inhibitors (66) and the possibility that toxicity could result from oxidation of ethanol through pyrazole-insensitive pathways. Both the protein and lipid changes have been linked to the consequences of ethanol oxidation in the liver, some essential features of which are summarized below.
Metabolism of ethanol

The oxidation of ethanol represents a metabolic burden for the liver. Though trace amounts of ethanol can be synthesized endogenously (67), it is essentially a foreign compound that originates as a waste product of microorganisms (yeasts). Moreover, ethanol has a high caloric value (7.1 Cals/g) and is readily absorbed from the gastrointestinal tract. Since only 2–10% of the amount absorbed is eliminated through lungs and kidneys, the rest must be oxidized and the bulk of its oxidation takes place in the liver. This organ specificity is further aggravated by the absence of storage and a high caloric value (7.1 Cals/g) and is readily absorbed from the gastrointestinal tract. Since only 2–10% of the amount absorbed is eliminated through lungs and kidneys, the rest must be oxidized and the bulk of its oxidation takes place in the liver. This organ specificity is further aggravated by the absence of storage and a feedback regulation to adjust its oxidation to cellular needs. Therefore, the oxidation of ethanol produces striking metabolic imbalances in the liver.

The main hepatic pathway for ethanol oxidation involves alcohol dehydrogenase (ADH), an enzyme of the cytosol, which catalyzes the conversion of ethanol to acetaldehyde. Hydrogen is transferred from ethanol to the cofactor nicotinamide adenine dinucleotide (NAD), which is converted to its reduced form (NADH). As a net result, ethanol oxidation generates an excess of reducing equivalents in the liver, primarily as NADH and, by transhydrogenation (68), probably also as NADPH, the reduced form of nicotinamide adenine dinucleotide phosphate (69). In addition, ethanol can also be oxidized to acetaldehyde by an accessory pathway that requires NADPH as a cofactor and is localized in the endoplasmic reticulum (70). Unlike ADH, this microsomal ethanol-oxidizing system (MEOS) increases in activity during chronic alcohol consumption (70). There is also debate on a possible role of catalase (an enzyme of peroxisomes) in the conversion of ethanol to acetaldehyde.

It was reported more than 10 years ago that alcohol feeding results in proliferation of the smooth membranes of the hepatic endoplasmic reticulum (50). This finding was subsequently confirmed (71–73) and established on a biochemical basis by the demonstration of an increase in both phospholipid and total protein content of the smooth membranes (74). The mechanism of these microsomal alterations is unknown. By analogy with the proliferation of this organelle induced by other drugs or foreign compounds that utilize microsomes during their metabolism, it has been linked to the fact that ethanol can be oxidized at this site.

The various metabolic effects of ethanol can be attributed either to the excess of reducing equivalents or to the interaction of other microsomal functions in association with the development of the MEOS pathway. Since microsomes utilize rather than produce reducing equivalents, the enhancement in microsomal activities produced by chronic alcohol consumption tends to reduce the changes in redox state generated by the ADH-dependent alcohol oxidation.

Some of the effects can also be due to metabolites of ethanol, such as acetaldehyde and acetate. Acetaldehyde is the first major oxidation product of ethanol when the latter is oxidized by any of the previously described pathways. Acetaldehyde oxidation proceeds via aldehyde dehydrogenase, 80% of the activity of which has been located in the mitochondria (75). Under normal conditions, the rates of ethanol and acetaldehyde oxidations are similar, and the concentration of acetaldehyde in liver and blood remains very low. However, the increased rate of ethanol oxidation that follows prolonged alcohol consumption results in increased acetaldehyde levels (76), particularly because the oxidation of acetaldehyde in the mitochondria is not increased, but is actually decreased after ethanol consumption (77). Part of the metabolic effects of acetaldehyde may result from generation of NADPH during its oxidation, as discussed before in the case of ethanol. Acetaldehyde, however, is a very reactive compound which may exert some toxic effects of its own.

Finally, most of the ethanol oxidized is recovered as free acetate in the hepatic vein (78). It is uncertain whether acetyl-CoA results directly from acetaldehyde oxidation or is formed from free acetate, but its presence is indicated by the incorporation of labeled ethanol in a variety of metabolites (such as fatty acids and cholesterol) that use acetyl-CoA as a precursor. Although, in vitro, the liver readily utilizes acetate, in vivo most of the acetate is utilized by peripheral tissues (79). During ethanol oxidation, hepatic oxidation of acetate is further decreased as a result of the inhibition of the citric acid cycle (80). The possible consequences of this abnormal generation of free acetate in the liver are largely unknown.

Origin of the increased liver lipids and mechanisms for their accumulation

Ethanol-induced fatty liver could result from an increased supply of lipids to the liver from three main sources: dietary lipids, adipose tissue lipids, and lipids synthesized in the liver itself. Alternatively, lipids can accumulate if their disposal by oxidation, lipolysis, or secretion into serum or bile is inadequate. Depending on the experimental condition, the various sources and possible mechanisms can be implicated. The mechanisms for liver fat accumulation are illustrated in Fig. 4.

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Fig. 4. Theoretical mechanisms for alcoholic fatty liver. Ethanol intake could result in fatty liver either by enhancing the pathways illustrated by wide arrows or by blocking those illustrated by broken lines (307).

a) Increased supply of lipids from the small intestine. After consumption of ethanol for several days with diets containing a characteristic fatty acid composition, the fatty acids that accumulate in the liver derive primarily from dietary fat (38, 81, 82). Acute alcoholic fatty liver is exaggerated by oral administration of fat (83) and diminished by diversion of the lymph carrying lipids from the intestine (84). As emphasized before, after chronic alcohol feeding, the degree of hepatic lipid accumulation is also influenced by the fat content of the diet. However, even the restriction of dietary fat to only the required essential fatty acids does not fully prevent the development of ethanol-induced fatty liver (21).

Acute administration of ethanol to naive rats increases the intestinal lymph flow and output of both dietary (85) and nondietary (86) lipids. The major determinant of this increase in lymph lipid output appears to be the stimulatory effect of ethanol on splanchnic circulation (87–89) and the mesenteric lymph flow (85). However, the lymphagogue effect of ethanol decreases in rats fed ethanol chronically and, after several weeks of ethanol feeding, the lymph lipid output of ethanol-fed rats was similar to that of pair-fed controls despite persistence of the fatty liver (85). The in vivo changes in lymph lipid output did not correlate with in vitro changes in intestinal lipid metabolism. After 12–20 hr of administration of a high ethanol dose or after chronic alcohol feeding, there was increased ability of the gut to oxidize fatty acids (90) and to synthesize triglycerides (90–93) and cholesterol (94), but this increased ability was not associated with significant increases in the output of lipid in the lymph. By contrast, 1–3 hr after administration of an acute ethanol dose, fatty acid oxidation and esterification by intestinal slices were depressed (90), despite the fact that the net output of lipids in the lymph increased (85).

Thus, dietary lipids play a permissive role for the full development of ethanol-induced fatty liver, but the fatty liver is not due primarily to increased lipid absorption or production by the gastrointestinal tract.

b) Increased mobilization of fatty acids from the adipose tissue. The fatty acids that accumulate in the liver after administration of one large dose of ethanol resemble those of adipose tissue (81, 95). Mobilization of pre-labeled fatty acids from epididymal fat pads and concentrations of plasma FFA were found either increased (95, 96) or unchanged (97, 98). The effects of ethanol on plasma FFA depend upon the dose. Administration of a moderate dose of alcohol to man (0.5–1 g/kg body weight per hr) produces a rapid fall of short duration in the plasma concentration of FFA (99), decreased FFA turnover (100), and a concomitant reduction in circulating glycerol (101). This inhibitory effect of ethanol on FFA mobilization was found to be mediated by acetate (102), the major final product of hepatic ethanol oxidation. The metabolic consequences of this substitution of acetate for FFA, the major normal fuel for many tissues, are unknown. By contrast, the ad-
ministration of doses that result in blood ethanol levels over 200 mg/100 ml produces a significant increase in plasma FFA (24, 103). Since stressful doses of ethanol probably stimulate fatty acid mobilization (via catecholamine release) and depress it (via acetate production), the net effect may depend on the particular experimental condition. An initial fall in FFA followed by a secondary rise has been confirmed (104, 105), the stimulatory response being inhibited by administration of β-adrenergic blocking agents (105).

c) Increased uptake of fatty acids by the liver. Although the flux of plasma FFA is not affected by a moderate alcohol dose, the fraction of the total flux that is taken up by the liver is increased (106) because of the stimulatory effects of ethanol on hepatic blood flow (87–89, 107). After intraperitoneal injection of a very small dose (0.7 g/kg body weight), the increased uptake of FFA accounted for the rate of hepatic fatty acid accumulation and release; this contribution decreased at higher but still moderate doses of ethanol (106). The contribution of this mechanism to the fatty liver that follows chronic ethanol ingestion remains to be determined.

d) Increased synthesis of fatty acids in the liver. When ethanol is given with a low-fat diet, endogenously synthesized fatty acids are deposited in the liver (38, 42, 81). This could derive from normal carbohydrate precursors or from ethanol itself. It is noteworthy that in vitro a large fraction of the carbon skeleton of ethanol is incorporated into fatty acids (108, 109). Although the liver can utilize two-carbon fragments from ethanol, in vivo, most of them are released into circulation as acetate (78), which completes its oxidation to CO2 in peripheral tissues. Therefore, the possibilities of in vivo conversion of ethanol into liver fat appears limited. The incorporation of acetate (110, 111) and pyruvate (111) into liver fatty acids increases in the presence of ethanol. However, the total rate of fatty acid synthesis from precursors other than ethanol has been found unaffected or decreased (109, 112–114), suggesting that the increased acetate incorporation takes place through the fatty acid elongation pathway of the mitochondria. This may represent a way to dispose of the excess of reducing equivalents generated by ethanol oxidation, but the contribution of enhanced fatty synthesis to the hepatic accumulation of fat remains unsettled.

e) Increased esterification of fatty acids in the liver. The increased NADH/NAD ratio generated by the oxidation of ethanol via ADH also favors the production of α-glycerophosphate from dihydroxyacetone phosphate (115). This increase facilitates the formation of glycerides such as triglycerides and phospholipids in isolated hepatocytes incubated with ethanol (116). Also, in vivo, ethanol-fed rats incorporate significantly more labeled glycerol into hepatic triglycerides, phosphatidylcholine, and (to a lesser extent) phosphatidylethanolamine (117), despite ethanol-induced inhibition of glycerol uptake by splanchnic organs (118) and possible dilution by greater hepatic concentrations of α-glycerophosphate (115). For the calculation of rates of glyceride synthesis in ethanol-fed rats, the size of the precursor pools needs assessment. However, the postulated increased triglyceride synthesis in keeping with observations that indicate enhanced activity of enzymes involved in these processes during chronic alcohol feeding. Indeed, ethanol consumption enhances the activity of hepatic microsomal 1-α-glycerophosphate acyltransferase (119), as well as other microsomal acyltransferases involved in phospholipid synthesis (120), though the latter effect depends on the type of fatty acid given together with ethanol (120). These increases in microsomal enzyme activities have been linked to the proliferation of the endoplasmic reticulum induced by chronic ethanol feeding.

f) Increased synthesis of cholesterol in the liver. Another microsomal process that is enhanced by chronic ethanol feeding is cholesterol synthesis. Indeed, liver slices obtained from ethanol-fed rats display increased ability to incorporate labeled acetate (but not mevalonate) into cholesterol, even when incubated in the absence of ethanol (121). Enhanced cholesterogenesis also occurs with other drugs (such as phenobarbital) that induce proliferation of the endoplasmic reticulum (122), but (unlike ethanol feeding) this is not associated with significant accumulation of cholesterol in the liver. Ethanol feeding mainly increases cholesterol esters (19). However, cholesterol esterification has been found unaffected after acute (116) or chronic (123) ethanol administration, suggesting that the accumulation of esters may be due to decreased disposal rather than to increased production, as discussed later.

g) Decreased oxidation of fatty acids in the liver. Decreased fatty acid oxidation produced by ethanol has been demonstrated in liver slices (110, 124), perfused liver (40), isolated hepatocytes (116), and in vivo (125). This reduction offers the most likely explanation for the deposition in the liver of dietary fat (when available) or fatty acids derived from endogenous synthesis (in the absence of dietary fat) after chronic alcohol consumption. Fatty acid oxidation occurs in the mito-
chondria. This process is impaired by both functional and structural changes that take place in this organelle.

Reducing equivalents resulting from ethanol oxidation by ADH in the cytosol are transferred into the mitochondria by various "shuttle" mechanisms. The activity of the citric acid cycle is depressed (40, 126), in part, because of the slowing of the reactions of the cycle that require NAD. The electron transport chain will use reducing equivalents originating from ethanol, rather than from the oxidation through the citric acid cycle of two carbon fragments derived from fatty acids. Thus, fatty acids that normally serve as the main energy source of the liver (127) are supplanted by ethanol. In addition, the increased NADH/NAD ratio in the mitochondria consequent to ethanol oxidation restricts β-oxidation at the acyl CoA dehydrogenase and β-hydroxyacyl CoA dehydrogenase sites, promoting accumulation of long-chain fatty acyl CoA. Indeed, β-oxidation is depressed when isolated hepatocytes are incubated with ethanol (116).

In addition to these functional changes in the mitochondria as a consequence of the metabolism of ethanol, chronic alcohol abuse results in persistent alterations of this organelle. Indeed, swelling and disfiguration of the mitochondria, disorientation of the cristae, and intramitochondrial crystalline inclusions are prominent in alcoholics (128, 129). Similar alterations were reproduced by isocaloric substitution of ethanol for carbohydrate in otherwise nutritionally adequate diets given to rats (50), baboons, (22) or man, with (25, 71) and without (26) a history of alcoholism, indicating a direct effect of ethanol rather than of malnutrition. Degenerated mitochondria are conspicuous and the debris of these degraded organelles is also found within autophagic vacuoles and residual vacuolated bodies (130). These ultrastructural changes of the mitochondria are associated with increased fragility and permeability (131-133), decreased phospholipid content (134), and altered fatty acid composition (135). Essential fatty acid deficiency partially protects the mitochondria from developing the increased fragility induced by chronic ethanol feeding (136). Increased serum activity of the mitochondrial enzyme, glutamate dehydrogenase, is found in alcoholics and has been proposed as a good indicator of the degree of liver cell necrosis in these patients (137). The mechanism of the alteration of the mitochondrial membranes is unknown, but could possibly be linked to the depression of mitochondrial protein synthesis induced by ethanol consumption (132).

The striking structural changes of the mitochondria are associated with corresponding functional abnormalities. These altered mitochondria have a reduction in cytochrome a and b content (132) and in succinic dehydrogenase activity (132, 138). The respiratory capacity of this organelle is depressed (139-142), including the oxidation of two-carbon fragments from fatty acids (143, 144). By contrast, β-oxidation of the fatty acids is enhanced (144). The accumulation of acetyl-CoA could result in increased ketogenesis, which indeed develops after chronic alcohol ingestion (145). In man, alcohol ingestion is associated with a progressive increase in ketonemia and ketonuria, which are most pronounced in the fasting state, in the absence of ethanol in the blood (145). Moreover, enhanced ketogenesis occurs in liver slices from ethanol-fed rats in the absence of ethanol (145). This contrasts with the inhibition of ketogenesis reported in isolated hepatocytes when incubated with ethanol (116).

These metabolic alterations of the mitochondria from ethanol-fed animals can be reproduced, at least in part, by incubation of normal mitochondria with acetaldehyde (146), a product of ethanol metabolism, the oxidation of which is in turn impaired in mitochondria from ethanol-fed animals (77). Furthermore, altered mitochondria from ethanol-fed animals develop increased susceptibility to the injurious effects of acetaldehyde (147). Therefore, it has been postulated that generation of acetaldehyde may play a pathogenetic role in the progressive impairment of the mitochondria and consequent alterations in fatty acid oxidation beyond those produced acutely by the altered redox state during ethanol oxidation.

h) Decreased hydrolysis of fatty acid esters in the liver.

The increase in triglycerides, phospholipids, and cholesterol esters that follows chronic alcohol consumption could result from decreased hydrolysis of these esters, an activity that is predominantly carried out by acid lipase and esterase of the lysosomes (148-150). Inhibitors of lysosomal lipase (such as sulfonylureas) promote accumulation of liver triglycerides (151). Though there is no information of the activity of this lipase after ethanol, other lysosomal enzymes are variably affected by acute and short-term ethanol administration (152-154) and they are increased after prolonged alcohol consumption (155). In addition to this acid lipase of the lysosomes, there are other lipase and esterase activities (which are optimal at alkaline pH) in other subcellular fractions. Some of these activities are involved in the hydrolysis of triglycerides and cholesterol esters from chylomicron or lipoprotein remnants. Hydrolysis of cholesterol esters, (a step prior to their secretion into blood or bile, or to their conversion to bile salts) has been found decreased after chronic ethanol administration to rats (123).
i) Decreased excretion of liver fat into the bile. The major lipids excreted in the bile are phospholipids (mainly lecithin) and free cholesterol, the concentration of which was unaffected in the bile of ethanol-fed rats (156) despite the increases of lecithin and esterified cholesterol in the liver. Most of the cholesterol is, however, excreted as bile salts. Bile salt concentration was also found unaffected after chronic ethanol consumption (156). After acute ethanol administration or when blood ethanol levels are elevated in rats chronically fed alcohol, bile secretion was depressed (137, 158). By contrast, in rats fed ethanol-containing diets for 2–4 weeks, but withdrawn from alcohol for several hours, the flow of bile, and the secretion of both bile salt-dependent and the bile salt-independent fractions of the bile increased (156, 158). However, the increased secretory rate of bile salts may merely reflect their enlarged hepatic pool, since turnover rates and daily excretion of both cholic and chenodesoxycholic acid were significantly depressed in ethanol-fed rats (121). Moreover, ethanol feeding suppresses the normal increase in bile acid production that follows cholesterol feeding, leading to a striking increase in the concentration of esterified cholesterol in the liver (121). The mechanism of this inhibition has not been clarified. The reduction in bile acid excretion could be secondary to a decrease in cholesterol 7-α-hydroxylase activity (159). Regardless of the mechanism, the block of this major catabolic pathway of cholesterol may be responsible for the hepatic accumulation.

j) Decreased release of serum lipoproteins. A block in the hepatic synthesis and/or release of serum lipoproteins has been proposed as the most likely mechanism of the fatty liver produced by a variety of toxic agents (such as carbon tetrachloride (160), ethionine (161), or orotic acid (162)) as well as that produced by dietary deficiencies in choline (49) and protein (163, 164). However, in contrast with these fatty livers, that produced by chronic alcohol consumption develops in association with hyper- rather than hypolipemia, both in man (24, 165) and experimental animals (48, 166). During the initial stages of liver damage, alcoholic hyperlipemia involves increased hepatic release of serum lipoproteins, excluding a block of this pathway as a primary mechanism of the steatosis. However, lipoprotein retention could result if the increased production is not matched by efficient secretion. Ethanol has been shown to interfere with secretion of other export proteins with an associated decrease in liver microtubules (56). Microtubule alterations induced by other drugs (such as colchicine) interfere with the secretion of lipoproteins as well as other proteins (167, 168).

PATHOGENESIS OF ALCOHOLIC HYPERLIPEMIA

Etiologic role of ethanol

Alcoholic hyperlipemia follows diabetes as the second major cause of nonfamilial hyperlipemias (169). It was first recognized as a transient or recurrent condition following bouts of excessive drinking (170), generally associated with fatty liver (171) and sometimes with pancreatitis (172) or hemolytic anemia (173).

Hyperlipemia is more prominent in alcoholics with fatty liver than in patients with well-established cirrhosis (174, 175). Particulate fat behaves mainly as very low density lipoproteins (VLDL) on ultracentrifugation and as pre-β lipoproteins on electrophoresis. Thus, alcoholic hyperlipemia is usually classified as Type IV (176). Increased chylomicron or chylomicron-like particles are also found in some alcoholics even in the fasting state (169). In addition to the hypertriglyceridemia, there are also increased concentrations of serum cholesterol and phospholipids with corresponding increases in β- and α-lipoproteins. The serum lipid pattern changes rapidly after alcohol withdrawal. Triglyceride clearance is the fastest, whereas the clearance of cholesterol and phospholipids is slower (Fig. 5). Initially, the hypercholesterolemia is mainly due to an increase in free cholesterol. After the hypercholesterolemia has subsided, the esterified fraction may increase, probably reflecting an improvement of hepatic function and plasma lecithin:cholesterol acyl transferase (LCAT) activity; the total cholesterol may be secondarily raised (177).

In addition, it has been recently recognized that some individuals manifest unusual sensitivity to the hyperlipemic effects of ethanol. In these subjects, hyperlipemia may adopt a chronic course indistinguishable from other forms of chronic hyperlipemia, the role of alcohol being suspected mainly because of the lack of response to the usual treatment and the improvement after alcohol withdrawal. It is now apparent that factors other than ethanol itself can contribute to the development of alcoholic hyperlipemia, including subclinical abnormalities of lipid metabolism which can be exaggerated or unmasked by alcohol consumption.

Acute effects of ethanol on serum lipids

The oral or intravenous administration of ethanol to volunteers, resulting in inebriating concentrations
TRIGLYCERIDES

PHOSPHOLIPIDS

FREE CHOLESTEROL

TOTAL CHOLESTEROL

CHOL ESTERS

Fig. 5. Changes in plasma lipid fractions of a patient during recovery from alcoholic hyperlipemia (177).

(140–250 mg/100 ml of blood) produced a rapid increase in serum triglycerides (104, 165, 178) whereas smaller doses did not produce consistent changes in either pre- or postprandial triglyceride values (179). The administration of a high ethanol dose (180 g of ethanol in 6 hr) to chronic alcoholics produced hypertriglyceridemia due to increases in pre-β lipoproteins, chylomicron-like particles in the fasting state and in some of the lipoproteins, with characteristics intermediate between VLDL and low density lipoproteins (LDL) (180), which may represent VLDL remnants (181).

The effects of ethanol on serum triglycerides are greatly enhanced if alcohol administration is followed or accompanied by a fat-containing meal (182–185). Under these conditions, doses of ethanol that result in blood ethanol concentrations of less than 100 mg/100 ml usually produce a several-fold increase in serum triglycerides which persists for more than 12 hr. The lipemic response to the combination of fat and ethanol is significantly higher than the sum of the individual changes due to fat or ethanol given alone (185). The hypertriglyceridemia occurs mainly in the VLDL fraction, which exhibits a pre-β mobility on electrophoresis, but increases in chylomicrons and other lipoprotein fractions are also found in the postprandial state (185). Blood cholesterol and phospholipids remain unchanged (177, 178, 180, 186) unless alcohol administration is continued for several days in which case a moderate increase was observed (24, 187).

Unlike its effect on man and in rabbits (188, 189), acute ethanol administration to rats does not produce consistent hyperlipemia. After administration of high doses of ethanol to fasting rats (over 5 g/kg body weight), a lipemic response has been described after a few hours of administration, especially in male rats (190). Other investigators, however, found either no changes in serum triglycerides (97, 191–194) or even a decrease 3–4 hr after alcohol administration (195, 196). Increases, no change, and decreases in plasma esterified fatty acids have been produced in mice by changing the dose of ethanol administered (197). In general, high ethanol concentrations in the blood are associated with hypolipemia, whereas moderate levels have variable results after acute ethanol administration to animals. This difference in species susceptibility may be due to differences in the ability to remove lipids from the blood.

Another type of hyperlipemia occurring 10–16 hr after administration of an acutely large dose of ethanol to rats has been reported by some (83, 198), but not by others (97, 191, 192, 194). This late hyperlipemia is enhanced if a lipid load is given simultaneously with ethanol (83, 199) and has been used as a model for the study of postprandial alcoholic hyperlipemia (199). However, the same effect is observed whether the lipids are administered orally, intraperitoneally, or intravenously (83). In every case this enhancement of lipemia was associated with increased accumulation of fat in the liver. Since at the time when hyperlipemia occurs, hepatic lipid accumulation has reached its maximum and the blood alcohol levels are greatly decreased, this hyperlipemia probably reflects recovery from a transient fatty liver. The relevance of this model to human alcoholic hyperlipemia is therefore questionable, since the latter is a prompt response to alcohol administration and subsides rapidly after ethanol withdrawal (177, 200).

In contrast to man, the rat develops only mild hyperlipemia or none after an oral load of fat (166, 201) and this effect is not changed significantly if ethanol (3 g/kg body weight) is administered together with the fat-containing meal (166).

Chronic effects of ethanol on serum lipids

Experiments both in human volunteers and in animals indicate that prolonged exposure to ethanol is a key factor in the development of hyperlipemia. Administration of ethanol together with nutritionally
adequate diets for several weeks to alcoholic volunteers produces striking modifications of serum lipids (24, 103, 177, 178). The ingestion of 200–300 g of ethanol per day, resulting in blood ethanol concentrations between 100 and 200 mg/100 ml, produces a 4-fold increase in serum triglycerides and some increases in cholesterol and phospholipids (Fig. 6). This effect does not result solely from caloric overload, since comparable hyperlipemia was produced when ethanol was given in substitution for other calories (177). In these patients, the lipemic response is self-limited and, after 2–3 weeks of ethanol administration, serum triglycerides return to normal levels despite continuation of or even an increase in the alcohol intake. The same (but less marked) trend is followed by phospholipids and cholesterol. Plasma FFA concentration remains unchanged until high blood alcohol levels (over 250 mg/100 ml) are achieved by increasing the dose (24, 95, 103). The mechanism of the transient nature of the hyperlipemia remains unknown. Progressive deterioration of liver function or an effect of high ethanol concentrations have been incriminated as possible causes for the disappearance of the hyperlipemia upon continued ethanol drinking.

It has recently been shown (202) that chronic alcohol consumption promotes an increased ability to develop postprandial hyperlipemia even after a meal containing no alcohol. After administration of a fat-containing meal, alcoholics develop a higher and more prolonged elevation of serum triglycerides than nonalcoholics. This effect is prominent in alcoholics with fatty liver but not as marked in those with cirrhosis (Fig. 7). This suggests that the hyperlipemic response fades as liver function deteriorates. The addition of alcohol to the fat-containing meal increases postprandial hyperlipemia in nonalcoholics (185, 202), but only slightly in alcoholics with fatty liver and actually decreases it in alcoholics with cirrhosis (202).

Similarly, in the rat, the administration of alcohol-containing diet for several weeks results in a prompt and intense postprandial hyperlipemia (166), which contrasts with the lack of effect of an acute dose. As in alcoholics, striking postprandial lipemia occurs after administration of diet without ethanol, though the enhanced response fades relatively rapidly after ethanol withdrawal (166). In these rats, ethanol (36% of total calories) was administered in nutritionally adequate liquid diets for 3–4 weeks and its effects were studied by comparison to controls pair-fed with diets in which ethanol calories were replaced by carbohydrate. The action of carbohydrates on serum lipids (203) may have actually minimized the effects of ethanol. Never-
theless, serum lipids (mainly as VLDL) increase and reach their peak 90 min after an acute administration of diet. At this time, marked turbidity or even lacescence of the serum is observed (48, 166). When ethanol is administered chronically in drinking water (a method that results in much lower ethanol intake), the lipemic response is variable. Dajani and Kouyoumjian (195) showed that a large dose of ethanol decreases serum triglycerides within a few hours, both in control rats and in rats fed alcohol in drinking water for 6 weeks. However, it is apparent from their results that the ethanol-pretreated rats had higher serum triglycerides than the controls after the acute administration of either ethanol or water. In rabbits, the lipemic effect of acute ethanol administration can also be enhanced by chronic ethanol feeding (204). On the other hand, the lipemia observed in rats 16 hr after a high dose of ethanol was not different from that of rats given alcohol chronically in the drinking water (205).

Influence of other factors on the development of alcoholic hyperlipemia

Ethanol-induced hyperlipemia is usually moderate and requires relatively high ethanol doses. However, some patients develop marked hyperlipemia with moderate alcohol intake and the hyperlipemia promptly disappears after alcohol withdrawal. This suggests that some associated conditions in these patients may enhance the effect of ethanol on the development of hyperlipemia.

A possible role of a defect in postheparin lipoprotein lipase activity (PHLA) was reported to contribute to the hyperlipemia in 6 of 8 alcoholics with marked hyperlipemia (177). Similar patients were found to have a decrease in fractional turnover rate of intravenously injected exogenous triglycerides (169, 206). However, in either case, these alterations in lipid removal persisted after abstinence from alcohol. Thus, one factor in the development of marked hyperlipemia in some alcoholics could be the co-existence of alcoholism with a defective removal of serum lipids. Furthermore, some of the reported alcoholic patients with marked hyperlipemia had other conditions, such as diabetes (177, 207) or pancreatitis (172), which can contribute to hyperlipemia. An inhibitor of PHLA has been reported in association with pancreatitis (208, 209).

It has also been observed (207) that some patients (with normal PHLA) have unusual susceptibility to the lipemic effects of ethanol: they developed hyperlipemia with doses of ethanol (120–160 g per day) that did not affect serum lipids of normal subjects or individuals with endogenous type IV hypertriglyceridemia. However, one must wonder whether the difference in response to ethanol between these groups is secondary, at least in part, to the prior alcohol consumption, which by itself increases the ability of ethanol to promote hyperlipemia.

Another factor that may contribute to exacerbation of alcoholic hyperlipemia is the pre-existence of a type IV hyperlipemic defect. Alcoholics with a primary type IV hyperlipemia show striking elevation of serum triglycerides during alcohol consumption, whereas only moderate increases are found in patients with a similar degree of alcoholism but without the pre-existing lipoprotein disorder (210). Moreover, when the lipemic effect of ethanol is compared in nonalcoholics, patients with pre-existing type IV hyperlipemia develop higher hypertriglyceridemia than controls and this was manifested with amounts of ethanol within the boundaries of social drinking (211). This raises the possibility that moderate alcohol consumption may unmask subclinical hyperlipemic states which are relatively common (212, 213).

Mechanism of the ethanol-induced hyperlipemia

While serum triglycerides are hydrolyzed extensively in the body and their component fatty acids are oxidized by many tissues or stored in adipose tissue, they are produced at two main sites: the intestine and the liver. By contrast, cholesterol is produced in many tissues and transported to the liver for excretion into the bile, either as neutral sterols or as bile salts. To fulfill these transport needs, the liver produces very low density (VLDL) and high density (HDL) lipoproteins. VLDL serves to carry fat from the liver to other tissues. The major function of HDL appears to be the transport of extrahepatic cholesterol back to the liver for excretion (214). It is not clear whether there is a net transport of phospholipids from or to the liver, or whether phospholipids merely play a functional and/or structural role in serum lipoproteins.

Accumulation of lipids in the blood occurs when their rate of entry into the blood exceeds their rate of removal. Thus, hyperlipemia can be produced either by an excessive production and release of lipids into circulation or by defective removal from the blood or by combination of these mechanisms. The various possible mechanisms of ethanol-induced hyperlipemia are illustrated in Fig. 8.

Increased production of serum lipids from the intestine

The enhancement of post-alimentary lipemia by ethanol has long been suspected to be due to alterations in the rates of gastric emptying and/or fat absorption. The effects of ethanol on gastric emptying are concentration dependent. Concentrations of ethanol of about 8–10 g/100 ml are needed to exhibit significant changes in gastric emptying. At these concentra-
Fig. 8. Theoretical mechanisms for alcoholic hyperlipemia. Ethanol intake could result in hyperlipemia either by enhancing the pathways illustrated with wide arrows or by blocking those illustrated with broken lines.

At higher concentrations, ethanol effects on gastric emptying are consistently inhibitory (216, 218). It has been argued that this inhibition of gastric emptying may account for the delayed appearance of hyperlipemia in rats given a high dose of ethanol acutely (219). However, a similarly delayed hyperlipemia occurs in the fasting state (83). Moreover, hyperlipemia was produced in ethanol-fed rats, despite equal rates of fat disappearance from the gastrointestinal tract (48).

Ethanol could also increase the release of nondietary lipids from the intestine into the lymph. Indeed, it was shown that infusion of rat intestine with ethanol at a high concentration (10 g/100 ml) increased the output of VLDL in the intestinal lymph (198). It was postulated that this increase in nondietary lymph lipids, though small, may contribute to alcoholic hyperlipemia. This appears unlikely, however, because the much greater increase in lymph lipid output (including VLDL) induced in rats by acute administration of ethanol and fat was insufficient to produce significant hyperlipemia (166). Moreover, the administration of orotic acid, an agent that blocks the hepatic but not the intestinal release of triglycerides, abolishes ethanol-induced hyperlipemia in rats (220). Thus, the increased output of lipids into the lymph after ethanol does not seem to play a major role in the development of alcoholic hyperlipemia.

Increased production of serum lipids from the liver

Alcoholic hyperlipemia can be reproduced experimentally even if the participation of the intestine is excluded, leaving the liver as the major possible source of the increased serum lipids, particularly triglycerides. Indeed, in rats diverted of their own mesenteric lymph, intravenous administration of equal loads of lymph lipids to both ethanol-fed and control rats reproduces the alcoholic hyperlipemia in the ethanol-fed rats (166). If lymph depletion is not prevented by intravenous replacement, hepatic and plasma lipids decrease and alcoholic hyperlipemia does not occur. This indicates that although an adequate supply of dietary lipids represents a permissive factor needed to induce alcoholic hyperlipemia in the rat, the major site of origin of the increased serum lipids is a nonintestinal one, most likely the liver.

To determine whether the accumulation of serum lipids results from increased release of triglycerides from the liver, three major types of assessment have been used: the rate of lipid entry into the blood after blocking its removal with Triton WR 1339, the incorporation of labeled precursors into serum lipoproteins, and the release of triglycerides by isolated liver preparations.

Triton WR 1339 inhibits lipoprotein lipase by complexing with the lipid moiety of serum lipoproteins (221). The accumulation of serum lipids following a Triton block has been used to measure the rate of entry of lipids into the blood (222). As might be expected from the difficulty in producing consistent hyperlipemia after acute ethanol administration to rats, the effects of the Triton block have also been extremely variable in this experimental model. Triton-induced hypertriglyceridemia has been reported in-
creased (220, 223), decreased (195), or unchanged (224) by acute ethanol administration to rats. The type of response has been found to depend on the dose of ethanol administered, higher doses resulting in inhibition and smaller doses in stimulation of hepatic lipid secretion (197). The effects of a large acute dose of ethanol may be complicated by the effects of stress on triglyceride secretion, since acute stress inhibits Triton-induced lipemia (225). Finally, the same group of investigators have found inhibition (226) and stimulation (106) in different series of rats submitted to apparently the same acute administration of ethanol. After several weeks of feeding liquid diets containing ethanol, rats develop marked hyperlipemia after administration of dietary lipids with or without ethanol. Complete block of the blood lipid removal by Triton produces greater accumulation of serum lipids in the ethanol-fed rat than in pair-fed controls (48), despite the fact that neither Triton nor ethanol affects lipid absorption. This indicates that alcoholic hyperlipemia induced by chronic administration of ethanol is due to excessive production of lipids from the liver.

The incorporation of labeled fatty acids into serum lipoproteins increases during induction of hyperlipemia by acute ethanol administration to man (100, 227) and rabbits (188) or by chronic ethanol administration to rats (48, 166). However, these changes in triglyceride labeling are not conclusive evidence of enhanced synthesis because they may reflect changes in the fatty acid pools preceding serum triglyceride formation. Better evidence has been obtained by using amino acid precursors of triglyceride-carrying lipoproteins. Indeed, the incorporation of intravenously injected [14C]lysine into the protein moiety of VLDL increases in rats rendered hyperlipemic by chronic ethanol administration (48, 166), whereas the incorporation into other liver or serum proteins was not similarly affected. This indicates that the increased incorporation was not due to different degrees of isotopic dilution in amino acid pools preceding protein synthesis. Moreover, in ethanol-fed rats, the increased incorporation of lysine into VLDL-protein was associated with increased specific activity, suggesting that the increased labeling is not due to retention of the protein in the plasma but rather to increased synthesis.

The studies in isolated liver preparations have not reproduced the conditions under which alcoholic hyperlipemia develops. Livers from naive rats have been perfused with solutions containing ethanol. The effects on triglyceride secretion depend upon the concentration of ethanol. With very high concentrations (400 mg/100 ml) the hepatic release of triglycerides in the perfusate is inhibited (157). At lower concentrations, however, this process remains unaltered (111). When livers from ethanol-fed rats were perfused with ethanol (200 mg/100 ml), a marked stimulation of triglyceride release was observed (228). However, the ability of these livers to secrete triglycerides was greatly diminished in the basal state (without ethanol) compared to that of controls. It must be pointed out that the technique of chronic ethanol administration used in this study did not result in the production of either fatty liver or hyperlipemia.

**Increased removal of serum lipids by extrahepatic tissues**

Plasma and tissue lipoprotein lipase activities are not affected by ethanol in vitro (177, 178, 229). In man, acute administration of ethanol does not change post-heparin lipoprotein lipase activity (178, 185, 207), postheparin plasma clearing activity (183), and the triglyceride extraction by the forearm tissues (227). In the rat, the lipolytic activity that can be measured in the fasting plasma even in the absence of heparin, has been found decreased after an acute large dose of ethanol (229). However, the much greater activity that follows the injection of heparin is not changed by the administration of ethanol (229, 230). Tissue lipoprotein lipase activities have also been found either unchanged (230) or increased (231) after ethanol.

The clearance of chylomicron-triglycerides from the blood remains unchanged after acute (224) or chronic (48, 81) ethanol administration regardless of whether these particles are obtained from normal or ethanol-treated rats (48). In alcoholics, as emphasized before, there are individual variations in lipoprotein lipase activity (177) and blood lipid clearance (169, 232). When these activities are decreased, the alteration persists after alcohol withdrawal (177, 206), suggesting an associated defect rather than a direct effect of ethanol.

**Decreased removal of serum lipids by the liver**

Although most of the triglyceride removal from chylomicrons and VLDL is carried out in extrahepatic tissues, part of the triglyceride removal and most of the cholesteryl ester removal is carried out by the liver. A triglyceride lipase, located in the plasma membrane of the hepatocyte, can hydrolyze triglyceride-rich remnants of chylomicrons and VLDL. A decrease in this activity could cause hypertriglyceridemia. After injection of heparin, this enzyme is released into the plasma where it can be separated from lipoprotein lipase. In patients with various types of liver damage, including alcoholic hepatitis, the activity of the hepatic triglyceride lipase has been found decreased, whereas lipoprotein lipase activity remained unaffected (233, 234). Possible accumulation of intermediate density lipoproteins, considered to be remnants of partially metabolized chylomicrons and VLDL, has been sug-
gested in alcoholics with liver disease (235, 236). Recently, it has been shown that, contrasting with the lack of significant changes in the removal of chylomicron-triglycerides, the removal of chylomicron-cholesterol esters was delayed in ethanol-fed rats (237), suggesting that accumulation of remnants also contributes to alcoholic hyperlipemia.

**INTERRELATIONSHIPS BETWEEN SERUM AND LIVER LIPIDS DURING PROGRESSION OF ALCOHOLIC LIVER INJURY**

The association of fatty liver development with enhanced hepatic production of serum lipoproteins suggests that alcoholic hyperlipemia may play a compensatory role in counteracting fat accumulation, since the other major pathway of disposition for liver fat (fatty acid oxidation) is inhibited. Intrahepatic lipid availability is one of the major determinants of lipoprotein synthesis (238, 239). However, in the rat, the increased availability of fatty acids in the liver that follows a single ethanol ingestion does not appear to be sufficient to increase lipoprotein production to a degree resulting in consistent hyperlipemia, which develops only after chronic alcohol consumption (166). In other species, including man, hyperlipemia can be elicited after a single dose of ethanol, but even in man this lipemic response is enhanced by chronic alcohol ingestion (202). This suggests an adaptation of the hepatic capacity for lipoprotein production in response to the increased availability of liver fat. In keeping with this interpretation, the activity of key enzymes involved in the synthesis of the lipid (119) and carbohydrate (240) moieties of serum lipoproteins have been shown to increase after chronic alcohol feeding. Moreover, this increased capacity is manifested even after a load of fat without ethanol (166, 202).

The full development of alcoholic hyperlipemia after approximately one month of feeding 36% of calories as ethanol to rats coincides with the cessation of the accumulation of fat in the liver, and this situation remains unaltered for most of the animal life span despite continuation of the alcohol administration (21). Although the enhancement of the hepatic capacity to dispose of liver lipids as serum lipoprotein could contribute to the stabilization of the steatosis, it is also obvious that this compensatory effort is only partially effective and fatty liver is not fully prevented. This relative inefficiency may be due, at least in part, to other effects of ethanol that tend to decrease serum lipoprotein production. After acute administration, ethanol exerts inhibitory effects on protein synthesis (241–250), which might involve lipoproteins as well. Although protein synthesis is restored or even enhanced after chronic alcohol intake, (241, 251, 252) the secretion of protein from the liver into the plasma remains altered (56). It is reasonable to anticipate that such a defect in export might also involve serum lipoproteins. In keeping with this possibility, marked accumulation of VLDL-like particles in the Golgi apparatus is observed in ethanol-fed rats (50). The operation of mechanisms that produce opposite effects on lipoprotein release probably explains also the marked variations in the lipemic effects of ethanol that can be produced by apparently minor changes in experimental design.

When alcoholic fatty liver progresses to more advanced stages of liver damage, the relative inefficiency of the lipemic response becomes an absolute one, namely, hyperlipemia decreases whereas hepatic steatosis is exaggerated. This has been recently documented in baboons fed 50% of calories as ethanol for several years (202), an animal model that reproduces the entire spectrum of alcoholic liver disease (37). Inverse correlations between the degree of alcoholic hyperlipemia and the severity of the liver damage have been long recognized (174, 175). The incorporation of labeled fatty acids into serum triglycerides is decreased in cirrhosis and the degree of impairment varies directly with the severity of the liver damage (253). The inability has been proposed to differentiate hepatocellular from obstructive jaundice (254). More recently, it has been observed that the changes in serum lipids, particularly the decrease in VLDL or pre-β lipoprotein, could be an early indicator of the transition from fatty liver to more advanced lesions (202), and could be an important consideration in the recognition of those alcoholics prone to progress to severe hepatic lesions upon continued drinking. At this stage of liver damage, the decrease of VLDL from previously elevated values is associated with proportional decreases in pre-β lipoproteins, but as the severity of the hepatic injury increases, profound alterations in the structure of serum lipoproteins occur. These changes are indistinguishable from those observed in nonalcoholic forms of liver disease and reflect a marked impairment of hepatic function. Some degree of hypertriglyceridemia can be observed in alcoholics with severe liver damage. Although the mechanism of this alteration is not fully elucidated, the present evidence suggests that it is probably due to decreased hepatic removal of triglyceride-rich remnants of chylomicrons and/or VLDL (233, 234). Characteristically, serum cholesterol decreases at the expense of the esterified fraction with relative or absolute increase in free cholesterol and lecithin. These lipid changes are associated with alterations in the
electrophoretic mobility of serum lipoproteins that reflect striking changes in apoprotein composition. There is a decreased concentration of both $\alpha$ and pre-$\beta$ lipoproteins, and a single broad band in the $\beta$ position can be seen on electrophoresis in patients with severe liver disease (255). Both VLDL and HDL isolated by ultracentrifugation manifest a $\beta$ or close to $\beta$ mobility on electrophoresis. The alterations in VLDL seem to be secondary to the alterations in HDL, since pre-$\beta$ mobility can be restored by incubation with normal HDL, especially with added LCAT (256). Since the liver is the major site of synthesis of both LCAT and HDL, which provides the activator (mainly apoA) and the substrates for this enzyme, it could be anticipated that liver disease would affect the function of this system. Indeed, decreased LCAT activity has been reported in a variety of liver diseases (257), including those due to alcohol (258). This deficiency can be responsible for the increases in free cholesterol and lecithin (which are the substrates for LCAT), commonly observed in severe liver disease, with corresponding decreases in the products of LCAT reaction, namely cholesterol ester and lysolecithin. Much less clear is the relationship between the decreased LCAT activity in liver disease and the profound structural abnormalities of serum lipoproteins, including the appearance of discoidal bilamellar structures (236). These alterations are similar to those observed in familial LCAT deficiency (258) and similar discoidal lipoproteins are produced by 5,5'-dithionitrobenzoic acid (DTNB) (259), an inhibitor of LCAT. In patients with extra- or intrahepatic cholestasis, there are also similar abnormalities in the lipoproteins, which culminate in the formation of lipoprotein-X (a very abnormal lipoprotein which carries albumin as apoprotein) (260). Although a lipoprotein very similar to lipoprotein-X is also found in familial LCAT deficiency (261), cholestasis is not generally associated with LCAT deficiency unless marked hepatocellular damage coexists (262). Thus, these associations do not exclude the possibility that the protein abnormalities and the LCAT deficiency might be independent manifestations of the liver injury.

**ALCOHOL, ATHEROSCLEROSIS AND HIGH DENSITY LIPOPROTEINS**

The role of alcohol on the development of atherosclerosis and coronary heart disease (its most severe complication) has been a matter of lively controversy since the beginning of the century. The opinion that alcohol could be a major etiologic factor in these diseases prevailed during the last century. This opinion was challenged by the observation of Cabot (263), who showed that the incidence of atherosclerotic lesions in autopsies from patients with a history of alcoholism was remarkably low. Thereafter, the opinion that alcohol was “protective” against atherosclerosis was expressed in a number of studies (264, 265). A dissenting position was adopted by Wilens (266), who indicated that the low incidence of atherosclerosis in chronic alcoholism could be due to the younger age of the alcoholics in the necropsy material and to a lesser degree of association between alcoholism and other conditions known to favor the development of atherosclerosis, such as hypertension, diabetes, and obesity. He noted however that alcoholics had a lower tendency to develop lesions in the heart and brain in the presence of hypertension. It should be emphasized that coronary heart disease is the result of thrombosis as well as atherosclerosis. While some further studies confirmed the low incidence of coronary heart disease in alcoholics who died with cirrhosis of the liver (267–269), others failed to observe significant correlations (270) or showed negative correlation with cirrhosis, but not with alcoholism (271).

Both Ruebner, Miyai, and Abbey (272) and Parrish and Eberly (273) interpreted the rarity of myocardial infarction in cirrhotics as a statistical fallacy, resulting from the low likelihood of two fatal diseases occurring together and from the heavy contribution of cases with atherosclerotic and hypertensive heart disease to autopsy series, making any study group appear to exhibit some “protective” effect. In autopsy series composed of victims of automobile accidents or other sudden and unexplained deaths, either there was no correlation between alcoholism and atherosclerosis (274) or even a positive correlation was reported (275). These series, by contrast, are heavily weighed with cases of alcoholism (276). Prospective clinical studies have shown either positive correlations (277–279) or no correlation (280–283). Alcoholism is frequently associated with smoking, a recognized risk factor of coronary heart disease. When the role of smoking has been accounted for, a negative correlation between alcohol consumption and coronary heart disease has emerged in several, but not all, recent studies (284–287), reviving the possibility of a protective effect of alcohol on atherosclerosis and ischemic heart disease.

It must be pointed out, however, that alcoholism involves a variety of psychological, sociological, and probably biochemical and genetic determinants, and the validity of the controls used in epidemiologic studies is likely to be the subject of criticism. One factor that may be particularly relevant to this problem is the variable ability of the alcoholics to develop hyperlipemia, depending upon the degree of hepatic injury.
and the presence of other associated conditions. Although hyperlipemia is consistently produced by administration of ethanol to man and animals, especially in the postprandial state, this change is mild, decreases with progression of the liver damage, and disappears rapidly after alcohol withdrawal. Thus, clinically significant hyperlipemia (which is measured in the fasting state and generally after alcohol withdrawal) occurs only in a minority of alcoholics. Moreover, since ethanol enhances (and perhaps unmasks) other subclinical hyperlipemic states, it is likely that patients of this type contribute heavily to those classified as hyperlipemic alcoholics. If this were the case, one would expect that when the frequency of ischemic heart disease is studied in alcoholics with hyperlipemia (288), alcohol hyperlipemia appears to represent a risk with regard to ischemic heart disease similar to that of hyperlipemias of other origins.

The difficulty in selecting adequate controls in epidemiologic studies makes the experimental approach highly desirable. However, thus far this type of assessment has been hampered by the lack of development of animal models that can be considered relevant to either human alcoholism or atherosclerosis. This is witnessed by the paucity of experimental work on the possible relationships between these two leading causes of death. The most classical experimental study was performed by Eberhard (289) in rabbits fed a high cholesterol diet for approximately 20 weeks. He found that the inclusion of 25% ethanol in the drinking water produced a significant increase in the concentrations of cholesterol in the liver and the plasma, whereas both lesions of the aorta and the cholesterol content of the vessel were reduced. Using a similar model (but over a much shorter time), Feller and Huff (290) confirmed the hypercholesterolemic effect of ethanol, but found neither significant changes in the aortic content of nonsaponifiable lipids (the major fraction of which is cholesterol) nor in the incorporation of labeled acetate into these lipids. More recently, however, similar experiments have been repeated by Goto et al. (291), using a larger number of rabbits fed cholesterol-enriched diets for a period more akin to that used by Eberhard, and again a protective effect of ethanol on this cholesterol-induced atherosclerosis was indicated. Daily intragastric administration of 0.5 g of ethanol per kg body weight to rabbits on atherogenic diet failed to prevent electrocardiographic changes following hypoxia, exercise, and other strains (292).

Using other animal species, other investigators failed to detect any protective effect. Nichols et al. (293) found no effect of ethanol or wine on either the spontaneous variety of atherosclerosis observed in fowls (cockerels) or the stimulation of this process by stilbes-

trol. Since a large proportion of these birds did not develop lesions at all, the severity of the alteration was increased by adding cholesterol and low-protein diets (294). Under these conditions, 7% alcohol in the drinking water did not significantly influence the experimentally induced lesions (295). Furthermore, in rats made atherosclerotic by administration of diets containing both 1% cholesterol and 0.3% cholic acid, Gottlieb et al. (296) found that the addition of 20% alcohol to the drinking water increased the extension of the vascular sudanophilia (a morphological counterpart of the lipid deposition).

The major problem of the experimental work performed thus far is the question of relevance with regard to both alcoholic and atherosclerotic diseases. With regard to alcoholism, we have learned that the technique of alcohol administration used in these studies results in minimal or no elevations of blood ethanol concentrations because of the natural aversion of the animals to the consumption of alcohol. Moreover, ethanol administration reduces the intake of food and can produce protein malnutrition, which, by itself, exaggerates cholesterol-induced atherosclerosis (294).

With regard to atherosclerosis, the criticism has been made that the lesions produced by high cholesterol feeding in these species resemble human essential xanthomatosis (with lipid-laden histiocytes and monocytes) more than human atherosclerosis (297). In the latter, initial lipid deposition occurs in smooth muscle cells proliferating in the intima of the vessels (298). Moreover, striking differences in cholesterol metabolism exist between these species and man (299), particularly with regard to the ability of dietary cholesterol to produce hypercholesterolemia and to increase cholesterol pools. In view of these facts, it is not surprising that the concept that human atherosclerosis is related to the consumption of cholesterol or predictable by the levels of cholesterolemia (extrapolations from animal experiments) has been discredited (300). The problem of clinical relevance has switched the interest toward the use of nonhuman primates as experimental models of atherosclerosis. Indeed, primates share relative inability to absorb cholesterol, stong mechanisms of negative feedback of cholesterol synthesis, and the ability to dispose cholesterol excesses into bile salts (299). Moreover, atherosclerosis has been produced in rhesus monkeys (Macaca mulatta) by a “table-prepared” average American diet, with a rather low degree of hypercholesterolemia (301).

Though atherosclerosis is a multifactorial process, in which not only lipid disturbances but also vascular and hematological abnormalities contribute to the pathogenesis, accumulation of cholesterol esters is a key factor in its pathogenesis (302). The effect of etha-
Thus, mitochondrial damage perpetuates fatty acid chronic alcohol consumption, decreases fatty acid oxidation by interfering with citric acid cycle activity. This block is partially compensated for by increased ketone body production, which results in ketonemia. Thus, mitochondrial damage perpetuates fatty acid accumulation even in the absence of ethanol oxidation. Alcohol facilitates esterification of the accumulated fatty acids to triglycerides, phospholipids, and cholesterol esters, all of which accumulate in the liver. The accumulated lipids are disposed of in part as serum lipoprotein, resulting in moderate hyperlipemia. In some individuals with pre-existing alterations of lipid metabolism, small ethanol doses may provoke marked hyperlipemia which responds to alcohol withdrawal. Inhibition of the catabolism of cholesterol to bile salt may contribute to the hepatic accumulation and hypercholesterolemia. The capacity for lipoprotein production and hyperlipemia development increases during chronic alcohol consumption, probably as a result of the concomitant hypertrophy of the endoplasmic reticulum and Golgi apparatus. However, this compensation is relatively inefficient in ridding the liver of fat. This inefficiency may be linked to alterations of hepatic microtubules induced by ethanol or its metabolites, which interfere with the export of protein from liver to serum, promoting hepatic accumulation of proteins as well as fat. As liver injury aggravates, hyperlipemia wanes and liver steatosis is exaggerated. Derangements of serum lipids similar to those found in other types of liver disease also become apparent. The changes in serum lipids may be a sensitive indicator of the progression of liver damage in the alcoholic.

SUMMARY

Alcohol promotes accumulation of fat in the liver mainly by substitution of ethanol for fatty acids as the major hepatic fuel. The degree of lipid accumulation depends on the supply of dietary fat. Progressive alteration of the mitochondria, which occurs during chronic alcohol consumption, decreases fatty acid oxidation by interfering with citric acid cycle activity. This block is partially compensated for by increased ketone body production, which results in ketonemia. Thus, mitochondrial damage perpetuates fatty acid accumulation even in the absence of ethanol oxidation. Alcohol facilitates esterification of the accumulated fatty acids to triglycerides, phospholipids, and cholesterol esters, all of which accumulate in the liver. The accumulated lipids are disposed of in part as serum lipoprotein, resulting in moderate hyperlipemia. In some individuals with pre-existing alterations of lipid metabolism, small ethanol doses may provoke marked hyperlipemia which responds to alcohol withdrawal. Inhibition of the catabolism of cholesterol to bile salt may contribute to the hepatic accumulation and hypercholesterolemia. The capacity for lipoprotein production and hyperlipemia development increases during chronic alcohol consumption, probably as a result of the concomitant hypertrophy of the endoplasmic reticulum and Golgi apparatus. However, this compensation is relatively inefficient in ridding the liver of fat. This inefficiency may be linked to alterations of hepatic microtubules induced by ethanol or its metabolites, which interfere with the export of protein from liver to serum, promoting hepatic accumulation of proteins as well as fat. As liver injury aggravates, hyperlipemia wanes and liver steatosis is exaggerated. Derangements of serum lipids similar to those found in other types of liver disease also become apparent. The changes in serum lipids may be a sensitive indicator of the progression of liver damage in the alcoholic.

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