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Alcohol-related Diseases and Carcinogenesis[1]

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Abstract

Possible mechanisms whereby alcohol abuse and alcohol-related diseases may promote the development of cancer are analyzed. The mechanisms discussed include: (*a*) contact-related local effects on the upper gastrointestinal tract; (*b*) the presence of low levels of carcinogens in alcoholic beverages; (*c*) induction of microsomal enzymes involved in carcinogen metabolism; (*d*) various types of cellular injury produced by ethanol and its metabolites and their relationship to cancer, particularly in the liver; (*e*) the nutritional disturbances frequently associated with alcohol abuse. The relationship between alcohol-induced cirrhosis and hepatocellular carcinoma is also discussed, and case histories of patients seen at the Bronx Veterans Administration Medical Center with hepatocellular carcinoma in the absence of cirrhosis are reviewed. Data are presented demonstrating the induction, by chronic ethanol consumption, of microsomal enzymes which convert procarcinogens to carcinogens. These data were derived from experiments in which the ability of microsomes isolated from liver, intestine, and lung tissues of ethanol-fed and control rats to activate several test carcinogens was examined in the Ames *Salmonella*-mutagenicity test. The hypothesis is presented that ethanol-mediated induction of enzyme systems which activate procarcinogens to carcinogens in various tissues contributes to the enhanced incidence of cancer in the alcoholic.

Introduction

The epidemiology of the link between cancer and alcoholism has been discussed in detail in another paper of this workshop (371). Detailed evidence has been presented of the increased incidence of cancer of the upper alimentary tract and the colon in the alcoholic. This aspect therefore will not be reviewed in detail here. Hepatocellular carcinoma also has been associated with alcoholism. However, this has usually been attributed not to ethanol but rather to the ensuing cirrhosis. Recently, some clinical evidence has accumulated that, even in the absence of cirrhosis, hepatocellular carcinoma of the liver is linked to alcoholism. Some observations will be presented to illustrate this possibility. The thrust of this paper, however, will be the discussion of possible mechanisms whereby alcohol abuse may promote the development of cancers. Our considerations will focus on: (*a*) local effects of ethanol in the gastrointestinal tract; (*b*) induction, by chronic ethanol consumption, of microsomal enzymes which convert procarcinogens to carcinogens; and (*c*) general mechanisms of cellular injury produced by ethanol. The relationship of the latter effect to cancer is based on the generally held belief, supported by experimental and clinical evidence, that repeated cell injury may eventually promote neoplastic transformation, possibly through inappropriate regenerative responses. The interaction of alcohol, nutrition, and cancer will also be discussed.

Cancer of the Alimentary Tract and Local Effects of Ethanol

Clinically, an association between heavy drinking and certain types of cancer has been observed for many years. One of the first suggestions that there might be a relationship between alcohol and cancer was made in France when Lamu (194) showed that the chronic intake of absinthe (a beverage containing wormwood as well as alcohol) was associated with carcinoma of the esophagus. In 1950, the relationship between cigarette smoking and lung cancer was widely recognized, and this led investigators to take a closer look at personal habits in relation to the development of cancer. In 1964, the World Health Organization surveyed the research on alcoholism and cancer and concluded that an association existed between excessive drinking of alcoholic beverages and cancer of the mouth, the larynx, and the esophagus (400). In a series of studies (405–408), heavy drinkers were found to have roughly a 10-fold increased risk of developing cancer of the mouth. Subjects who drink heavily often also smoke heavily. This latter fact was first taken into account by Flamant *et al.* (103), who assessed both factors and reported that there was a "strong" or "very strong" association of alcohol intake with cancer of those sites that come most directly in contact with alcohol (tongue, hypopharynx, larynx, esophagus). More recent studies such as those of Keller and Terris (170), Rothman and Keller (317), and Martinez (244), which were included in the Third National Cancer Survey (394), substantiate this association. All these studies provided evidence for a cooperative role between tobacco and alcohol in tumor formation of the oral cavity, the larynx, and the esophagus. It was calculated that 76% of these cancers in males could be eliminated if exposure to alcohol and tobacco were avoided (317). More recent studies in North America and Canada have confirmed those findings. The risk for a heavy drinker who smoked to develop oral cancer was 6 to 15 times greater than for nondrinkers and nonsmokers (98, 108). Also, women who drink and smoke heavily develop cancer of the tongue and buccal cavity some 15 years earlier than do women abstaining from both (5). It appears that alcohol plays a more important role than smoking with respect to cancer of the esophagus whereas smoking seems to be more strongly associated with cancer of the mouth and pharynx (103). Recently, additional sites of cancer associated with alcohol were detected in the pancreas (39, 138, 149), the cardia of the stomach (237), and the colon (394).

How cancers of the gastrointestinal tract relate to alcohol is not known. One mechanism that has been suggested is the decreased salivary secretion which might result in both higher

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local concentrations of carcinogens and lesser rinsing of the mucosal surfaces (180). Another mechanism may be related to the tissue damage produced by the high local ethanol concentration to which some of these tissues are subjected. Indeed, ethanol has been shown to have various local effects on the upper gastrointestinal tract. In Ontario, Canada (4), an oral cytology service identified dyskaryotic cells in 3.0% of the 3445 smears of dental patients examined; there were twice as many such lesions (6.5%) in 276 smears from alcoholics. In the esophagus, functional abnormalities have been described in the alcoholic. Changes in peristalsis, without clinical complaints of dysphagia, have been detected in a majority of alcoholics who presented with peripheral neuropathy (102, 395). Prominent changes included a decrease in frequency of primary peristaltic waves in the distal two-thirds of the esophagus as well as an increase in tertiary, nonpropulsive activity after swallowing; there was no change in lower esophageal sphincter pressure. Little is known about the direct effects of alcohol on esophageal functions, although ethanol apparently does not promote luminal loss of hydrogen ions and does not alter the electrical resistance of the mucosa (113). Thus, the changes documented by Winship *et al.* (395) may represent either direct neuropathic or myopathic effects of alcohol. The role played by malnutrition has not been defined.

Alcohol is also known to exert striking effects on the stomach. The occurrence of gastritis after the acute administration of alcohol has been clinically well established since William Beaumont's observations (179). These classic studies were confirmed more recently by endoscopy in binge drinkers. Hemorrhagic lesions also involve the duodenum. Although blind peroral biopsies of the duodenum had failed to reveal duodenitis during acute episodes of chronic alcoholism (303), fiberoptic duodenoscopy detected gross and microscopic duodenitis in a majority of subjects in a more recent study (118).

The incidence of chronic gastritis in alcoholics is a more controversial issue. Whereas some studies detected no relationship between alcohol intake and histological evidence of atrophic gastritis (287, 398), other investigators have suggested a connection (77, 163, 304, 312). In the study by Dinoso *et al.* (77), 50% of the alcoholics examined had either superficial or atrophic gastritis which was present even after 2 months of abstinence. Maximal acid output in these patients tended to increase with improvement in histology. Patient selection (nutritional status, definitions of alcoholism), biopsy techniques (blind *versus* directed), and length of abstinence before examination may, at least in part, explain the lack of agreement in the literature regarding the relationship between alcoholism and chronic gastritis. An increased incidence of atrophic gastritis would be of particular importance since this lesion appears to be a precursor of human gastric carcinoma.

The acute and chronic effects of alcohol on gastric acid secretion and gastric mucosal integrity have been studied extensively both *in vivo* and *in vitro*. In the intact animal, the acute i.g.[2] administration of ethanol results in an augmented secretion of hydrochloric acid (81). It appears that this effect is mediated by the release of antral gastrin (25, 399), an increased vagal tone (120), and enhanced synthesis or release of gastric histamine (80, 81). Chronic administration of ethanol increases the gastrin response to a meat meal (368) and, at least initially, increases the maximal acid output of dogs (49). In the latter study, the maximal acid output returned to normal levels after approximately 6 months, suggesting that the parietal cell response to endogenous gastrin might be gradually impaired by injury.

Other work has focused on the direct effects of alcohol on gastric permeability and on active transport of various ions. Davenport (70) demonstrated that the direct application of ethanol to the Heidenhain pouches of dogs resulted in the insorption of hydrogen ions and the exsorption of sodium ions. This observation was confirmed and extended by Dinoso *et al.* (78), who found that these ion fluxes and the accompanying mucosal injury could be increased or decreased by the addition of acid or buffer, respectively, to the alcohol perfusate. Using lithium as an ionic analog of H^+, the same conclusion was reached in a human study (348). On the basis of these results, it was concluded that alcohol alters the permeability of the gastric mucosal "barrier" and that the subsequent ingress of hydrogen is potentially injurious. More recently, others have suggested that the deleterious effects of alcohol are mediated only in part by a change in mucosal permeability (113, 338, 340). It was found that topical ethanol increases electrical resistance across the mucosa and diminishes the transmucosal electrical potential. Such findings are more consistent with a change in active transport of ions, particularly Cl^-, rather than a simple increase in permeability (which should decrease electrical resistance), although the net ionic changes, namely, an increase in luminal Na^+ and a decrease in luminal H^+, would be the same. A Mg^{2+} and $Mg^{2+} + HCO_3^-$-ATPase is postulated to link the active transport of Cl^- and HCO_3^-. It has been shown that ethanol may reduce active transport of chloride by inhibiting this ATPase activity (359).

In summary, alcohol both stimulates and inhibits gastric acid production by different mechanisms. In the dog, topical ethanol causes stimulation of acid secretion (70). In humans, however, while i.v. ethanol stimulates acid secretion (139), p.o. ethanol produces no change or perhaps mild inhibition of acid secretion (59, 60). Thus, the importance of H^+ in ethanol-mediated gastric injury is not known.

Ultrastructural changes have been described in the gastric mucosa after topical ethanol administration, including disruption of tight cell junctions (83). Changes, however, were most marked in the cytoplasm and nuclei. Dinoso *et al.* (80) studied the effect of ethanol in buffered solution on canine gastric mucosa and found that with ethanol concentrations greater than 20% v/v there was disruption of the apical cell membrane and focal separation of tight junctions. Underlying atrophic gastritis in humans causes increased blood loss during ethanol ingestion which suggests an injurious effect of ethanol (79), but electron microscopic studies have not been done in this situation; therefore, the underlying lesion is not well defined. Furthermore, the relationship, if any, between cancer of the stomach and local injurious effects of ethanol is not known.

In addition to the local effects of ethanol itself, some congeners in alcoholic beverages may play an etiological role in the development of cancer. Indeed, esophageal cancer has been

[2] The abbreviations used are: i.g., intragastric; BP, benzo(*a*)pyrene; DMN, dimethylnitrosamine; SER, smooth endoplasmic reticulum; ADH, alcohol dehydrogenase; MEOS, microsomal ethanol-oxidizing system; PB, phenobarbital; 3-MC, 3-methylcholanthrene; BP hydroxylase, benzo(*a*)pyrene hydroxylase; DMN *N*-demethylase, dimethylnitrosamine *N*-demethylase; i.r., intrarectally.

produced in animals by administering relatively large amounts of nitrosamines which may be congeners in some alcoholic beverages (227, 235). Furthermore, the presence of ethanol seems to catalyze the production of nitrosamines from nitrites and secondary amines *in vitro* (301). An increased incidence of esophageal cancer has been described in the alcoholic (58, 369). In France, the risk of esophageal neoplasia is particularly pronounced in alcoholics who drink apple brandy whisky but is less apparent in those consuming beer or wine (369). Similarly, in eastern and southern Africa, beverages derived from maize have been implicated as risk factors (58). A variety of polycyclic hydrocarbons (phenanthrene, fluoranthrene, benzanthracene, BP, chrysene) have been found in alcoholic beverages (248), and asbestos fibers (derived from filters) have been found in beer, wine, sherry, and vermouth (31, 32, 65, 390).

Induction of Enzyme Activities by Ethanol in Microsomes of Liver, Intestine, and Lungs and Its Possible Link to Carcinogenesis

Relationship of the Microsomal Cytochrome P-450 System to Carcinogenesis

It is well established that the microsomal cytochrome P-450-dependent biotransformation system is involved in the metabolic conversion of many structurally diverse chemicals to electrophiles capable of reacting with nucleic acids, proteins, and lipids (392). It is these electrophilic metabolites that are generally responsible for the carcinogenic effects of the parent compounds. Activation of many procarcinogens by microsomal enzymes is an obligatory event for mutagenesis in microbiological assays and cell transformation in tissue culture (391). Induction of microsomal enzyme activity increases the mutagenic effect of many compounds in the Ames *Salmonella*-mutagenesis assay (3,125, 305). Since the extent of metabolic activation of various secondary carcinogens can be correlated with microsomal enzyme activity (57, 67, 82, 99, 114, 126), factors such as environmental pollutants, drugs, and diet which can influence the activity of this enzyme system are also expected to affect tumor formation in animals exposed to carcinogens (189, 246, 256, 298, 316, 389). Although there are experiments which appear to contradict this expectation, there are a number of studies which have shown a positive correlation between induction of microsomal enzyme activity and chemical carcinogenesis (90, 171, 189). There are several possible explanations for these seemingly contradictory observations. Since many inducers of microsomal enzymes are also substrates for these enzymes, the simultaneous presence of both inducer and carcinogen may result in competition for enzyme-binding sites. Furthermore, the components of the cytochrome P-450-dependent system and associated enzymes such as epoxide hydrase and glutathione transferases also are involved in the detoxification of many of the same chemicals which require activation for carcinogenesis. These enzymes not only respond differently to various inducers but also vary with respect to their relative basal concentrations from tissue to tissue and may vary with respect to their relative inducibility in different tissues. This latter point may prove to be particularly important with respect to ethanol as a microsomal inducer, as will be illustrated by data presented subsequently. Ultimately, what is probably most critical with respect to the relationship between induction of microsomal enzyme activity and carcinogenesis is the relative effect of the inducer on carcinogen activation and inactivation.

Reactions which are cytochrome P-450 dependent and are known to play a role in chemical carcinogenesis include: (*a*) *N*-hydroxylation of aromatic amines [such as the potent procarcinogens 2-aminofluorene and 2-acetylaminofluorene (265)], hydrazine compounds [such as 1,2-dimethylhydrazine which produces tumors of the colon and rectum (391)], and some substituted hydrazines (including isoniazid) which produce pulmonary tumors in specific strains of mice (172, 339, 366); (*b*) dealkylation of alkylnitrosamines, *e.g.*, *N*-demethylation of DMN (66, 125, 126, 265); (*c*) epoxide formation from polycyclic hydrocarbons (69, 347), aflatoxins (265), and furosemide (396).

In humans, microsomal biotransforming activity and/or cytochrome P-450 have been found in the liver (33, 71, 192, 333), lymphocytes (393), skin (206), placenta (57), intestine (140), colon (12), and lungs (354). Although the liver is the principal organ of xenobiotic metabolism, metabolism in other tissues, especially in those which have direct contact with the environment (intestine, lung, skin), is also important since such metabolism can influence absorption, biological reactivity, and systemic distribution of these compounds. Many procarcinogens do not produce tumors at the site of application (391). They may be activated in tissues other than where they finally act with activated metabolites being released into the blood stream and affecting special target organs (22, 391).

Effect of Alcohol Consumption on Hepatic Microsomal Enzymes

Changes in Ethanol and Drug Metabolism. The first indication of an interaction of ethanol with the microsomal fraction of the hepatocyte was provided by the morphological observation that in rats ethanol feeding resulted in a proliferation of the SER (151, 152). This increase in SER resembled that seen after the administration of a wide variety of xenobiotic compounds including known hepatotoxins (257), numerous therapeutic agents (56), and food additives (197). Most of the substances which induce a proliferation of the SER are metabolized, at least in part, in the microsomal fraction of the hepatocyte which comprises the SER. The observation that ethanol produced proliferation of the SER raised the possibility that, in addition to its oxidation by ADH in the cytosol, ethanol may also be metabolized by the microsomes. A microsomal system capable of methanol oxidation had been described (285), but its capacity for ethanol oxidation was extremely low. Furthermore, this system could not oxidize long-chain aliphatic alcohols such as butanol and was sensitive to catalase inhibitors, azide, and cyanide. Therefore, Ziegler (413) concluded that this system is clearly different from the cytochrome P-450-dependent system and involves the H_2O_2-mediated ethanol peroxidation by catalase. However, a MEOS with a rate of ethanol oxidation 10 times higher than reported by Orme-Johnson and Ziegler (285) has been described (212, 214). The system requires NADPH and O_2 and is relatively insensitive to catalase inhibition. Furthermore, the MEOS was differentiated from the system reported by Orme-Johnson (285) and from catalase by its ability to oxidize long-chain aliphatic al-

cohols (362) which are not substrates for catalase (48). The striking increase in the non-ADH fraction of ethanol metabolism with increasing ethanol concentrations (123, 252, 363) is consistent with the known K_m for MEOS and ADH. Whereas the former has a value of 8 to 10 mM (214), the latter has a K_m varying from 0.26 to 2 mM (100, 230, 241, 310). In perfused liver, the K_m of ADH is even lower (230). The *in vitro* K_m of MEOS agrees well with *in vivo* K_m measurements from experiments utilizing the ADH inhibitor pyrazole and from experiments with isolated hepatocytes (254), suggesting that MEOS may play a significant role in ethanol metabolism.

Reconstitution of MEOS from the 3 microsomal components (cytochrome P-450, NADPH-cytochrome *c* reductase, lecithin) (Chart 1) has been demonstrated by Ohnishi and Lieber (277) and confirmed by Miwa *et al.* (266). The activity of the reconstituted MEOS showed a dependency upon cytochrome P-450 and the reductase and required synthetic phospholipids (such as lecithin) for its maximal activity. The involvement of reduced cytochrome P-450 in MEOS activity was further shown by the action spectra (93). How cytochrome P-450 mediates ethanol oxidation has not been clarified. It could contribute to the generation of a hydroxyl radical which in turn might be "scavenged" by ethanol since other compounds that interact with hydroxyl radicals inhibit MEOS activity (43, 278).

Following chronic ethanol consumption, there is a significant increase in MEOS activity (212, 214). This is associated with an increase in various constituents of the smooth fraction of the membranes involved in drug metabolism, such as phospholipids, cytochrome P-450 reductase, and cytochrome P-450 (153, 159). The increase of cytochrome P-450 was associated with the appearance of a distinct form of cytochrome P-450 which exhibited a high affinity for cyanide (54, 133, 158). Further evidence in favor of an increase of a distinct species of cytochrome P-450 after ethanol treatment was derived from inhibitor studies (375). More direct proof was obtained from studies of microsomal proteins (277) which indicated that the rise in cytochrome P-450 involved a hemoprotein different from those induced by PB or 3-MC. Moreover, sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed an induction of microsomal protein with a molecular weight of 53,400 which was presumed to be an apoprotein of cytochrome P-450. The partially purified cytochrome P-450 from ethanol-fed rats was more active for alcohol oxidation than was the control preparation in the presence of an excess of NADPH-cytochrome *c* reductase and L-α-dioleoyl lecithin. These findings are in keeping with recent demonstrations of the heterogenicity of hepatic cytochrome P-450. Although components of microsomal mixed-function oxidases have previously resisted many attempts at purification, recently, several groups (129, 135, 147, 326) succeeded in purifying multiple forms of cytochrome P-450 from rat and rabbit hepatic microsomes. One form, for example, is inducible by PB and another is inducible by polycyclic aromatic compounds such as β-naphthoflavone and 3-MC. These forms have different molecular weights as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and different spectral, immunological, and catalytic properties. The PB-inducible form is relatively more active for benzphetamine *N*-demethylation than is the other, whereas the 3-MC-inducible form is more active for benzopyrene hydroxylation. We have observed at least 3 forms of cytochrome P-450 (determined by continuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis with heme staining) in chronic ethanol-fed rats. One of these forms is distinct from those induced by PB or 3-MC treatment. Partially purified cytochrome P-450 from ethanol-fed rats was more active for alcohol oxidation than was the control preparation in the reconstituted system (276).

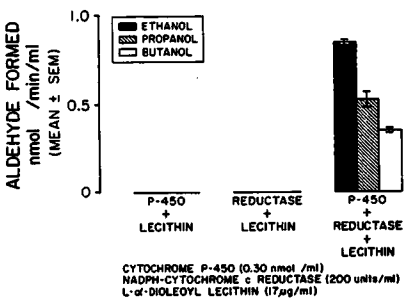

Chart 1. Ethanol, propanol, and butanol oxidation by the reconstituted MEOS. Active oxidation of alcohols is achieved by the combination of the 3 components. Acetaldehyde, propionaldehyde, and butyraldehyde in incubates were measured directly by gas-liquid chromatography (276).

In addition to MEOS activity, repeated ethanol administration also results in increased activities of a variety of microsomal drug-detoxifying enzymes (8, 42, 159, 264, 322). Some effects are readily detectable after a single ethanol dose (306). The increase in the activity of hepatic microsomal drug-detoxifying enzymes and in the content of cytochrome P-450 induced by ethanol ingestion offers a likely explanation for the observation that ethanol consumption enhances the rate of drug clearance *in vivo*. The tolerance of the alcoholic to various drugs has been generally attributed to central nervous system adaptation (164). However, there is occasionally a dissociation in the time course of the decreased drug sensitivity of the animals and the occurrence of central nervous system tolerance, the former preceding the latter (308). Thus, in addition to central nervous system adaptation, metabolic adaptation must be considered. Indeed, it has been shown that the rate of drug clearance from the blood is enhanced in alcoholics (166). Of course, this could be due to a variety of factors other than ethanol, such as the congeners present in alcoholic beverages and the use of other drugs so commonly associated with alcoholism. Controlled studies have shown, however, that administration of pure ethanol with nondeficient diets either to rats or humans (under metabolic ward conditions) results in a striking increase in the rate of blood clearance of meprobamate and pentobarbital (264) (Chart 2). Similarly, increases in the metabolism of aminopyrine (379), tolbutamide (42) and rifamycin (119) were found. Furthermore, the capacity of liver slices from animals fed ethanol to metabolize meprobamate was also increased (264), which clearly showed that ethanol consumption affects drug metabolism in the liver itself, independent of drug excretion or distribution. Failure to verify such an effect (150) was probably due to the very low dosage of ethanol administered.

The stimulation of microsomal enzyme activities also applies to those enzymes which convert exogenous substrates to toxic compounds. For instance, CCl_4 exerts its toxicity only after conversion in the microsomes. Alcohol pretreatment dramatically stimulates the toxicity of CCl_4 (131, 242). The experiments of Hasumura *et al.* (131) were carried out at a time when the ethanol had disappeared from the blood to rule out the increase of the toxicity of CCl_4 due to the presence of ethanol (367).

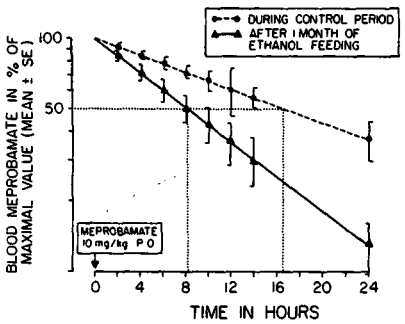

Chart 2. Effect of ethanol consumption on clearance of meprobamate from blood. Four volunteer alcoholics were tested before and after 1 month of ethanol ingestion; half-lives are shown by the *dotted lines* on *x* and *y axes* (264).

The potentiation of the CCl_4 toxicity by ethanol pretreatment may be accounted for by the increased production of toxic compounds of CCl_4 since the conversion of $^{14}CCl_4$ to $^{14}CO_2$ and covalent binding of CCl_4 metabolites to protein were significantly accelerated in microsomes of ethanol-pretreated rats (131). It is likely that a larger number of other toxic agents will be found to display a selective injurious action in the alcoholic.

Hepatic Activation of Procarcinogens after Alcohol. It has been shown that there is a good relationship between BP hydroxylase activity and tumor formation *in vivo* (90, 171). It is of interest to note therefore that, after administration of adequate liquid diets containing either 36% of total calories as ethanol or isocaloric carbohydrates to female Sprague-Dawley rats for 15 days, BP activity was elevated by 57% in the ethanol-fed animals (319). Recently, we extended these results on the effects of ethanol on BP metabolism in the liver (336) to include hepatic microsomal activation of BP to its mutagenic derivatives using the *Salmonella typhimurium* His^- strain TA 100 to assay mutagenic activity (3). Over the entire range of BP tested, the mutagenic effect of BP was enhanced when hepatic microsomes from ethanol-fed female rats were used for activation. The difference in the extent of BP activation was similar to the difference in BP hydroxylase activity. Hepatic microsomes from ethanol-fed and control male Sprague-Dawley rats which did not show any significant difference in BP hydroxylase activity also did not differ with respect to BP-mediated mutagenesis.

A decrease in hepatic cytochrome P-450, benzo(*a*)pyrene monooxygenase activity, and DNA binding of BP after acute injection of different ethanol concentrations (0.7 to 1.8 mg/kg body weight) into mice was reported by Capel *et al.* (41), who also reported a decrease in BP hydroxylase activity and DNA binding of BP after chronic ethanol administration for 2 to 8 weeks. However, in these latter experiments, ethanol was given together with the drinking water (10% v/v); therefore, alcohol consumption must have been relatively low and nutrient intake must have been uncontrolled.

In addition to polycyclic hydrocarbons (248), a number of nitrosamines have been detected in alcoholic beverages (227). It is generally agreed that the initial step in the bioactivation of DMN to a methylating agent is microsomal *N*-demethylation. There are different isoenzymes of DMN demethylase. Czygan *et al.* (66) characterized a DMN *N*-demethylase which has a K_m of 40 mM and is inducible by PB, 3-MC, or Aroclor 1254 as a cytochrome P-450-dependent enzyme system. On the other hand, Venkatesan *et al.* (378) described a DMN *N*-demethylase with a K_m of 0.2 mM, which is inhibited by the "inducers" used by Czygan *et al.* (66).

It was shown by Maling *et al.* (242) that administration of an acute dose of isopropyl alcohol or ethanol to rats enhances the activity of DMN *N*-demethylase at a DMN concentration of 10 mM. This increase of enzyme activity was accompanied with a potentiation of DMN-induced hepatotoxicity. We have found (336) that chronic ethanol administration in a liquid diet to Sprague-Dawley rats results in a significant enhancement of DMN *N*-demethylase activity measured at both high (100 mM) and low DMN concentrations (5 mM) regardless of the sex of the animals.

Effect of Ethanol Consumption on Intestinal Microsomal Enzymes and Associated Enhanced Mutagenicity

In the last decade, the importance of intestinal xenobiotic metabolism has been recognized, although it has been realized that its activity is relatively small compared to that of the liver. Because of the relatively high concentrations of environmental dietary carcinogens in the small bowel and because of the great variety of those compounds in the intestine, their intestinal activation may be of obvious clinical importance, especially since this activation may be linked to absorption and biological availability.

Cytochrome P-450, NADPH-cytochrome P-450 reductase, and mixed-function oxidase activities have been demonstrated in the gastrointestinal tract of several species (50, 352, 353, 387). Recent studies also have shown that this system may mediate the intestinal metabolism of drugs and carcinogens and can be induced by dietary constituents (142, 288, 386). For example, the intestinal metabolism of phenacetin is greatly increased in rats fed a diet containing charcoal-broiled ground beef (288). Moreover, in the rat, a number of vegetables (*e.g.*, spinach, broccoli, and cabbage) enhance the intestinal metabolism of BP (387), and dietary iron has prominent effects on other drug-metabolizing activities (141, 142). Finally, dietary constituents such as polycyclic hydrocarbons and flavones contained in animal chow (387) are capable of inducing the intestinal enzymes involved in the oxidative metabolism of xenobiotics. The oxidative enzymes involved in drug metabolism have been localized in the rat small intestine (142). Two gradients exist for the content of P-450 and activities of BP hydroxylase, *p*-nitroanisole *O*-demethylase, and NADPH-cytochrome P-450 reductase. Along the length of the small bowel, the activity and content of these drug-metabolizing components are highest in the upper part. Within an individual villous structure, these components are highest in the upper villous tip cells and decrease progressively toward the crypt areas (141, 142).

Since ethanol is a common dietary constituent and produces ultrastructural changes in intestinal mucosa (*e.g.*, proliferation of the endoplasmic reticulum and mitochondrial distortion) similar to those in the liver (324), it was reasonable to expect that the intestinal metabolism of xenobiotics may also be altered by ethanol consumption. Indeed, we have reported elevated cytochrome P-450 levels and microsomal enzyme activities in the upper small intestine after chronic ethanol ingestion in rats (335, 337), a finding that has been confirmed recently (157). As shown in Charts 3 and 4, chronic ethanol ingestion increases BP hydroxylase activity by 200% and also enhances

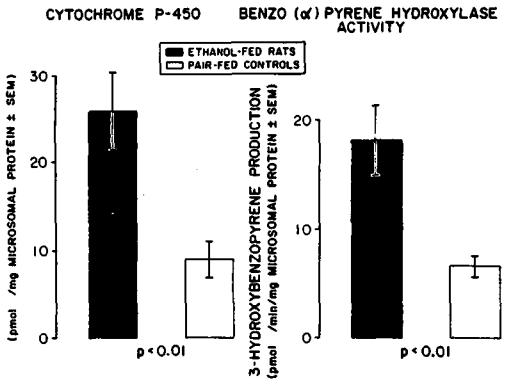

Chart 3. Microsomal cytochrome P-450 content and BP hydroxylase activity in the mucosa of the proximal small intestine after 25 days of ethanol administration in 7 pairs of rats (335).

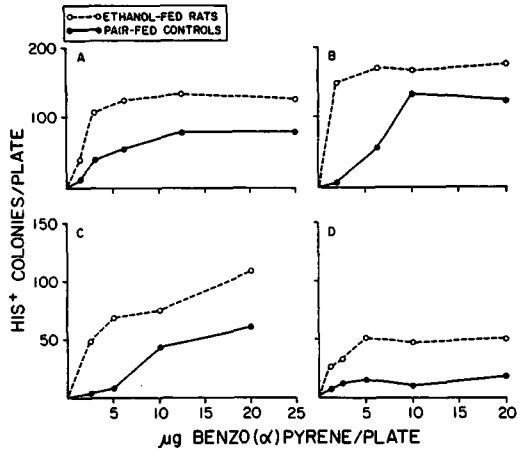

Chart 4. Metabolic activation of BP to a mutagen by intestinal microsomes isolated from ethanol-fed and control rats. Each *point* is an average of the number of His$^+$ colonies of *S. typhimurium* strain TA 100 present on duplicate plates. The levels of spontaneous His$^+$ revertants have been subtracted. In each case, the microsomes were from pools of 3 animals pair fed either the ethanol containing or the control diet. The microsomal protein concentrations per plate were: *A*, 0.5 mg; *B*, 0.5 mg; *C*, 1.0 mg; *D*, 1.4 mg (335).

the capacity of intestinal microsomes to activate BP to a mutagen in the *S. typhimurium* test system. In the same test system, 2 other compounds, namely, 2-aminofluorene and tryptophan pyrolysate, both of which need microsomal activation to become mutagenic, were studied (336). The capacity of intestinal microsomes to activate both compounds to mutagens also was significantly enhanced after chronic alcohol ingestion.

Changes in Microsomes of Lungs and Possible Relationship Between Cancer of Lungs and Other Tissues and Alcoholism

Although an association between alcoholism and lung cancer has not been established, xenobiotic metabolism in the lungs seems to be important since, as in the case of the intestine, lungs represent a major site of entry into the body. Since alcohol and tobacco play a synergistic role with respect to carcinogenesis in the oropharynx, larynx, and esophagus (412), we wondered whether chronic ethanol administration to rats may increase pulmonary activation of procarcinogens contained in tobacco pyrolysates, which may be inhaled by smoking. Indeed, tobacco pyrolysates and smoke condensates, when activated by microsomes, have been shown to be mutagenic (329).

Tobacco pyrolysate was prepared from commercially available cigarette tobacco which was heated under vacuum at 500° for 1 min (250). No difference was detected in benzo(*a*)pyrene monooxygenase activity between alcohol-fed and control animals [22.4 ± 3.2 (S.E.) *versus* 21.5 ± 4.2 pmol 3-hydroxybenzo(*a*)pyrene per mg microsomal protein per min]; lung microsomes from both groups of animals activated BP to the same degree in the *S. typhimurium* test. However, when lung microsomes from ethanol-fed rats were incubated with tobacco pyrolysate, an enhanced activation to mutagenic derivatives was observed (Chart 5). Since no difference was seen in BP activation, compounds in tobacco pyrolysate other than BP must be incriminated for the difference in mutagenicity. The active compounds have not been identified but may include pyrolysis products of peptides (251) or of some amino acids (250) or nitrosamines which have been detected in trace amounts in tobacco (227). At the present time, further observations are needed to assess the clinical relevance of our findings of enhanced pulmonary activation of tobacco derivates to mutagenic compounds after chronic ethanol consumption. Similar microsomal studies should also be carried out in other tissues in which an association of cancer and alcoholism has been described. For instance, in addition to cancer of the colon and rectum, cancer of breast and thyroid gland have been associated by Breslow and Enstrom (37) with beer consumption and they also showed an association between kidney and bladder cancer and alcohol. Some investigators (296, 357) have found an association between alcohol and prostatic cancer, but others (334, 411) have not. Still others have noted an association between alcohol consumption and melanoma (394). A summary of epidemiological data is presented in various reviews on alcohol and cancer (1, 180, 370) and elsewhere in this issue (371).

The inductive effect of chronic ethanol ingestion on the capacity of isolated liver, intestine, and lung microsomes to activate procarcinogens to mutagens suggests a possible link between this effect of ethanol and the enhanced incidence of cancer in the alcoholic. These speculations are particularly relevant since in various animal experiments ethanol has been shown to have syncarcinogenic effects. The carcinogenic effect of 7,12-dimethylbenzanthracene applied to either the cheek epithelium of young Syrian hamsters (88, 89) or the skin of mice (351) was enhanced by ethanol. Numerous papillomas

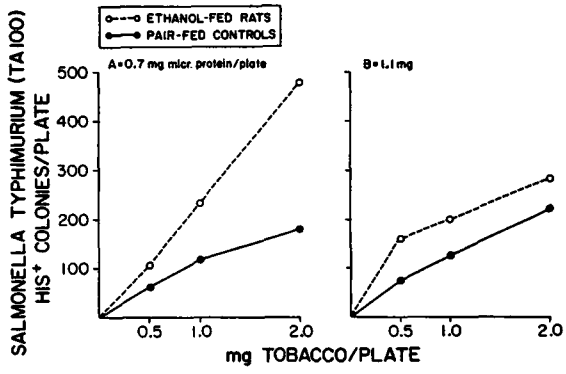

Chart 5. Metabolic activation of tobacco pyrolysate to mutagens by lung microsomes (*micr.*) isolated from ethanol-fed and control rats. Assays were performed as described in Chart 4.

and 2 carcinomas of the esophagus were obtained by the administration of BP to mice in dilute alcohol (145), while no carcinomas were detected when ethanol was given alone or when BP was given in other solvents. Furthermore, the simultaneous administration of ethanol and DMN in rats has resulted in a significantly elevated number of papillomas and epidermoid carcinomas (116). It should be noted, however, that in another study the simultaneous administration of ethanol (25 and 38% in drinking water) did not modify the hepatocarcinogenesis effect of diethylnitrosamine (330, 331). The results of this latter study must be questioned, however, since when administered in drinking water ethanol is consumed in only small amounts by rodents.

It should also be pointed out that acetaldehyde, the product of ethanol metabolism, was also found to induce sister chromatid exchange and cross-links in DNA in human cells (311). Consistent with this observation is the finding that ethanol causes chromosomal changes in *vivo* but not in *vitro* (275).

Hepatocellular Carcinoma and Mechanisms of Ethanol-induced Liver Injury

Among the various theories of hepatocellular cancer, it has been proposed that repeated cell injury promotes the development of tumors. Experimentally, liver cancer can indeed be induced more readily if superimposed on a regenerating liver (62); clinically, as discussed before, a majority of hepatocellular tumors are associated with cirrhosis of the liver. It is therefore germane to review and examine mechanisms whereby alcohol injures the liver, including the pathogenesis of cirrhosis.

Pathogenesis of Alcoholic Fatty Liver

In its milder form, alcoholic liver disease is characterized by accumulation of excess fat in the liver, so-called fatty liver.

Alcohol as a Direct Cause of the Fatty Liver. Each g of ethanol provides 7.1 calories, which means that 20 oz (or 586 ml) of 86 proof (43%, v/v) beverage (not an unusual intake for the alcoholic) represent about 1500 calories or one-half to two-thirds of the normal daily caloric requirement. Therefore, the alcoholic has a much reduced demand for food to fulfill his caloric needs. Since alcoholic beverages do not contain significant amounts of protein, vitamins, and minerals, the intake of these nutrients may become readily borderline or insufficient. Economic factors may also reduce the consumption of nutrient-rich food by the alcoholic. In addition to acting as "empty" or "naked" calories, alcohol can result in malnutrition by interfering with the normal processes of food digestion and absorption (229). For all these reasons, deficiency diseases readily develop in the alcoholic. In rodents, severely deficient diets result in liver damage even in the absence of alcohol. Extrapolation from these animal results to humans led to the belief that in alcoholics the liver disease is due not to ethanol but solely to the nutritional deficiencies and that, given an adequate diet, alcohol is merely acting by its caloric contribution and is not more toxic than a similar caloric load derived from fats or starches (30). This opinion prevailed despite some statistical evidence gathered both in France (297) and in Germany (203) which indicated that the incidence of liver disease correlated with the amount of alcohol consumed rather than with deficiencies in the diet. A major challenge to the concept of the exclusively nutritional origin of alcoholic liver disease arose from an improvement in the method of alcohol feeding to experimental animals. Indeed, when the conventional alcohol feeding procedure is used, namely, when ethanol is given as part of the drinking water, rats usually refuse to take a sufficient amount of ethanol to develop liver injury, if the diet is adequate. This aversion of rats to ethanol was counteracted by the introduction of the new technique of feeding ethanol as part of a nutritionally adequate, totally liquid diet (72, 219, 220). With this procedure, ethanol intake was sufficient to produce a fatty liver despite an adequate diet. This technique is now widely adopted for the study of the pathogenesis of the fatty liver in the rat. In addition to the fatty liver, ethanol dependence developed in these rats, as witnessed by typical withdrawal seizures after cessation of alcohol intake (215).

Having established an etiological role for ethanol in the pathogenesis of the experimental fatty liver, the question of its importance for the development of human pathology remained. To determine whether ingestion of alcohol in amounts comparable to those consumed by chronic alcoholics is capable of injuring the liver even in the absence of dietary deficiencies, volunteers (with and without a history of alcoholism) were given a variety of nondeficient diets under metabolic ward conditions, with ethanol either as a supplement to the diet or as an isocaloric substitution for carbohydrate (219, 220, 223). In all these individuals, ethanol administration resulted in fatty liver development which was evident both on morphological examination and by direct measurement of the lipid content of the liver biopsies, which revealed a rise in triglyceride concentration up to 15-fold.

Origin and Mechanism of Fat Deposition in the Liver. Lipids which accumulate in the liver can originate from 3 main sources: dietary lipids (which reach the blood stream as chylomicrons); adipose tissue lipids (which are transported to the liver as free fatty acids; and lipids synthesized in the liver itself (Chart 6). These fatty acids of various sources can accumulate in the liver as a result of a large number of metabolic disturbances, primarily (*a*) decreased lipid oxidation in the liver, (*b*) enhanced hepatic lipogenesis, (*c*) decreased hepatic release of lipoproteins, (*d*) increased mobilization of peripheral fat, and (*e*) enhanced hepatic uptake of circulating lipids. Depending on the experimental conditions, any of the 3 sources and the various mechanisms can be implicated.

During consumption of ethanol with lipid-containing diets,

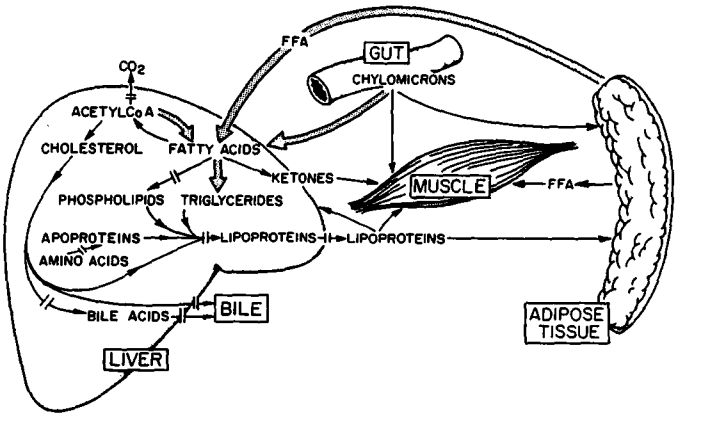

Chart 6. Possible mechanisms of fatty liver production through either increase (→) or decrease (⊣→) of lipid transport and metabolism. *FFA*, free fatty acids.

the fatty acids which accumulate in the liver are derived primarily from dietary fatty acids; whereas when ethanol is given with a low-fat diet, endogenously synthesized fatty acids are deposited in the liver (224–226). Some of these effects can be considered as consequences of the metabolism of ethanol in the liver (Chart 7). Depending on the metabolic state of the animal, both decreased lipid oxidation and enhanced lipogenesis can be linked to ethanol oxidation and the associated increased generation of NADH as discussed elsewhere (208). In addition to the functional changes which are a direct consequence of the metabolism of ethanol, chronic ethanol abuse results in more persistent changes in the mitochondria (209). The striking structural changes of the mitochondria are associated with corresponding functional abnormalities including a decreased capacity to oxidize fatty acid (253). Thus, decreased fatty acid oxidation, whether as a function of the reduced citric acid cycle activity (secondary to the altered oxidation-reduction potential) or as a consequence of permanent changes in mitochondrial structure, offers the most likely explanation for the deposition of fat in the liver, especially fat derived from the diet. Contrasting with fed rats, in which there is evidence for both accelerated lipogenesis and increased utilization of dietary fat (7), ethanol did not stimulate fatty acid synthesis in fasted rats (283). Moreover, in adequately fed rats given one large, sublethal dose of ethanol, it was observed that fatty acids resembling those of adipose tissue accumulate in the liver (130, 225). Experimental procedures or agents which reduce the normal rate of peripheral fat mobilization, *i.e.*, adrenalectomy, spinal cord transection, or ganglioplegic drugs, prevented or decreased this type of hepatic fat accumulation (38, 243, 309). More direct approaches, however, such as studies in rats with prelabeled epididymal fat pads, yielded conflicting information, with evidence for increased (173) or unchanged (160) fatty acid mobilization. Similarly, in rats, one large dose of ethanol has been reported to result in increased (38, 243) or unchanged (87) circulating levels of free fatty acids. In humans, even with amounts of ethanol as large as 300 g/day, the concentration of circulating free fatty acids did not increase; it rose only after ingestion of very large doses of ethanol (400 g/day) (220). In short-term studies, ethanol administration produced a fall in the level of circulating free fatty acids in humans (160, 219) with reduced peripheral venous-arterial differences in free fatty acids (221), decreased free fatty acid turnover (161), and concomitant reduction in circulating glycerol (96). This effect of ethanol upon free fatty acid mobilization from adipose tissue was found to be mediated by acetate (63). Acetate is the end product of ethanol metabolism in the liver (Chart 6) and is released into the blood stream. Since stressful doses of ethanol probably both stimulate fatty acid mobilization (via catecholamine release) and depress it (via the acetate produced), the net effect may depend upon the particular experimental conditions. This may account for some of the apparent contradictions of the literature.

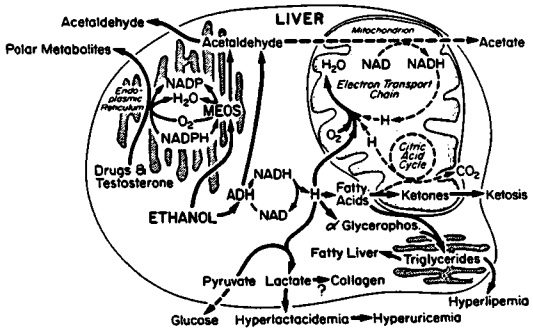

Chart 7. Oxidation of ethanol in the hepatocyte and its link to fatty liver, hyperlipemia, hyperuricemia, hyperlactacidemia, ketosis, hypoglycemia, and drug and acetaldehyde metabolism. - - - -, pathways that are depressed by ethanol (210); α *Glycerophos.*, α-glycerophosphate.

Thus, the main events leading to the development of the alcoholic fatty liver can be summarized as follows. Ethanol, which has an almost "obligatory" hepatic metabolism, replaces the fatty acids as a normal fuel for the hepatic mitochondria. This results in fatty acid accumulation, directly because of decreased lipid oxidation and indirectly because one way for the liver to dispose of excess hydrogen generated by ethanol oxidation is to synthesize more lipids. Fatty acids derived from adipose tissue accumulate in the liver only when very large amounts of ethanol are given. The lipids increase in the liver despite the fact that the transport mechanism via release of lipoproteins from the liver into the blood stream is stimulated by ethanol, at least during the initial state of intoxication.

With progressing liver injury, as discussed subsequently, the hypersecretion of lipoprotein fades away and may actually fall below normal levels. At that stage, instead of offsetting, in part, the lipid accumulation, altered lipoprotein secretion may actually contribute to it and thereby aggravate liver injury even further (see below).

The Influence of Dietary Factors. *Role of Dietary Fat.* As discussed before, alcohol ingestion leads to deposition in the liver of dietary fat. This observation prompted an investigation into the role of the amount and kind of dietary fat in the pathogenesis of alcohol-induced liver injury. Rats were given liquid diets containing a normal amount of protein for rodents (18% of total calories), with varying amounts of fat. Reduction in dietary fat to a level of 25% (or less) of total calories was accompanied by a significant decrease in the steatosis induced by ethanol (213). The importance of dietary fat was confirmed in volunteers. For a given alcohol intake, much more steatosis developed with diets of normal fat content than with a low-fat diet (224). In addition to the amount, the chain length of the dietary fatty acid is also important for the degree of fat deposition in the liver after alcohol feeding. Replacement of dietary triglycerides containing long-chain fatty acids by fat containing medium-chain fatty acids markedly reduced the capacity of alcohol to produce a fatty liver in rats (222). The propensity of medium-chain fatty acids to oxidation rather than to esterification is the most likely explanation of the reduction in alcoholic steatosis upon replacement of dietary long-chain fatty acids by medium-chain fatty acids.

Role of Protein and Lipotropic Factors (Choline and Methionine). In perfused liver, ethanol was shown to increase choline uptake (16), but this was found to be unrelated to lipid accumulation (259). In growing rats, deficiencies in dietary protein and lipotropic factors (choline and methionine) can produce fatty liver (30), but primates are far less susceptible to protein and lipotrope deficiency than are rodents (143). Clinically, treatment with choline of patients suffering from alcoholic liver injury has been found to be ineffective in the face of continued alcohol abuse (284); experimentally, massive supplementation with choline failed to prevent fatty liver produced by alcohol in volunteer subjects (323). Unlike rat liver, primate livers contain

very little choline oxidase activity, which may explain the difference with regard to choline deficiency. The phospholipid content of the liver represents another key difference between the ethanol- and choline-deficient fatty liver. After the administration of ethanol, hepatic phospholipids increase (219); whereas in the fatty liver produced by choline deficiency, they decrease (10). Moreover, orotic acid, which prevents the phospholipid decrease and the development of fatty liver due to choline deficiency, had no such effects after ethanol (86). Furthermore, hepatic ATP decreased after chronic ethanol feeding (29, 112, 385) but was unaffected in choline deficiency (346). Conversely, hepatic carnitine is decreased by choline deficiency (61) but increased after ethanol feeding (185). Moreover, ethanol-induced fatty liver is associated with increased circulatory lipoproteins and enhanced incorporation of [^{14}C]lysine into lipoproteins (19), whereas the opposite occurs with choline deficiency (232). Ultrastructurally, the lesions also differ (152, 325). Thus, hepatic injury induced by choline deficiency appears to be primarily an experimental disease of rats with little, if any, relevance to alcoholic liver injury in humans. Even in the rats, massive choline supplementation failed to prevent fully the ethanol-induced lesion, whether alcohol was administered acutely (76) or chronically (211). Alcohol has been reported either to aggravate (360) or attenuate (292) choline-induced liver injury.

Protein deficiency may affect the liver, but this has not yet been clearly delineated in human adults. In children, protein deficiency leads to steatosis, one of the manifestations of kwashiorkor. In adolescent baboons, however, protein restriction from 20% to 7% of total calories (as part of a low-fat diet, 14% of calories) did not result in liver injury on either biochemical analysis or light and electron microscopic examination even after 19 months (217). Conversely, an excess of protein was not capable of preventing ethanol from producing fat accumulation in human volunteers (223). In that study, dietary protein represented 25% of total calories, or 2.5 times the recommended amount. Thus, even in the absence of protein deficiency, ethanol is capable, in humans, of producing striking changes in the liver. Severe protein deficiency (4% of total calories) also produced steatosis in the baboon (217). Similar lesions were reported in the rhesus monkey (191). When protein deficiency is present, it could potentiate the effect of ethanol. Indeed, administration of ethanol with a diet deficient in protein and lipotropic factors had more pronounced effects than that of either factor alone (182, 226), at least in rodents. Possible interactions of ethanol and protein nutrition were also suggested by the observation that ethanol feeding increases the activities of hepatic cystathionine synthetase and 5-methyltetrahydrofolate-homocysteine methyltransferase, which may impair mechanisms for methionine conservation in protein deficiency (101). To what extent such abnormalities contribute to the ethanol-induced liver lesions remains to be assessed. In any event, the ultrastructural abnormalities produced by protein deficiency (91, 294) differ from those resulting from alcohol (152, 196). Furthermore, clinical protein malnutrition is associated with characteristic plasma amino acid abnormalities, including a depression of branched-chain amino acids, whereas the alcohol-induced liver injury in the well-fed baboon was associated with opposite amino acid changes (341). Of course, in alcoholic patients, plasma amino acid abnormalities may reflect a complex interaction of many factors: nutrition; alcoholic liver disease; alcohol-induced injury in other organs; and associated disease states. The frequent concurrence of chronic alcoholism and nutritional deficiency makes the separation of these variables especially difficult. However, whereas branched-chain amino acids and α-amino-*n*-butyric acid were found to be increased 2- to 3-fold and 7-fold, respectively, in the plasma of baboons fed alcohol as 50% of total calories (341), these amino acids are all depressed when measured in protein deficiency. Following intestinal bypass for obesity, decreased absorption of dietary protein is observed (271). In such patients, plasma α-amino-*n*-butyric and branched-chain amino acids, phenylalanine, threonine, and lysine are decreased while plasma serine and glycine, both nonessential amino acids, are increased. By contrast, relatively increased α-amino-*n*-butyric acid was considered to be characteristic of changes of plasma amino acids in alcoholics (344).

Transitional Lesions from Reversible Alcoholic Fatty Liver to Irreversible Liver Injury

At the 2 extremes in the spectrum of alcoholic liver disease, one has the fully reversible fat accumulation which can occur after a few days of alcohol abuse and the irreversible cirrhosis characteristic of end-stage alcoholic liver disease. In humans, the evolution from fatty liver to cirrhosis may take 5 to 20 years. In the sequence of events, a number of lesions occur, which can be considered as transitional stages between the alcoholic fatty liver and cirrhosis. When these lesions are particularly florid, with manifest extensive necrosis and polymorphonuclear inflammation, they are sometimes considered to be characteristic of a stage of "alcoholic hepatitis" which is believed by many to be "the" precursor lesion of cirrhosis, intermediate between the fatty liver stage and the end-stage fibrosis. In practice, delineation of such a hepatitis stage is not always easy. In fact, extensive hepatitis is relatively uncommon in an alcoholic population, as compared to the frequency of fatty liver and cirrhosis. Fatty liver, however, is also associated with milder forms of necrosis and inflammation, in which case the term "severe" fatty liver or "mild" alcoholic hepatitis is being used. In fact, at the early so-called simple or uncomplicated fatty liver stage, as discussed before, striking lesions in the mitochondria and the rough endoplasmic reticulum can be detected. These in a sense might also be considered as transition lesions between "simple" fat accumulation and more severe liver injury. In fact, the injurious effects of alcohol in the liver are multiple, and the classic distinction between "simple fatty liver," "alcoholic hepatitis," and "cirrhosis" is arbitrary. In reality, there is a continuum of lesions, and the more sensitive the mode of investigation, the earlier signs of such injury can be found. However, 2 types of lesions stand out as key stages in the evolution of alcoholic liver disease, namely, the swelling or "ballooning" of the hepatocyte and the necrosis with associated inflammation.

"Ballooning" of the Hepatocyte and Associated Impairment of Protein and Lipoprotein Export. In addition to fat accumulation, the alcoholic fatty liver is characterized by striking deposition of protein (17, 18). In the early stages of fatty liver development, this protein accumulation was found to be as important quantitatively as that of fat and contributed to a similar extent to the hepatomegaly which developed after chronic alcoholism. Although enhanced organelle proteins (mitochondria and microsomes) do contribute to the total in-

crease, the major fraction of the proteins is deposited in the cytosol. The nature of all the proteins which accumulate in the cytosol has not been elucidated, but up to now increases have been found in export proteins such as albumin and transferrin (18) but not in constituent proteins of the cytosol. This observation led to the hypothesis that one of the early lesions induced by chronic alcoholism may be the interference with the capacity of the liver to export proteins. Consistent with this concept was the finding of a decrease in hepatic microtubulin believed to be implicated in the export of proteins from the liver (18) and delayed serum albumin labeling after administration of labeled amino acids (18). Metabolites of ethanol may be responsible for this effect (249), particularly acetaldehyde, a known hepatotoxin (see below). The retention of protein may contribute to the "ballooning" of the hepatocytes, a common morphological alteration found in alcohol liver injury, and the decrease in plasma transferrin in alcoholics with or without cirrhosis (195).

In addition to protein release, lipoprotein secretion is also affected. Contrasting with the hyperlipemia, which is commonly associated with the administration of moderate to large amounts of ethanol (21, 233, 253), an extremely high dose has been reported to decrease serum triglycerides (68), very-low-density lipoproteins (240), high-density lipoproteins (184), and the incorporation of glucosamine into the carbohydrate moiety of serum lipoproteins (267) in the rat.

In volunteers, chronic ethanol administration resulted in initial hyperlipemia (220). However, blood lipid content declined after 2 to 3 weeks, implying that lipoprotein output falls with progressing alcoholic liver injury. This concept is supported by the study of Marzo *et al.* (247), who correlated serum lipids with various histological stages in 90 alcoholics. Peak serum lipid values were found during the stage of fatty metamorphosis. During the succeeding stages of steatosis and interstitial chronic hepatitis, a progressive decrease in serum lipids occurred. The decrease was predominantly in the triglyceride and cholesterol fractions. In well-established cirrhosis, circulating lipoproteins are generally low (24, 40). In addition, α-lipoproteins are absent by electrophoresis in sera from patients with some alcoholic liver injury (289, 327), associated with the appearance of abnormal lipoproteins (13, 327). Thus, after the initial development of fatty liver accompanied by hyperlipemia, the blood lipids return toward normal and even decrease (36). Progressive deterioration of liver function, including lipoprotein production and secretion, could be responsible and may secondarily aggravate fat accumulation in the liver and the swelling of the hepatocyte. It is reasonable to postulate that excessive swelling in turn leads to cell death or necrosis, a key feature of alcoholic hepatitis.

Development of Mitochondrial Injury. In addition to alterations of the rough endoplasmic reticulum, as previously noted, alcoholics are known to have profound hepatic mitochondrial changes (358) which are associated with increased serum activity of the intramitochondrial enzyme glutamate dehydrogenase (186). From these clinical observations, however, it was impossible to assess whether the mitochondrial changes were a direct result of chronic ethanol intake or were secondary to other factors such as dietary deficiencies. Recent studies have incriminated alcohol itself as the responsible agent and have clarified some functional counterparts of the ultrastructural lesions.

Ultrastructural Changes of Mitochondria. In humans chronic alcohol consumption results in striking mitochondrial alterations which include swelling and disfiguration of mitochondria, disorientation of the cristae, and intramitochondrial crystalline inclusions (178, 358). Similarly, in the rat, isocaloric substitution of ethanol for carbohydrate in otherwise adequate diets leads to enlargement and alterations of the configuration of the mitochondria (152), indicating that ethanol itself or one of its metabolites causes the alterations rather than dietary deficiencies. Mitochondrial changes similar to those seen in chronic alcoholics were also produced by isocaloric substitution of ethanol for carbohydrate in baboons (217), and in humans, both in alcoholics (196, 223) and in nonalcoholics (323). Degenerated mitochondria were conspicuous, and the debris of these degraded organelles was also found within autophagic vacuoles and residual vacuolated bodies (321). The striking structural changes of the mitochondria are associated with corresponding functional abnormalities.

Alterations of Mitochondrial Functions. These injured mitochondria have a reduction in cytochrome *a* and *b* content (320) and in succinic dehydrogenase activity (286, 320), although in one study (380) succinic dehydrogenase activity measured in total liver homogenates was reported to be increased in ethanol-fed rats. The respiratory capacity of the mitochondria was found to be depressed (117, 132, 177) using pyruvate succinate and acetaldehyde as substrates. Oxidation of other substrates was also found to be reduced in mitochondria of ethanol-fed rats, except for α-glycerophosphate, the oxidation of which was reported by some to be increased (176) or unchanged (302), whereas others found it to be decreased (134).

Oxidative phosphorylation was found to be selectively altered at Site 1 (46). Since the structural changes of the mitochondria persist, the question arose as to whether these in turn could be responsible for some alterations in lipid metabolism beyond those which were attributed to the altered oxidation-reduction change. The first indication that ethanol consumption may result in more persistent metabolic changes came from the observation that alcohol ingestion is associated with a progressive increase in ketonemia and ketonuria, which was most pronounced in the fasting state (202). The ketonemia may aggravate the acidosis of the hyperlactacidemia (220) and on occasion may lead to severe alcoholic ketoacidosis (156, 207). The capacity for ethanol to produce ketonemia was found to be greater than that of fat itself, provided, however, that fat was present in the diet. Thus, fat seems to play a permissive role (202). Mitochondria obtained from ethanol-fed rats, when incubated *in vitro*, even in the absence of ethanol, display decreased capacity to oxidize fatty acids but enhanced β-oxidation, which is possibly responsible for the increased ketogenesis (44). Decreased fatty acid oxidation, whether as a function of the reduced citric acid cycle activity (secondary to the altered oxidation-reduction potential) (208) or as a consequence of permanent changes in mitochondrial structure (as emphasized in this paragraph), offers the most likely explanation for the deposition of fat in the liver after chronic alcohol ingestion, especially fat derived from the diet (224–226, 258). It is noteworthy that high concentrations of acetaldehyde, the product of ethanol metabolism, mimic the defects produced by chronic ethanol consumption on oxidative phosphorylation at Site 1 (45). One may wonder to what extent chronic exposure

to acetaldehyde is the cause for the defects observed after chronic ethanol consumption, especially since it was found recently that, after chronic ethanol consumption, mitochondria become more susceptible to the toxic effects of acetaldehyde. These toxic effects occur even at 0.2 mM acetaldehyde (253), a concentration reported in the liver *in vivo* after ethanol administration. Alcoholics may exhibit higher acetaldehyde levels than do nonalcoholics for a given ethanol load and blood level (188). It is therefore not unreasonable to speculate that exposure to high acetaldehyde levels may in turn affect mitochondrial function and result in a vicious cycle, especially since mitochondria of alcohol-fed animals were found to have a reduced capacity to oxidize acetaldehyde (134).

Alcoholic Hepatitis. Alcoholic hepatitis is a morphological entity characterized by hepatocellular necrosis and an inflammatory reaction of polymorphonuclear leukocytes. The lesions are characteristically localized in the centrolobular area but may be more diffusely distributed in more severe cases. Intracellular irregular clumps of hyalin, so-called Mallory bodies, may be present and, although helpful in establishing an etiological diagnosis, are not pathognomonic for the disease. Indeed, similar structures have occasionally been described in primary biliary cirrhosis (115, 238), in hepatocellular carcinoma (168), in Indian childhood cirrhosis (318), after griseofulvin treatment (75), in patients who underwent bypass operation for morbid obesity (127), and in tissue cultures of cells derived from rats treated with diethylnitrosamine, a chemical carcinogen (35). Although the severity of the lesions is usually somewhat greater in cases with Mallory bodies (51, 299), no significantly worse prognosis was associated with their presence.

The exact nature and pathogenesis of alcoholic hyalin has not been elucidated, but the fact that, experimentally, hyalin bodies can be produced by agents which interfere with protein secretion supports the concept that these bodies may be sequela of the impaired protein secretion discussed before (74). Immune responses have been attributed to hyalin. The role of this and other "autoimmune" reactions in the pathogenesis of alcoholic liver injury are discussed in detail in another review (109). The clinical picture of alcoholic hepatitis was originally restricted to severely ill patients with jaundice, fever, ascites, right upper quadrant pain, and hepatomegaly, sometimes progressing to coma and death (300). However, it soon became obvious that the typical morphological lesions also could be observed in patients with minimal and nonspecific complaints of anorexia, weight loss, or nausea (26, 121). Thus, the clinical expression of alcoholic hepatitis may range from nearly asymptomatic to a fulminant fatal disease (231). Severe clinical symptoms are usually associated with severe histological lesions (181), but the reverse does not hold true, and extensive necrosis may be seen in patients with minimal complaints (300). Characteristically, serum transaminases are only moderately elevated in alcoholic hepatitis in contrast to viral hepatitis, and there is a considerable overlap between the values in patients with and without hepatitis (376). Serum alkaline phosphatase is often elevated, and leukocytosis may be present. Other tests reflect the degree of liver insufficiency, and elevated bilirubin, low serum albumin, and prolonged protime may be present to a variable degree. The combination of fever, jaundice, and right upper quadrant pain together with moderately elevated transaminases, leukocytosis, high alkaline phosphatase, and high bilirubin may mimic extrahepatic obstruction (15, 262). The differentiation of alcoholic hepatitis, a medical condition, and extrahepatic obstruction, a surgical entity, is important since an increased mortality has been observed in open liver biopsies of patients with alcoholic hepatitis (122).

Thus, it appears that clinical symptoms and biochemical tests, although sometimes suggestive, do not permit a definitive diagnosis and that correct staging depends on morphological criteria.

Although liver biopsy is the most accurate way of determining the degree of liver cell necrosis, this is obviously not a practical tool for routine follow-up of a patient or for mass screening purposes. Therefore, spillover in the blood of liver enzymes, especially transaminases, is commonly used as a marker of liver cell necrosis. However, blood transaminase values are a poor reflection of liver cell necrosis as revealed on biopsy (165), especially in alcohol-induced liver cell injury. In alcoholic hepatitis, for example, levels of transaminases are only moderately elevated, and normal values can occasionally be found. γ-Glutamyltranspeptidase is no more reliable, although some correlation with liver cell necrosis exists (401). Elevation from nonhepatic origin is common, and in some alcoholics elevated levels may only reflect microsomal induction (111, 154, 361), although some have attributed this effect to lack of carbohydrate (270). However, more recent studies showed this not to be the case (343). In contrast with transaminases and γ-glutamyltranspeptidase, which show a considerable overlap between patients with and without significant cell necrosis on biopsy, in a recent study it was found that glutamate dehydrogenase reflected more accurately the degree of underlying cell necrosis (376) (Chart 8). The high liver specificity of the enzyme (332), its solely mitochondrial origin (mitochondrial lesions associated with alcoholic liver disease have been discussed before), and its predominantly centrolobular localization (124) (the area which suffers the major impact of alcoholic liver injury) could explain the superiority of the enzyme as an index of liver cell necrosis in alcoholic patients.

The mechanisms whereby alcohol abuse leads to necrosis of the hepatocyte have not been fully elucidated. It is likely that a variety of mechanisms are involved, such as toxic effects of acetaldehyde; consequences of injury to the endoplasmic reticulum, mitochondria, and microtubules; as well as the physical damage resulting from the accumulation of fat and protein and the distention of the hepatocyte.

As mentioned before, there is a continuum of graduations of liver necrosis and inflammation ranging from its virtual absence in the initial fatty liver stage to extensive necrosis and inflammation in the case of florid hepatitis. In the absence of liver biopsy, a determination of glutamate dehydrogenase may help define the evolution of the patient from one stage of alcoholic liver injury to another.

Collagen Accumulation, Fibrosis, and Cirrhosis

There is not only a wide spectrum of inflammation and necrosis in alcoholic liver injury, but there is also a great variability in the magnitude of collagen deposition. At the earlier stages in the so-called simple or uncomplicated fatty liver, collagen is detectable by chemical means only (97, 293). When collagen deposition is sufficient to become visible by light microscopy, usually it appears first around the central vein,

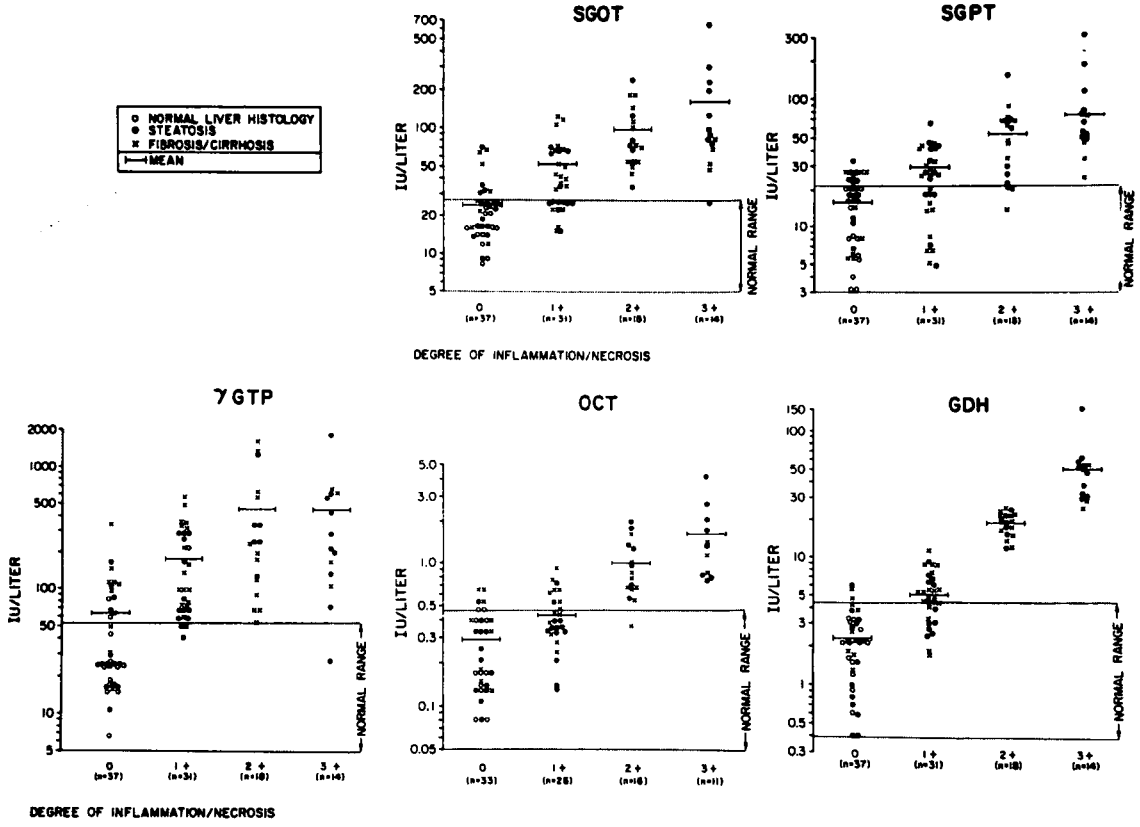

Chart 8. Correlation of serum glutamate dehydrogenase (*GDH*), serum glutamic-oxaloacetic transaminase (*SGOT*), serum glutamic-pyruvic transaminase (*SGPT*), γ-glutamyltranspeptidase (γ-*GTP*), and ornithine carbamyl transferase (*OCT*) with degree of liver cell necrosis in 100 alcoholics (376).

resulting in so-called "perivenular" sclerosis, (previously called "pericentral" sclerosis). This lesion is usually described in association with full-blown alcoholic hepatitis. Less well recognized is the fact that this perivenular sclerosis frequently may be seen in the absence of overt hepatitis (84). In a retrospective study of liver biopsies from alcoholic patients that had been read by the pathologists as simple fatty liver, appropriate staining disclosed perivenular sclerosis in 40% (377). Experimental studies in the baboon model for alcoholic liver injury have shown that, in animals that progressed to cirrhosis, perivenular sclerosis invariably occurred at the fatty liver stage in the absence of alcoholic hepatitis or preceding it, before the development of the irreversible lesions of fibrosis and cirrhosis; by contrast, animals that did not develop the lesion did not progress beyond the stage of fatty liver. These experimental data suggest that, at least in the baboon, perivenular sclerosis in the absence of hepatitis is a common and early warning sign of impending cirrhosis, if drinking continues (377). The concept of perivenular sclerosis as a precirrhotic lesion in alcoholics is further strengthened by similar observations in patients with fatty livers after bypass operation for morbid obesity (245) and in diabetic individuals (94).

In view of these observations, one must now reconsider the classic concept according to which a major precursor lesion for cirrhosis is the extensive inflammation and necrosis of fulminant alcoholic hepatitis, which in turn triggers the scarring. Indeed, collagen deposition occurs already prior to the development of full-blown alcoholic hepatitis. Furthermore, in experiments in the baboons, full-blown cirrhosis developed without a mandatory stage of full-blown hepatitis but with only moderate inflammation and necrosis (216, 218). Moreover, there is a relative paucity of cases of alcoholic hepatitis as compared to fatty liver and cirrhosis in many populations. It appears now that collagen deposition, perivenular sclerosis, and even cirrhosis can develop in the presence of minimal inflammation and necrosis, which may suffice to trigger the fibrosis (209). On the other hand, it is also possible that alcohol may have some direct effect on collagen metabolism independent of the necrosis and inflammation. In support of this possibility is the observation of possible enhanced collagen production after alcohol (97, 260). Theoretically, interference by alcohol of collagen breakdown could also contribute to collagen accumulation. Thus far, however, there is no experimental evidence to support such a mechanism. There is actually evidence for enhanced rather than decreased collagen degradation (261, 279).

In the discussion of the pathogenesis of the alcoholic fatty liver, reference was made to the debate of the respective role of malnutrition and alcohol itself as an etiological factor. A similar debate has been raging with regard to the development of alcoholic cirrhosis. Recently, this long-standing discussion whether the liver injury observed in the alcoholic is due to malnutrition rather than alcohol *per se* was rekindled by a report of Patek *et al.* (292). In this epidemiological study of 304 alcoholic patients, alcohol intake and dietary habits were evaluated. There were 195 patients with hepatic cirrhosis, 40 precirrhotics, and 69 noncirrhotics. By history, alcohol contributed 50 to 59% of total calories. Two-thirds of the patients

drank for more than 20 years. Duration and degree of alcohol abuse was comparable in all 3 groups. Dietary intake, however, differed. Over the 2-year period preceding the presenting illness, noncirrhotics had higher food calorie intake and higher protein intake than did the cirrhotics. This type of retrospective study, however, is complicated by the difficulty in differentiating cause and effect. It is well known that complications of severe liver disease, particularly cirrhosis, can by themselves be reasons for poor dietary intake. It is therefore not clear whether the difference in dietary intake between cirrhotics and noncirrhotics is the cause rather than a consequence of that disease.

The etiological role of alcohol *per se* (in the absence of dietary deficiency) in the pathogenesis of alcoholic liver injury was assessed in experimental studies carried out in the baboon. The dose of alcohol given was comparable to that of alcoholics, namely, 50% of the total calories, taking advantage of the liquid diet technique first developed in the rat and now applied to the baboon (216). With this diet, alcohol intake was sufficient to result in periods of obvious inebriation. Upon interruption of alcohol administration, some withdrawal symptoms (such as seizures) were observed. Twenty-three baboons fed the isocaloric control diet retained normal livers, whereas the animals given ethanol all developed excessive fat accumulation. In addition, 5 showed mild inflammation, 9 developed fibrosis, and in 6 baboons studied for 2 to 5 years cirrhosis evolved (Fig. 1).

Not all baboons fed ethanol progressed to cirrhosis; similarly, not all alcoholics develop this complication: the incidence varies depending on duration and dose of alcohol intake (204). Regardless of possible predisposing factors, the baboon study clarifies the respective role of malnutrition and alcohol itself in the pathogenesis of cirrhosis. Thus, one may conclude from our studies that, although earlier evidence suggested that malnutrition can cause liver damage, alcohol itself can result in the development of the typical complications observed in the alcoholic. An important corollary of this finding is the fact that adequate diet did not prevent the development of the alcoholic lesions. The therapeutic implication of this observation is that alcoholics cannot fully prevent the development or the aggravation of liver injury by maintaining an adequate diet unless they also control the degree of alcohol intake. It has been shown in the past by others and our own group that alcohol ingestion results in impaired digestion and malabsorption and that it produces intestinal injury (20). It is unlikely, however, that the effects described are of sufficient magnitude to offset the large excess of nutrients present in our baboon diet. Moreover, under our experimental conditions in the baboon, there was absence of protein and fat malabsorption.[3] Furthermore, as discussed before, plasma amino acid changes observed in these baboons were the opposite of those produced by protein malnutrition (enhanced as opposed to depressed branched-chain amino acids) (341). The possibility that nutritional deficiencies may potentiate the effect of alcohol is presently being investigated in the baboon, since such a phenomenon was observed for the fatty liver in the rat (226). Experimentally, drastic dietary alterations (such as cholesterol overload) may favor the development of cirrhosis (291). Whether this applies to clinical conditions and particularly the development of cirrhosis in humans is less clear. Epidemiological studies of Lelbach (203, 204) did not detect dietary insufficiency as a precondition for alcoholic cirrhosis. The incidence of cirrhosis correlated with the amount of alcohol consumed, not with history of dietary deficiencies; similar results were observed in France by Pequignot (297). Some have even suggested a lowered incidence of cirrhosis with dietary insufficiency (193), whereas others as discussed before reached the opposite conclusion (292). Of course, regardless of the controversy surrounding the role of malnutrition in the pathogenesis of liver injury in the alcoholic, in our present state of knowledge, one is still justified in stressing the importance of correcting nutrient deficiencies when present, especially in view of the general interaction of alcoholism, nutrition, and cancer to be discussed in a subsequent section.

Hepatocellular Carcinoma in the Alcoholic

Hepatocellular carcinoma in alcoholics is commonly thought to occur in association with cirrhosis of the liver. Indeed, the incidence of cirrhosis in patients with hepatocellular carcinoma has been reported to vary between 16 and 80% (47), with most reports indicating a 55 to 80% association. Numerous etiologies have been proposed for carcinoma in cirrhotic patients, especially in conjunction with the regeneration associated with cirrhosis. Of special interest concerning the possible role of alcohol in carcinogenesis is the fact that hepatocellular carcinoma can also occur in noncirrhotic alcoholics. The occurrence of primary hepatocellular carcinoma is world-wide (6, 47, 64, 85, 95, 136, 144, 174, 187, 190, 198, 239, 268, 269, 280–282, 290, 295, 307, 345, 350, 364, 365), but reports specifically characterizing hepatocellular carcinoma in noncirrhotic patients in Western populations are scarce. Because little information is available for this noncirrhotic group of patients, we have retrospectively undertaken a review of autopsy cases seen at the Bronx Veterans Administration Medical Center since 1947.

Personal Series of Hepatocellular Carcinoma at the Bronx Veterans Administration Medical Center. All autopsy files were reviewed, and clinical records were obtained for all cases of hepatocellular carcinoma in patients who did not manifest hepatic cirrhosis in the nontumorous liver.

Since 1947, there have been 48 cases of hepatocellular carcinoma, and of these 15 (31%) have been in patients without cirrhosis in the nontumorous liver. The age range of this group of noncirrhotic patients was 22 to 79 years; the average age was 53.4 years.

Alcoholism was diagnosed in 7 patients, but quantitation of intake and duration were seldom recorded. Three patients were considered "social" drinkers. Of the remaining 5 patients, 4 patients had no statement recorded concerning alcohol consumption, and one patient is reported to have consumed no alcohol.

No subject had a prior history of hepatitis or jaundice, although 3 patients had undergone previous surgical procedures. The presence of Australia antigen was not recorded in any patient. No patient had a history of parasitic infestation. Results of stool examinations for parasites were negative in the one patient tested. Four patients had 5 associated neoplasms (2 with colonic lesions, 1 with an oropharyngeal lesion, and 1 with an esophageal lesion and a meningioma). Only one patient,

[3] J. Lindenbaum and C. S. Lieber, unpublished observations.

a painter, had a known chemical exposure.

Two patients had been diagnosed at outside hospitals prior to Bronx Veterans Administration Medical Center admission. Of the remaining 13 patients, the distribution of symptoms is shown in Table 1. Physical findings in these 13 patients are listed in Table 2. The 2 patients who had been diagnosed previously at other hospitals were clinically severely ill at the time of admission.

In 5 patients, the diagnosis was completely unsuspected at the time of admission. Admission diagnoses included one each of inguinal hernia, psychiatric disease, oropharyngeal carcinoma, metastatic colon carcinoma, and congestive heart failure.

Laboratory parameters for this group of patients are shown in Table 3. Of note is the mild anemia as well as elevation of serum glutamic-oxaloacetic transaminase and alkaline phosphatase. Only one patient had documented hypercalcemia. α-Fetoprotein determinations were performed in 2 patients: one was positive; one was negative.

Table 1

Presenting symptoms in 13 patients with hepatocellular carcinoma without cirrhosis at the Bronx VA Medical Center

Symptoms	No. of patients
Abdominal pain	5
Epigastric (2)	
Right upper quadrant (2)	
Retrosternal (1)	
Wt loss	4
Anorexia	4
Mass	2
Right upper quadrant (1)	
Epigastric (1)	
Constipation	2
Low back pain	1
Lethargy	1
Leg edema	1
Hematemesis	1
Rectal bleeding	1
Behavioral change	1
Nonreducible hernia	1

Table 2

Physical findings in 13 patients with hepatocellular carcinoma

Signs	No. of patients
Cachexia	8
Hepatomegaly	6
Nodular liver	4
Peripheral edema	3
Mass	3
Epigastric (2)	
Right upper quadrant (1)	
Ascites	3
Vascular prominence (abdominal)	2
Icterus	1
Spider angiomata	1

Table 3

Laboratory tests in 13 patients with hepatocellular carcinoma

Test	Av. value
Total protein	7.0 g/100 ml
Albumin	3.8 g/100 ml
Total bilirubin	1.0 mg/100 ml
Serum glutamic-oxaloacetic acid transaminase	160 IU/100 ml
Alkaline phosphatase	225 IU/100 ml
Hematocrit	33%

Two patients underwent chemotherapy. One patient, who received Cytoxan, succumbed 3 months after diagnosis. The other, who received methotrexate and vinblastine, succumbed 8 months after diagnosis. Seven patients in whom the diagnosis was made at the time of admission survived an average of 2.5 months. One patient, misdiagnosed as having metastatic adenocarcinoma, survived 4 months. In 5 patients, the diagnosis of hepatocellular carcinoma had not been anticipated before autopsy. Average survival of these 5 patients was 7 months.

Characteristics of Hepatocellular Carcinoma in the Absence of Cirrhosis. Although the majority of our patients had a heavy alcohol intake, quantitation was not possible from the hospital records. Only a few patients had been overseas, and there were no histories of a hepatitis-like syndrome or jaundice. The role of viral infection alone or in conjunction with alcohol remains to be elucidated as a causative factor in this disease.

Presenting symptoms were suggestive of malignant disease, although these complaints may also occur in any patient with a heavy alcohol intake. The pain was predominantly in the right upper quadrant or epigastric, without any particular distinguishing characteristics, as has been noted previously (282). Approximately 40% of patients had no symptoms directly related to carcinoma.

Physical findings are not specific for hepatocellular carcinoma, since they may be found in any chronic debilitating disease. Hepatomegaly, however, was frequently found in these patients, and a nodular liver was palpable in 31% of these patients at the time of admission.

Laboratory parameters were of no particular assistance in pointing toward the correct diagnosis. However, hypercalcemia in the presence of hepatomegaly and cachexia should suggest a malignant process.

The survival interval from time of diagnosis was remarkably brief, averaging 4.5 months overall. Chemotherapy did not enhance survival in 2 patients. Although cause of death was difficult to ascertain, the usual terminal event was abrupt and clinically unexplained.

Because of these dismal statistics, it is imperative to define this lesion earlier. Hopefully, this will be possible through increased clinical awareness and application of recent diagnostic advances (23, 92, 107, 110, 167, 175). Because of the noncirrhotic nature of the uninvolved liver, it is possible that many of these patients will be candidates for newer treatment modalities, such as partial hepatectomy (282).

In any event, our findings, as well as those of others (169, 372), of hepatocellular carcinoma in the alcoholic in the absence of cirrhosis are consistent with the possible role of alcohol as cocarcinogen. Further studies, however, are needed to determine whether there is a truly statistically significant increase and incidence of such liver tumors in the alcoholic in the absence of cirrhosis. Indeed, previous reports of enhanced incidence of hepatic cancer in the alcoholic (200, 290) did not differentiate those with and without associated cirrhosis, which, as discussed, may contribute to the development of cancer independently of alcohol.

Alcoholism, Nutrition, and Cancer

General Interaction of Alcohol, Nutrition, and Cancer

In the foregoing sections, we have discussed how alcohol

abuse, independent of malnutrition, can affect the liver, alimentary tract, and other tissues and possibly influence carcinogenesis. However, in addition to these direct effects of ethanol, carcinogenesis may also be influenced by some indirect consequences of alcoholism resulting from the malnutrition commonly associated with alcohol abuse. Indeed, deficient intake of thiamine, folic acid, magnesium, and iron are often present in alcoholics and deficiency of pyridoxine, pantothenic acid, riboflavin, and zinc may also occur (137). Alcohol also has been shown to affect the metabolism of various essential nutrients as well as the absorption, distribution, metabolism, activation, storage, and excretion of many dietary components (342). Each of these deficiencies and their relation to cancer will be discussed individually. In general, it is well known that diet modifies tumor formation in laboratory animals (391). Certain dietary habits also affect carcinogenesis in humans (402–404). Development in the field of azo dye carcinogenesis drew attention many years ago to the fact that diet can modify the effectiveness of chemical carcinogenesis. Rats fed a rice diet low in protein and riboflavin were highly sensitive to liver tumor formation when treated with 4-dimethylaminoazobenzene, but a diet containing adequate amounts of protein and vitamin B_2 reduced and in some cases prevented the carcinogenicity. These observations may have been related to alterations in the level of azo dye reductase, which is involved in the degradation of the carcinogen (263). Furthermore, the carcinogenicity to the liver of the mycotoxin aflatoxin B_1 was increased when rats were maintained on a low lipotrope diet (315). On the other hand, DMN was not carcinogenic when given with a protein-free diet, presumably because of the severe reduction of microsomal activating enzymes in the liver (255). However, under these conditions, rats exhibited tumors in the kidney after a fairly long latent period.

Whatever its role in promoting cancer, malnutrition is very common in cancer patients. The relationship of malnutrition in the pathogenesis of oral cancer in Swedish women is well established (410). It has been shown (180) that head and neck cancer patients had significantly poorer nutrition than do controls. Studies in Iran (183), in Puerto Rico (244), and in Sweden (155) showed a correlation of malnutrition with the development of esophageal cancer in humans. Deficiencies reported were mainly those of iron, zinc, vitamin A, and vitamin B. In a clinical study, Kissin and Kaley (180) determined blood levels of thiamine, riboflavin, and ascorbic acid in patients with head and neck cancer and found all levels to be significantly decreased.

Specific Nutritional Deficiencies in the Alcoholic and Their Impact on Oncogenesis

Vitamin A. Low serum carotene (148) and vitamin A (349) levels have been reported in alcoholics, and hepatic synthesis of retinol-binding protein also was found to be impaired (349). Acute ethanol administration results also in a lowered vitamin A (2). Vitamin A has been implicated in the differentiation of epithelial tissue, and it has been shown that vitamin A deficiencies affect the induction of pulmonary tumors (55, 73, 328), bladder tumors (53), colon tumors (274, 314), and cervical tumors (52). Moreover, a recent epidemiological study in over 8000 men in Norway suggests that relatively low dietary intake of vitamin A is correlated with a relatively high incidence of lung cancer (34). A study in India (384) showed that three-fourths of oropharyngeal cancer patients had subnormal vitamin A levels. Retinoid deficiency enhances the binding of BP metabolites to tracheal epithelial DNA. On the other hand, the absence of vitamin A in the media of cultured rat colon resulted in a lower binding of BP to DNA and protein and in a lower activity of BP hydroxylase (11). This could be explained by the fact that vitamin A deficiency decreases mixed-function oxidase activity at least in the liver (27). However, i.r. administration of *N*-methyl-N'-nitro-*N*-nitrosoguanidine, a primary carcinogen to vitamin A-deficient rats, also produced less tumors than in vitamin A-supplemented rats (273). Thus, the effect of vitamin A on chemical carcinogenesis may depend on the type of carcinogen chosen and on the tissue studied.

Vitamin B_1. The alcoholic is commonly deficient in thiamine, but thiamine deficiency has not been implicated in carcinogenesis and is not known to affect immunocompetency.

Vitamin B_2. Riboflavin deficiency is common among alcoholics especially in the lower socioeconomic groups. Both riboflavin and iron play important roles in the activity of respiratory enzymes, and deficiencies of both produce similar clinical features (glossitis, cheilosis, brittle nails). A Plummer-Vinson-like syndrome was observed among female patients with oral cancer (410). Wynder and Chan (409) found a significant hyperplasia on the skin of riboflavin-deficient mice, and the importance of riboflavin has been demonstrated by the fact that the skin of riboflavin-deficient mice had increased susceptibility to tumor formation. The effect of riboflavin deficiency on the carcinogenicity of azo dye compounds has been mentioned before.

Vitamin B_6. Pyridoxine deficiency occurs in the alcoholic, and acetaldehyde has been incriminated in the accelerated destruction of the vitamin (236). In addition to its key function in hematopoiesis, vitamin B_6 has been shown to play an important role in the production of antibody response to the administration of various antigens (14); this may influence tumor development indirectly. Wynder (403) has related pyridoxine deficiency to enhanced liver tumor formation.

Vitamin E. Abnormally low blood levels of vitamin E in alcoholics have been found by Losowsky and Leonard (234) and Myerson (272). Vitamin E and other antioxidants such as butylated hydroxytoluene, propyl gallate, and ethoxyquin have reduced the tumor induction with certain carcinogens in a number of target organs (128, 374, 388). In some cases, the effect was noted at low levels of those agents, and it can be assumed that this effect depended indeed upon antioxidant properties of the compound.

Vitamin C. Alcoholics have a decreased urinary excretion of ascorbic acid, which may reflect a general deficiency (205).

Folic Acid. It is well established that, in addition to a decreased dietary intake of folate, ethanol itself enhances requirements for folic acid (228). Indeed, chronic ethanol administration prevents the normal hematological response to folic acid (356). Folic acid deficiency has been shown to interfere with humoral and cellular immunity (201). Furthermore, the adverse effect of folic acid deficiency on the hemopoietic system as well as on the gastrointestinal tract is well known. Whether those morphological changes associated with folic acid deficiency alter the effect of chemicals or other carcinogenic agents is not known.

Iron. Alcoholics also may have severe long-standing iron

deficiency, and the role of iron in carcinogenesis may be significant. Iron deficiency without anemia produces profound defects in cell-mediated immunity (382). Chronic prolonged and severe iron deficiency produces gastric atrophia in rats (381). Recent studies from Colombia and from the National Cancer Institute Epidemiological Section indicate that chronic iron deficiency may have a possible role in the etiology of gastric cancer (383). In Colombia, there is a gastric cancer rate 4 times higher than in the United States. Furthermore, chronic iron deficiency and/or chronic riboflavin deficiency have an effect on the development of Plummer-Vinson syndrome (404). Plummer-Vinson syndrome is known to increase the risk of cancer of the upper alimentary tract among women (who are more prone to the syndrome than are men) (199, 410).

Minerals. Magnesium deficiency is common in alcoholic patients, probably because of its increased excretion in urine (105, 162, 355, 397). Magnesium-free diets have been reported to lead to leukemia in certain strains of rats (9). Also, zinc deficiency has been reported in alcoholics (106) and has been associated with a high incidence of esophageal carcinoma in Iran (183). Zinc serves as cofactor for a number of enzymes. Zinc is needed for RNA metabolism and DNA synthesis. It is of interest that zinc has a protective effect on the teratogenicity of cadmium (28). On the other hand, the absorption of cadmium is significantly increased at a low serum iron level, which is common in alcoholics (104). Cadmium serum levels were found to be elevated in patients with bronchial carcinoma (146). The deficiency of another trace element, molybdenum, has been associated with an increased incidence of esophageal cancer in certain parts of the world (373), but its status in the alcoholic is unknown. Rogers and Gawienowsky (313) reported that serum copper was elevated in alcoholics. There is a high incidence of lung cancer among coppersmiths (28); however, the lack of a significant correlation in experimental animals has led to the conclusion that copper cannot be classified as a carcinogen.

Summary and Recommendations

In this paper, some medical complications of alcoholism were described, particularly their interrelationships with carcinogenesis. Emphasis was placed on mechanisms whereby alcohol may contribute to carcinogenesis through (*a*) contact related local effects, (*b*) the induction of microsomal enzymes which activate procarcinogens, (*c*) general mechanisms of tissue injury and regeneration (particularly in the liver), or (*d*) associated nutritional disturbances. Because of limitation of space and/or lack of information, a number of significant questions were neglected. For instance, the potential impact of the alcohol-induced increase in microsomal metabolism and/or the effect of alcohol-related liver injury on chemotherapy and its complications have not been considered. There is an obvious need for additional information in this important area. In addition, more information is required concerning the possible impact of alcohol-induced disease status on carcinogen metabolism. Several important opposing factors may interact: increased activation of secondary carcinogens and deactivation of primary ones due to microsomal induction (particularly in the early phase of alcoholism), as opposed to decreased inactivation of primary carcinogens due to liver damage (particularly in later stages). The relationship between alcohol-induced necrosis in various tissues and carcinogenesis remains to be further documented experimentally, particularly the postulated link between regeneration associated with cirrhosis and hepatocellular carcinoma. The status of the immune system is important in carcinogenesis and appears to be also affected by alcohol, but the available information is still sketchy.

In any event, the recent description of the induction by alcohol consumption of microsomal systems which activate secondary carcinogens in various tissues raises the possibility that this cocarcinogenic action of alcohol contributes to the enhanced incidence of cancer in the alcoholic.

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