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ALCOHOL AND CANCER

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INTRODUCTION

Alcohol abuse has long been recognized as a major risk factor for cancers of the upper alimentary tract and upper respiratory tract (for recent reviews see 1–7). Cancers at several other sites including the large bowel (8–17), breast (8, 10, 18–29), pancreas (30–34), and stomach (8, 35) also have been correlated with alcohol consumption. For some of these sites, however, there are studies that failed to detect an alcohol-related increase in cancer risk, as for example in the large bowel (36), pancreas (37–39), and breast (40–44). The reports of an association between even moderate alcohol consumption (one to two drinks per day) and breast cancer in particular have attracted a great deal of attention. Although there have been unexplained inconsistencies, which in some studies may have been related to such confounding factors as age of first full-term pregnancy, age at menarche, family history of breast cancer, age at which drinking began, and other dietary variables, the majority of the case-control studies and four of the five cohort studies referred to above, as well as a recent meta analysis (45) have found an association between drinking and breast cancer. Given the large numbers of women who drink moderately (46) and the fact that approximately ten percent

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of women in the United States develop breast cancer, the relationship between drinking and breast cancer has important public health implications; recommendations are needed, particularly for women who may be at high risk because of other factors.

Hepatocellular carcinoma also has been associated with alcohol abuse. In contrast to the aforementioned cancers, however, this has been attributed not to alcohol consumption per se but to the ensuing cirrhosis. Nevertheless, there is some evidence that risk of hepatocellular carcinoma is increased in alcoholics even in the absence of cirrhosis (47, 48).

These epidemiological studies have stimulated interest in the mechanisms whereby alcohol consumption increases the risk of developing cancer. The purpose of this review is to discuss the biochemical, molecular, and immunologic mechanisms that have been suggested to explain the epidemiologic observations.

THE POTENTIAL SIGNIFICANCE OF CARCINOGENS IN ALCOHOLIC BEVERAGES

A variety of carcinogens including nitrosamines (49–51), polycyclic hydrocarbons (52) fusel oils (53), asbestos fibers (54–56), and aflatoxins (57) have been found in different types of alcoholic drinks. However, with the possible exception of regions such as Brittany and Normandy in France (58–61), several areas in Africa (62, 63), Puerto Rico (64), and the Southern United States (65), where a particular locally prepared drink is popular and where there is notably high incidence of alcohol-associated cancer, the significance of the low levels of carcinogenic congeners found in alcoholic beverages is still speculative. The regional beverage question has been examined in greatest detail in Brittany and Normandy where the incidence of esophageal cancer is particularly high. Walker et al (51) have demonstrated that the local, widely consumed apple ciders and apple brandies not only contain dimethlynitrosamine (DMN), which is also found in a variety of beers, wines, and liquors, but also contain relatively high concentrations of diethlynitrosamine (DEN). The presence of DEN could be significant because it has been shown to be an esophageal carcinogen in rodents. The possibility that these carcinogens are of some significance is at least partially supported by the epidemiological studies conducted by Tuyns et al (1, 58) in the Department of Calvados, Normandy. They found that whereas the consumption of any type of alcoholic beverage entailed an increased risk of esophageal cancer, the risk associated with apple cider and, in particular, with brandy was greater than that for other types of drinks.

If the carcinogens present in alcoholic beverages or ethanol itself contribute to the increased incidence of the types of cancers seen in heavy alcohol users,

one might expect to see an effect of drinking that is independent of other factors known to increase cancer risk, such as smoking. Such effects have been reported by Tuyns et al (66) for esophageal cancer in Normandy and by Rothman & Keller (67), Wynder & Stellman (68), Elwood et al (69), and Blot et al (70), for cancers of the mouth and pharynx in the United States. In all these studies, however, there also was a marked increase in cancer incidence at the same sites when tobacco use was added to alcohol consumption. With respect to sites other than the upper alimentary and upper respiratory tracts, Breslow & Enstrom (8), in a study that correlated per capita consumption of spirits, wine, beer, and cigarettes with geographic cancer mortality rates in the United States, reported an association between cancers of the stomach, large bowel, breast and thyroid with beer consumption. The strongest single association found in this study was between rectal cancer and beer consumption. Such an association has also been noted in several additional studies (11, 13–17, 71). Williams & Horm (10), using interview data compiled in the Third National Cancer Survey (TNCS), also noted an alcohol-associated increase in cancers of the stomach, colon, breast, and thyroid. In contrast to some of these studies, the TNCS data did not indicate that the association with colon cancer was limited to beer but was seen with wine and hard liquor as well. There has not been a consistent association between large bowel cancer and beer consumption and this is particularly true for studies conducted in different countries (8, 11, 36, 72–74). This inconsistency may be due either to the association between beer and bowel cancer being spurious, i.e. related to other factors (12, 75, 76) or perhaps to variations in carcinogens or carcinogen concentrations present in the local beers. This point can be illustrated by the different results obtained in similar studies of brewery workers conducted in Denmark (36) and Ireland (11). Both these cohorts regularly consume relatively large amounts of beer; the Danish workers (a group which does not exhibit an increased risk of large bowel cancer) consume a light pilsner beer whereas the Irish workers (a group which does exhibit an excess of bowel cancer) consume a dark beer. Although no specific information is available on the carcinogen content of the beers involved in the actual studies, it is of interest that in general Danish beers appear to have a significantly lower DMN content that Irish beers (51). The relevance of these differences is still open to question, however, because there is no indication from animal studies that DMN can act as a bowel carcinogen. Nevertheless, the effects of chronic low doses of DMN in man are not known, especially under the conditions of multifactoral carcinogen exposure that exists outside the laboratory.

In summary, the results of some studies are consistent with the possibility that carcinogens, present in some alcoholic drinks, may contribute to alcohol-associated cancers. However, in upper alimentary tract (UAT) and upper

respiratory tract (URT) cancers, which are seen primarily with joint use of alcohol and tobacco, the role of alcohol-associated carcinogens may be significant only in some specific locales.

The carcinogens present in alcoholic beverages may play a role in cancer induction for other sites where an alcohol-associated increased incidence of cancer has been reported independent of tobacco use. Yet even in these cases, it could be argued that the increased cancer incidences were due not to alcohol-associated carcinogens but rather to other consequences of high alcohol consumption.

ETHANOL AS A COCARCINOGEN

Epidemiologically, the earliest and most consistently recognized effect of alcohol consumption on cancer risk has been the increased incidence of upper alimentary and upper respiratory tract cancers produced by the combination of alcohol and tobacco use (67, 77–90). In a recent case-control study for example, the increase in oropharyngeal cancer risk, expressed as odds ratios for the combination of these two factors, was approximately 38 times background; heavy drinking in the absence of smoking yielded an odds ratio of 5.8 and heavy smoking without drinking resulted in a 7.4-fold increased risk (70). Some studies have indicated a differential risk for upper alimentary and upper respiratory tract cancers that depend on the type of alcoholic drink consumed, being greater for spirits relative to beer and wine (64, 91–93), whereas others related risk to the total amount of ethanol consumed rather than to any particular type of beverage (1, 10, 36). Even though drinking per se produces an increased risk of cancer in the upper alimentary and upper respiratory tract, the synergistic interaction seen between drinking and smoking has led to the hypothesis that the principal effect of ethanol on the carcinogenic process is that of a cocarcinogen. This hypothesis is supported by the animal studies described below.

Although there is no experimental evidence that ethanol per se is carcinogenic, numerous studies conducted over the last 25 years have shown that ethanol is capable of acting as a cocarcinogen at several different body sites with a variety of chemical carcinogens.

Because of the relationship between smoking and drinking in cancer, many of the early investigations employed polycyclic aromatic hydrocarbons, which are common constitutents of tobacco smoke, as the inducing carcinogens. In one of the first such studies, Protzel et al (94) found that rats fed ethanol in their drinking water exhibited a decreased latent period and increased frequency of buccal tumors induced by topically applied benzo(a)pyrene (BP). In a similar study, ethanol (as a 50% solution) painted over areas that had been pretreated with dimethylbenzanthracene (DMBA)

was reported to act as a promoter of DMBA-induced neoplastic transformation in hamster cheek pouches (95, 96). Although ethanol did not affect tumor latency or incidence in these experiments, it did increase the frequency of parabasilar budding and dyskeratoses in exposed animals. Ethanol, used as a solvent, also was reported to act as a cocarcinogen for DMBA in a mouse-skin painting study (97) and in a similar application was reported to enhance the induction of esophageal tumors in mice (98). Intraperitoneal injection of ethanol (1.5mg/kg) for 7 days prior to injection of BP also was reported to increase the frequency of muscle tumors in mice (99).

In more recent studies of tobacco-associated carcinogens, McCoy et al (100, 101) reported that ethanol administered either as part of an isocaloric pair-feeding regimen or in drinking water increased the incidence of nasal and tracheal tumors induced in hamsters by i.p.-administered N-nitrosopyrrolidine (NPY), but had no effect on tumor induction by N'-nitrosonornicotine (NNN). Similarly, Griciute et al (102) did not observe an effect of ethanol on the incidence of NNN-induced tumors when the NNN was administered as an alcoholic solution, but did observe a decreased latent period for tumor induction in the alcohol group. On the other hand, ethanol consumption was shown to enhance the frequency of NNN-induced nasal tumors in rats, but this effect was seen only in rats that were fed an ethanol-containing diet four weeks prior to and during the NNN exposure (103). Isocaloric pair-feeding of ethanol also has been shown to promote the progression of esophageal tumors initiated by N-nitrosomethylbenzylamine (MBN) in rats (104). Interestingly, in this latter study feeding ethanol either prior to or during carcinogen exposure decreased the incidence of esophageal lesions and tumors. In contrast, prefeeding ethanol (4% in drinking water) in combination with a zinc-deficient diet enhanced the induction of MBN-induced esophageal tumors (105, 106). Dietary ethanol administered as a 5% solution in drinking water also has been reported to promote the development of esophageal tumors induced in rats by N-methyl-N-amylnitrosamine (107).

Conflicting results have been obtained with the use of DMN and DEN in combination with ethanol. Simultaneous administration of ethanol and DMN increased the number of esophageal papillomas and epidermoid carcinomas in rats (108) and brain tumors in mice (109), but chronic feeding of ethanol had no observable effect on DMN hepatic tumor induction (110). Similarly, the reported effects of ethanol on DEN-induced hepatocarcinogenesis have ranged from enhancement (111), to no effect (112), to a protective effect (113). Finally, Radike et al (114, 115) reported that the tumorigenic effect of another hepatocarcinogen, vinyl chloride, was enhanced in rats by the administration of ethanol in drinking water.

Experimentally, ethanol also has been reported to enhance tumor induction at two other sites with which it has been associated as a risk factor in

epidemiological studies, namely, the large bowel (for a recent review see 116) and breast. Seitz et al (117) showed that prefeeding an ethanol-containing liquid diet (36% total caloric intake) enhanced the induction of rectal tumors in rats by 1,2-dimethylhydrazine. This effect was also seen with the direct-acting carcinogen azoxymethyl-methynitrosamine (AMMN) (118). Conflicting results have been seen with the experimental induction, by azoxymethane (AOM), of colonic cancer in rats. Prefeeding either a low-alcohol beer (12% of caloric intake) or a low dose of ethanol (9% of calories) for 3 weeks prior to AOM-administration increased the incidence and proportion of tumors in the left but not the right colon, whereas high-alcohol beer and a high-dose ethanol diet, 23% and 18% of total calories respectively, reduced tumors in the right colon and had no effect in the left (119). Similar inhibiting effects of ethanol prefeeding on AOM-induced colonic cancer have been observed in studies over a range of ethanol and AOM concentrations (120, 121).

With respect to ethanol's effect on breast cancer, two experimental models have shown an enhancement of tumors with ethanol feeding. Ethanol administered as a 12% solution in drinking water, commencing immediately after weaning, significantly reduced the latent period in the genesis of spontaneous mammary adenocarcinoma in C3H/St mice (122). Of particular interest in this latter study was the finding that serum prolactin levels were actually lower in the alcohol-fed mice relative to the controls, as this observation is incompatible with the hypothesis that ethanol promotes breast cancer by stimulating prolactin secretion (123). Ethanol administered by gavage for 3–5 weeks prior to carcinogen administration also was reported to enhance the initiation of mammary cancers induced either with DMBA or methylnitrosourea (124).

In summary, most animal data indicate that ethanol consumption can indeed enhance the carcinogenic activity of a broad spectrum of organ-specific carcinogens in several animal species. This cocarcinogenic effect may be influenced, however, by the dose and timing of the ethanol exposure.

BIOCHEMICAL BASES FOR ETHANOL'S ACTIVITY AS A COCARCINOGEN

In the context of a multistage theory of chemical carcinogenesis, initiation generally refers to the DNA-damaging effects of chemicals that either are direct-acting electrophiles, capable of reacting with nucleophilic centers on DNA, or are converted to electrophilic derivatives during the course of their metabolism (125, 126). The principal enzyme system involved in this metabolism is the microsomal cytochrome P-450-dependent mixed function oxidases. The inductive effect of ethanol on this enzyme system has focused attention on ethanol's capacity to influence carcinogenesis through its effects on P-450-mediated carcinogen and retinoid metabolism. Post initiation, pro-

motion, and progression stages of carcinogenesis have been identified through the effects of carcinogens that are neither DNA reactive nor give rise to DNA-reactive metabolites and that exert their effects after initiation has been completed. Examples of such compounds include the phorbol esters, which promote the development of skin tumors at sites that had previously been initiated with polycyclic hydrocarbons such as BP (for reviews see 127–129), and phenobarbital, which promotes hepatic tumor development in animals previously treated with hepatocarcinogens (130, 131).

In most of the animal studies reviewed above, no attempt was made to determine whether ethanol's cocarcinogenic activity was related to effects on initiation, promotion, or progression. Furthermore, in a number of cases that considered the phase at which ethanol was acting, conflicting results have been reported, particularly on ethanol's capacity to influence the post-initiation phase of carcinogenesis. For example, Stenback (97) indicated that ethanol did not act as a promoter when applied to mouse skin that had been pretreated with 9,10-DMBA, whereas Elzay (95, 96) reported that a topically applied 50% solution of ethanol promoted carcinogenesis by 7,12-DMBA, which had been painted on hamster cheek pouches. More recently, Mufti et al (104) reported that ethanol, administered in the diet as part of a pair-feeding study, affected the promotion but not the initiation of esophageal tumors initiated in rats by MBN.

The mechanisms whereby ethanol may act directly as a cocarcinogen in either the initiation or promotion phase of chemical carcinogenesis include: (*a*) cytotoxic and mitogenic effects of ethanol and its metabolites; (*b*) the induction of microsomal enzymes that in turn affect carcinogen and retinoid metabolism and lipid peroxidation; (*c*) diminished capacity to detoxify electrophiles; (*d*) inhibition of repair of carcinogen-DNA adducts and (*e*) suppression of immune responses. Indirect consequences of alcohol abuse include increased risk of hepatitis B virus infection and specific dietary deficiencies.

Cytotoxic and Mitogenic Effects

Replicating DNA, because of its partially single-stranded nature, is more reactive with many chemical carcinogens than resting DNA (132). Repeated cell injury and repair in the presence of carcinogens would be expected, therefore, to sensitize tissues to chemical carcinogens. Liver cancer, for example, is chemically induced more readily when carcinogen exposure is superimposed on a regenerating liver (133). In support of the idea that some direct effect of ethanol on the tissues it contacts plays a significant role in alcohol-associated cancers, Williams & Horm (10) noted that the Third National Cancer Survey data indicated a gradient of decreasing risk that paralleled the successive dilution of alcohol in the alimentary tract and portal circulation: highest in the oral cavity; lower in the larynx, esophagus, and

liver; and lowest in the stomach, pancreas, and rectum. Local effects of drinking on the alimentary tract have been demonstrated in numerous studies. In an oral cytology survey, Anderson (134) found that diskaryotic cells occurred with higher frequency in heavy drinkers relative to other patients, and Winship et al (135) noted the occurrence of alcohol-associated functional abnormalities in the esophagus, which may represent either direct myopathic or neuropathic effects of ethanol. There is, however, a lack of convincing experimental evidence to support the assumption that ethanol facilitates carcinogen penetration in the upper alimentary tract by affecting the permeability of the mucosal barrier. Fromm & Robertson (136), for example, did not find evidence for significant changes in mucosal permeability following ethanol. Also, the epidemiological association of upper alimentary- and upper respiratory-tract cancers with the consumption of weak alcoholic beverages such as beer does not support the idea that the cocarcinogenic effect of ethanol is due to its capacity to damage the esophageal mucosa. There are reports that ethanol does alter gastric permeability and active transport of various ions in the stomach (137–139), but even here the deleterious effects of ethanol may be mediated only in part by a change in mucosal permeability (136, 140, 141).

Even though acute ethanol administration has been associated with gastritis and this effect has been confirmed in binge drinkers (142), the incidence of chronic gastritis in heavy drinkers is a more controversial issue. Whereas some investigators have suggested a connection between this condition and alcohol abuse (139, 143–145), other studies failed to detect a relationship between alcohol intake and histological evidence of atrophic gastritis (146, 147). The reason for this inconsistency is not known but may be due, at least in part, to patient selection (nutritional status, definitions of alcoholism), biopsy techniques (blind vs directed), and length of abstinence before examination. A possible increase in the incidence of atrophic gastritis in heavy alcohol users would be of particular interest because this lesion appears to be a precursor of human gastric carcinoma.

In the liver, large numbers of autopsy studies have shown that the occurrence of hepatoma is closely associated with cirrhosis, which is generally related to alcohol consumption. The incidence of cirrhosis in patients with hepatoma varies from 60 to 90% in different studies (48, 148, 149). Furthermore, a few studies have indicated that in alcoholics hepatomas may occur even in the absence of cirrhosis (47, 48). This may indicate that alcohol-induced hepatic cell injury below the level of identifiable cirrhosis could act as a predisposing condition to carcinogenesis.

Ethanol also may stimulate cell proliferation in the absence of any marked antecedent cytotoxic effect. Chronic ethanol consumption has been reported to stimulate rectal cell proliferation in the rat (116, 150), possibly as a

consequence of acetaldehyde exposure (117). The ethanol-associated increase in dimethylhydrazine-induced cancer in this species may be related to this cell-proliferative effect (117). This proposal is further supported by the observation that chronic ethanol consumption is also cocarcinogenic in the rectum with the direct-acting carcinogen AMMN (118). Ethanol also has been shown to be mitogenic for esophageal epithelium (151–153) and potentiates tracheal squamous metaplasia caused by vitamin A deficiency in rats (154).

Microsomal Enzyme Induction and Carcinogen Metabolism

The association of alcohol consumption with cancers at sites that do not come into contact with high concentrations of alcohol suggests that mechanisms other than, or in addition to, the direct cytotoxic effects of ethanol play a role in carcinogenesis. One possible explanation for ethanol's ability to act as a cocarcinogen at remote sites as well as at ethanol-contact sites lies in ethanol's capacity to act as an inducer of the microsomal cytochrome P-450-dependent biotransformation system (155–158). It is well known that this enzyme system is involved in the metabolic conversion of many structurally diverse chemical carcinogens to highly reactive electrophilic intermediates capable of reacting with critical macromolecules, including nucleic acids and proteins (125, 126, 159). Furthermore, an association has been suggested between the levels and distributions of various types of P-450 isozymes and susceptibility to some cancers (160–165). Work conducted in this laboratory and by others has shown that dietary ethanol does indeed result in the induction of carcinogen-activating enzymes not only in the liver, the major site of xenobiotic metabolism, but also in a number of other tissues in which alcohol-associated cancers are observed. These tissues include the lungs and intestines, which are major portals of entry for tobacco smoke and dietary carcinogens, and the esophagus (for a recent review see 166). Induction of P-450 in the esophagus may be particularly relevant to carcinogenesis at this site because of the low concentrations of other detoxifying enzyme systems in this tissue (167).

The general approach used in these studies has been to isolate microsomal preparations from tissues of alcohol-fed and control-diet animals, generally rats, hamsters, or mice, and then assay these preparations for their ability to metabolically convert procarcinogens either to mutagens detectable in the Ames Salmonella mutagenesis assay (168, 169), or to other detectable end products.

Enhanced microsomal conversion of many structurally diverse carcinogens has been observed after an inductive pretreatment with ethanol. The carcinogens used in these studies have included compounds and mixtures found in tobacco smoke such as BP, NPY, NNN, and tobacco pyrolyzate (48, 170–174), models of dietary carcinogens such as DMN and tryptophan pyrolyzate

(109, 173, 175, 176), and other hepatotoxins and carcinogens such as carbon tetrachloride, 2-aminofluorene, 2-acetylaminofluorene, benzene, 4-aminobiphenyl, benzidene, and methylazoxymethanol (48, 175, 177–181). In some instances these inductive effects have exhibited tissue, substrate, gender, and species specificities. For example, in the intestine, ethanol increased microsomal activation of BP and tryptophan pyrolyzate but not tobacco pyrolyzate, whereas lung microsomes from ethanol-fed rats exhibited an enhanced capacity to activate the promutagens in tobacco pyrolyzate but did not exhibit any increased activity toward BP or tryptophan pyrolyzate (173). Although the mutagen or mutagens being activated in the tobacco pyrolyzate are not known, it is of interest that lung microsomes from alcohol-fed rats also exhibit an enhanced capacity to activate the tobacco mutagen NPY (174). Ioannides & Steele (180) also reported both inductive and inhibitory effects toward different PAHs; Seitz et al (182) demonstrated a gender-specific effect on induction of BP metabolism in the rat; and Anderson et al (183) reported that in contrast to rats, where ethanol induces DMN demethylase activity, no induction is seen in mice.

Ethanol's ability to induce DMN demethylase activity is of particular interest as it is detectable over a DMN concentration range of 0.3–100mM (176). This is in contrast to other microsomal enzyme inducers such as phenobarbital, 3-methylcholanthrene and polychlorinated biphenyls, which increase the activity of DMN demethylase isozymes whose activity is detectable only at relatively high DMN concentration (>40mM) and repress the activity of low Km DMN demethylases (184–195). This effect of ethanol appears to be due to the induction of a unique species of cytochrome P-450 (196, 197) that differentially affects the activation of various carcinogens; a selective affinity for DMN has indeed been demonstrated with the ethanol-induced cytochrome P-450 (198). Ethanol is also an effective competitive inhibitor of DMN demethylase activity (166, 183, 199–202). This capacity to act both as an inducer and inhibitor may explain the conflicting reports of ethanol's influence on DMN-mediated carcinogenicity, particularly when the route of exposure and the presence or absence of ethanol at the time of exposure are taken into account. As Swann et al pointed out (202), when DMN is administered orally the liver can effect a "first-pass clearance" up to a DMN dose of 30 μg/kg. At higher doses the hepatic enzymes become saturated and methylation of the kidneys and other organs occurs. Ethanol when given to rats in relatively low amounts, equivalent to a person drinking 0.5 liter of beer, prevents this "first-pass clearance" and can produce a fivefold increase in the methylation of kidney DNA.

In summary, ethanol consumption increases the capacity for microsomal activation of many classes of chemical carcinogens in different tissues. The significance of this effect of ethanol vis à vis actual cancer risk will be

influenced by other factors operating in vivo including: the carcinogen-detoxifying capacity of various tissues; the route of carcinogen exposure and, in the alcohol abuser in particular, the presence of ethanol in the circulation at the time of carcinogen exposure.

Effects on Retinol (Vitamin A) Metabolism

Ethanol consumption results in a severe depression in hepatic vitamin A levels through at least two mechanisms: (*a*) it increases mobilization of vitamin A from the liver to other organs (203, 204); (*b*) it induces a cytochrome P-450-mediated breakdown of both retinol and retinoic acid (205, 206). These effects of ethanol may be of importance in carcinogenesis as vitamin A plays an essential role in the maintenance of normal growth and control of cell differentiation in a variety of epithelial and mesenchymal tissues (see 207–209 for reviews). In addition, there is both epidemiological and experimental evidence that vitamin A has anticarcinogenic properties affecting both the initiation and promotion stages of carcinogenesis (for a recent review see 210).

Dietary caretenoids and retinyl esters are the major sources of vitamin A, which is stored in the liver in the form of retinyl esters (211). In animals, retinoic acid is just as effective as retinal as a dietary supplement (212) and retinoic acid is more effective than either retinol or retinal as an anti-carcinogen or inducer of cellular differentiation in vitro (213–215). Epidemiological studies involving different geographic locales have associated dietary-retinoid deficiency and low-serum vitamin A levels with increased cancer risk, particularly of the esophagus and lung (216–221). These epidemiologic studies have been supported by animal studies that have demonstrated the efficacy of retinoids in prevention of cancers at different body sites (222–225).

Ethanol consumption has been observed to interact synergistically with vitamin A deficiency in increasing the incidence of tracheal squamous metaplasia in the rat (152, 154). This is of particular interest for upper alimentary and upper respiratory tract cancers in that squamous metaplasia is one of the earliest stages preceding the development of carcinoma in situ, with the latter often found in association with invasive carcinomas (226–230). In addition, in the same ethanol-consuming, vitamin A-deficient rats, the tracheal epithelium, which was not as yet involved in the formation of squamous metaplasia, exhibited a number of morphologic abnormalities. The ciliated cells contained an increased number of lysosomes and had compound cilia (152, 154). Increased numbers of lysosomes also have been observed on ciliated tracheal cells following exposure to carcinogens (231) and compound cilia have been observed an animals exposed to carcinogens and in humans with bronchial cancer (232, 233). More recently, a study of the effects of vitamin A

deficiency and ethanol on esophageal mucosa showed that in contrast to the trachea, vitamin A deficiency altered cellular differentiation but this alteration was not influenced by ethanol and that ethanol, independently of the vitamin A deficiency, stimulated basal cell proliferation (153).

In light of ethanol's effect on vitamin A metabolism, it may prove useful in formulating hypotheses for the cocarcinogenic effect of ethanol in the upper alimentary and respiratory tracts to take into account what is known of the mechanisms of action of vitamin A on differentiation and carcinogenesis. On a molecular level, retinoids appear to control the expression of genes that are involved in the cytoskeleton matrix as well as some oncogenes (207). Retinoids may also influence carcinogen metabolism through the induction of specific P-450 isozymes (234–236) and by directly interfering with the P-450-mediated activation of procarcinogens (237). Finally, retinoids also may inhibit tumor development by stimulating various aspects of cell-mediated immune responses (238–244).

Effect on Lipid Peroxidation

Lipid peroxidation has been implicated in promoting the carcinogenic process (245, 246). This suggestion is based in large part on the antagonistic effect of dietary polyunsaturated fats and dietary antitoxidants in carcinogenesis. In general, dietary fats enhance tumorigenesis (247) whereas antioxidants inhibit the process (245, 248, 249). Although there are a number of explanations for the effects of dietary fat on carcinogenesis, including a nonspecific caloric effect, an increase in the levels of membrane peroxidation, which is inhibited by antioxidants, is another possibility (250).

Experimentally, microsomes from ethanol-fed rats have been shown to generate reactive oxygen intermediates such as superoxide, peroxide, and hydroxyl radicals at elevated rates compared with controls (251–255). This is associated with increased lipid peroxidation in ethanol-fed animals (256–259). Furthermore, there is evidence for ethanol-associated lipid peroxidation in man (260, 261).

Diminished Capacity to Detoxify Electrophiles

Glutathione (GSH) plays a key role in the detoxification of electrophiles and in the reduction of lipid peroxides. Acute ethanol consumption has been reported to produce a marked decrease in hepatic GSH levels (262–265). This effect of ethanol could therefore contribute both to an increase in the number of carcinogen-DNA adducts produced as a result of electrophile production from carcinogens and to increased levels of lipid peroxidation. Several mechanisms contribute to the decreased hepatic GSH levels, with the most significant apparently being an increased efflux of GSH from hepatocytes (266). Other contributing factors include the reaction of acetaldehyde both with GSH

itself (266, 267) and with cysteine, a GSH precursor (268). There also is a decrease in hepatic synthesis of GSH following acute ethanol treatment (266). In contrast, chronic ethanol consumption produces a transient increase in hepatic GSH (269) and does not affect esophageal GSH levels in the rat (167).

Inhibition of DNA-Alkylation Repair

DNA-repair processes are important in protecting cells from chemical carcinogens that alter DNA structure and sequences. Such alterations result either in somatic mutations or the expression of oncogenes and ultimately lead to the uncontrolled cellular growth characteristic of tumors. Cells possess a number of enzyme systems capable of repairing different types of DNA damage and patients born with DNA-repair deficiencies are at greater risk of developing cancer (270, 271). Chronic alcohol consumption may increase cancer risk by inhibition of the DNA-repair enzyme, O^6-methylguanine transferase (O^6-MeGT), which removes alkyl groups (methyl and ethyl) from the O^6 position of guanine (272–274). In rats, chronic and acute alcohol consumption causes an increased persistence of DMN-induced hepatic O^6-MeG DNA adducts and acetaldehyde has been shown to inhibit both rat and human O^6-MeGT enzyme activity (275–277).

The major-DNA-base alkylation products generated by exposure to alkylating nitroso compounds such as DMN, in order of frequency of occurrence, are N^7-MeG, O^6-MeG, and O^4-MeT (278, 279). Persistence of O^6-MeG in DNA of various organs has been associated with carcinogenicity of several alkylating agents (280–282) and alkylation of the O^4 position of thymine may also be significant (283, 284). O^6-MeG is an alkyl transferase which transfers methyl or ethyl groups from the O^6 position of guanine to a cysteine residue located in the enzyme that in turn inactivates the transferase (285–287).

The first indication that chronic alcohol consumption interfered with the repair of O^6-MeG adducts came from experiments designed to examine the effects of ethanol consumption on DMN-induced hepatic DNA alkylation. In these experiments it was observed that O^6-MeG, but not N^7-MeG, adducts persisted for longer periods in ethanol-fed rats relative to controls (275, 277). Moreover, this effect appeared to be specific for O^6-MeG repair as removal of acetylaminofluorene adducts, which are repaired by a separate excision pathway (288), was not affected (275). Isolation of O^6-MeGT from ethanol-fed and control-diet animals showed a loss of hepatic O^6-MeGT activity following ethanol consumption. This in vivo decrease in O^6-MeGT activity appears to be due primarily to acetaldehyde generated by ethanol metabolism. Pretreatment of animals with disulfiram (antabuse), which inhibits acetaldehyde dehydrogenase activity and leads to higher and more prolonged levels of acetaldehyde following ethanol administration, exacerbated the loss of O^6-MeGT activity following ethanol administration (276). Furthermore, both rat

and human O^6-MeGT were shown to be significantly inhibited in vitro by acetaldehyde at concentrations as low as 0.1μM. Ethanol also was observed to inhibit O^6-MeGT in vitro but at concentrations in the range of 10–50mM; this inhibition appeared to be due to trace levels of acetaldehyde that were generated spontaneously or produced by residual alcohol dehydrogenase activity in the O^6-MeGT preparations (276). However, some studies have failed to detect an effect of ethanol on the repair of DMN-induced O^6-MeG adducts (289, 290) or an inhibition of O^6-MeGT activity by acetaldehyde at concentrations up to 300 μM (291, 292). The reasons for these conflicting results are not known at present but may be due to methodological differences in the various studies (275, 276).

Induction of Sister Chromatid Exchange (SCE)

Acetaldehyde, the first metabolite of ethanol, induces SCEs in cells grown in tissue culture (293–297). Daily treatment of Chinese hamster cell cultures with concentrations of acetaldehyde ranging from 0.25×10^{-3} to 1.5×10^{-3}% v/v produced a dose-dependent increase in SCEs (297). Acetaldehyde also has been shown to induce SCEs in human lymphocytes exposed in vitro (298, 299). Along these sames lines, Obe & Ristow (300), from a comparison between their own data on chromosomal aberrations in peripheral blood lymphocytes from alcoholics and literature values, concluded that there is an elevation of chromosomal aberrations in alcoholics. The potential significance of these observations with respect to tumor promotion is related to the hypothesis that compounds with SCE-inducing activity could theoretically act as promoters (301). By increasing the frequency of SCEs such compounds could enhance the possibility that recessive mutations are expressed. In addition, stimulation of chromosome damage and rearrangement could foster the expression of latent oncogenes. Acetaldehyde has been shown to enhance the tumorigenicity of BP in hamster lung (302) and itself induces laryngeal tumors in hamsters and nasal tumors in rats (303, 304). Consistent with these observations, acetaldehyde, in the presence of ethanol, has been shown to form mixed acetal-nucleoside DNA adducts (305) and to be mutagenic in a number of test systems (see 306 for a recent review).

Immunosuppression

Many tumor cells display novel surface antigens that, theoretically, should lead to the recognition and elimination of these cells by the immune system. As there is both epidemiological and experimental evidence linking alcohol abuse and ethanol or its metabolites to the suppression of immune responses (see 7 for a recent review), alcohol-associated immunosuppression has been considered for some time as a possible contributing factor in the increased cancer incidence seen in alcohol abusers (307). For the most part, however,

the epidemiological studies in which decreased immune responses have been associated with alcohol abuse have involved patients who already had alcoholic liver disease (308–314). It is difficult to assess, therefore, whether the immunological defects observed were due directly to ethanol or reflected other aspects of the disease process including malnutrition, which is known to affect immune responses (315). Nevertheless, animal and in vitro studies involving isolated immuno-competent cells have shown that both acute and chronic exposure either to ethanol or some of its metabolites impair cell-mediated immune functions in the absence of marked liver dysfunctions. For example, Roselle & Mendenhall (316) reported a significant decrease in lymphocyte response to mitogens after chronic ethanol treatment in guinea pigs. Jerrells et al (317) demonstrated that acute ethanol administration in rats resulted in a rapid loss of lymphocytes from spleen and thymus. Mufti et al (318) showed a similar depletion of splenic lymphocytes following chronic ethanol consumption in rats and a change in T-helper to T-suppressor cell ratios. In contrast to these negative effects, the activities of NK and K cells, which are believed to play a role in defense against tumors, appear to increase following ethanol in both humans and rodents (319, 320). In vitro studies of NK activity were consistent with the in vivo observations showing a moderate increase at ethanol concentrations up to 0.2% followed by a decline in activity above 2% ethanol (318).

Even though there are clear immunological defects associated with alcohol abuse, of which some may be the direct consequences of pathologic effects of ethanol or its metabolites, there is reason to question the significance of these effects regarding general chemical carcinogenesis. Although immunosuppressed patients or animals do exhibit increased cancer incidences, the cancers observed are mostly lymphoreticular neoplasms, i.e. cancers of the immune system itself (321, 322). Furthermore, nude mice, which are genetically defective in T-cell-mediated immune responses, do not exhibit an increased incidence of spontaneous tumors in organs other than those of the immune system, nor are they more susceptible than normal mice to chemically induced cancers (323, 324). Nevertheless, the immune system may play a vital role in the defense against virally induced tumors, particularly in hepatitis B virus-associated hepatocellular carcinoma.

HEPATITIS B VIRUS (HBV) AND HEPATOCELLULAR CARCINOMA

HBV infection is associated with increased risk of hepatocellular carcinoma (325–329) and alcoholics have an increased incidence of HBV infection (330–334). HBV DNA is capable of integrating into host genomic DNA, particularly in chronic HBV carriers (335, 336), and as such may induce

chromosomal alterations important in hepatocarcinogenesis (337). In a study of patients with various stages of alcohol liver disease and alcohol liver disease with hepatocellular carcinoma, Brechot et al (338) found that whereas 8 out of 51 subjects with alcohol liver disease without carcinoma had integrated HBV DNA in their livers, all 20 out of 20 subjects with cirrhosis and hepatocellular carcinoma had integrated HBV DNA sequences. Further support for the interaction between alcohol, HBV infection and hepatocellular carcinogenesis has been provided by Ohnishi et al (339), who noted that hepatocarcinogenesis was hastened significantly in HBsAg carriers who continued to drink.

DIETARY DEFICIENCIES AND ALCOHOL ABUSE

In addition to the direct effects of alcohol consumption on vitamin A metabolism, which, as discussed earlier, may influence both the initiation and promotion of chemically induced cancers, other alcohol-associated dietary deficiencies may also contribute to cancer risk. In cases of chronic alcohol abuse, ethanol may account on the average for as much as 50% of an individual's daily caloric intake. For example, a case control study among black males in Washington, D.C. noted that poor nutritional status was an important risk factor in alcohol-associated esophageal cancer (340, 341).

Iron and Zinc Deficiencies

Chronic iron deficiency, which is seen in alcohol abusers, also has been associated with an increased risk of upper alimentary tract cancer both in women with Plummer Vinson syndrome (78) and in inhabitants of Central Asia (216). Chronic iron deficiency also may play a role in the etiology of gastric cancer (307) and may influence cell-mediated immune responses (342, 343). Furthermore, alcoholics have hyperzincuria and reduced zinc levels (344, 345) and experimentally zinc-deficient diets have been shown to enhance esophageal tumor induction in rats treated with MBN (105).

Riboflavin (Vitamin B2)

Riboflavin deficiency is common among alcoholics, especially in lower socioeconomic groups, and has also been implicated in the Plummer-Vinson syndrome (346). Experimentally, Wynder & Chan (347) reported that in mice, riboflavin deficiency is associated with epithelial hyperplasia and increased susceptibility of skin to chemically induced cancer.

Pyridoxine (Vitamin B6)

Pyridoxine deficiency also has been associated with alcohol abuse and appears to be related to the acetaldehyde derived from ethanol metabolism

(348, 349). The decreased hepatic transaminase activity that is associated with alcohol abuse (350) is apparently due to acetaldehyde's effect on pyridoxine (351), which acts as a cofactor for these enzymes. Pyridoxine plays a key role in hematopoiesis and in both cell-mediated and humoral immune responses (343, 352), which ultimately may affect viruses such as HBV. Wynder (353) has further suggested that pyrodoxine deficiency is associated with enhanced hepatocarcinogenesis.

Vitamin E

Alcoholics have been reported to have abnormally low blood levels of vitamin E (354, 355) and in at least one experimental system, vitamin E has been shown to interact synergistically with another antioxidant, selenium, in preventing mammary carcinogenesis in rats (356).

Lipotrope Deficiency

Chronic ethanol consumption in rats increases requirements for lipotropic factors such as choline and methionine (357, 358). Lipotrope-deficient diets also have been shown to enhance the hepatocarcinogenicity of chemical carcinogens in rats (250, 359) and ethanol further enhances this effect of a lipotropic diet (111). The relevance of these observations to humans is questionable, however, because the hepatic injury induced by lipotrope deficiency appears to be primarily a disease of rats. (See 166 for a discussion of this topic.)

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