

THE CARCINOGENIC EFFECT OF  
ETHANOL CONSUMPTION

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ETHANOL CONSUMPTION

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## CHAPTER I

### INTRODUCTION

At one time, man realized that when fruits and other plant materials were well mixed with water and allowed to stand, a unique product was formed. This substance became known as alcohol or, more properly, ethyl alcohol. The oldest alcoholic drinks were fermented beverages of relatively low alcohol content such as beers and wines. In about 800 A.D., an Arabian known as Jabir Iban Hayyan succeeded in developing the technique of distillation. The word alcohol is derived from the Arabic term, al-kohl, meaning "finely divided spirit," since the vapors of fermented products dissipated in an invisible form (Sardesai, 1969).

Alcohol (or alcoholic beverages) has been used in social, religious, and symbolic events. In its various forms, alcohol has been a part of the medical profession's armamentation since the Middle Ages. It was considered to be a remedy for practically all diseases, as evidenced by the word "whiskey" which in Gaelic usquebaugh means "water of life" (Sardesai, 1969).

Alcohol is prevalent in societies across the world. Recent statistics indicate that in per capita consumption



of alcoholic beverages, the United States ranks seventeenth among the world's nations. Two of three adult Americans (21 years of age and over) drink at least occasionally, one in eight drinks to excess, and one in sixteen drinks enough to be classified as a problem drinker (Sardesai, 1969).

Thus, alcohol's popularity and its long usage by man, makes it particularly important to discuss its relationship to human health. The health hazards involved for individuals were early defined and recognized, although the role played by alcohol in cancer is not nearly as well studied and publicized as the role of tobacco. Alcohol is mainly known for its effects on the central nervous system and liver, rather than for the role it plays in human cancer.

In animal experiments, the acute toxic effects of ethanol are predominant, and no investigator has yet been able to produce cancer in animals with ethanol alone. Recent reviews of both experimental and epidemiological data, however, have confirmed the overwhelming evidence of the role played by alcohol consumption in human cancers. The cancers in excess arise from the buccal cavity, pharynx, larynx, lung, esophagus, liver and also breast cancer in women (Tuyns, 1982).

If ethanol is not a carcinogen by itself, it might act as a cocarcinogen by enhancing the role of other substances. Current epidemiological evidence indicates that in the United States increased risk among smokers is associated with the amount and duration of alcohol consumption

and leads us to the conclusion that alcohol acts primarily as a cocarcinogen or promoter (Tuyns, 1982).

The key questions that need to be addressed experimentally are: Where does ethanol exert its effects? What are the effects, and how do the effects result in increased risk for cancer? There are several possible mechanisms which can be envisioned for the association between alcohol and cancer: alcohol-induced increases in carcinogen metabolism, alcohol-induced decreases in liver metabolism, alcohol as a solvent, and alcohol-induced cytotoxic and mytogenic effects, alcohol-suppression on retinol (Vitamin A) metabolism, effect of alcohol on lipid peroxidation, alcohol-inhibition of DNA-alkylation repair, alcohol-induced sister chromatid exchange (SCE), and alcohol-suppression of immune responses (McCoy and Wynder, 1979; Garro and Lieber, 1990). Before alcohol's role as a cocarcinogen, or its association with these mechanisms can be discussed, it is important to understand the general properties of alcohol as well as its administration into living systems.

## CHAPTER II

### GENERAL BIOCHEMICAL FEATURES OF ETHANOL

#### Absorption

Alcohol can enter the body by several routes. It can be administered parenterally and can even be given by enema. Alcohol in vaporized form may be inhaled and absorbed through the lungs in amounts sufficient to cause intoxication. The most common entry of alcohol in the body, however, is by the mouth. Alcohol is one of the few substances that may be absorbed directly from the stomach (Berggren and Goldberg, 1940; Karel and Fleisher, 1948). Unlike carbohydrates, proteins, and fats, it does not require prior digestion, and no active processes are involved in its absorption, which begins almost immediately after ingestion. About 30 per cent of the alcohol taken by the oral route is absorbed from the stomach and the rest from the intestine. It does not appear in the feces and, therefore, it is completely absorbed and eliminated by other routes (Sardesai, 1969).

The rate of absorption from the stomach and the rate of passage into the intestine are influenced by many factors such as, the presence of food in the stomach, the con-

centration of alcohol, the type of alcoholic beverage, and the emptying time of the stomach. The presence of food in the stomach slows the absorption and delays its passage into the intestine (Sardesai, 1969). Up to a maximum of 30 percent, the higher the concentration of alcohol, the faster is the absorption. In general, alcohol is absorbed most rapidly from distilled spirits such as whiskey, slowly from wines, and more slowly from beers (Newman, 1942).

Complete absorption occurs only after the stomach contents pass into the intestine (Haggard, 1940). From the intestine, alcohol is absorbed as rapidly as it enters. It does not remain or accumulate in the intestine, as may happen in the stomach. Absorption from the intestine is swifter and more complete than absorption from the stomach (Sardesai, 1969).

#### Distribution

After absorption has ceased and there is no more alcohol left in the gastrointestinal tract, a condition of equilibrium is said to have been reached. The absorbed alcohol dissolves in the body water and the alcohol content of different tissues and body fluids will be in proportion to their water content. The average water content of the body is 65 per cent (Gaebler, 1964) and that of blood about 83 per cent and so, after equilibrium, the concentration of alcohol in the blood will be approximately 1.27 times that of the whole body. It is, therefore, possible to calculate

the amount of alcohol in the body by determining alcohol levels in the blood or in other biological fluids (Sardesai, 1969).

Because of its ready volatility, alcohol can also be determined in the breath. There is a constant relationship between the amount of alcohol in the blood and in the alveolar air. The alveolar air/blood alcohol ratio is close to 1:2100 (Sardesai, 1969).

While the major amount of ingested alcohol is completely metabolized, between 2 and 4 per cent after moderate drinking and as high as 10 per cent after excessive drinking is eliminated as such primarily by way of the kidney and lungs and to some extent by way of tears and perspiration (Carpenter, 1940).

#### Metabolism

It is now well established that the major portion of alcohol metabolism takes place in the liver of most species, although the kidney may play a minor role in some animals (Ferguson, 1965). The initial oxidation of alcohol to acetaldehyde is catalyzed by alcohol dehydrogenase, a zinc-containing enzyme, present primarily in the soluble fraction of the hepatic cell. Acetaldehyde is then converted to acetate or acetyl-CoA, which can then be metabolized by a number of routes such as biosynthesis of fatty acids, cholesterol formation, oxidation by way of the citric acid cycle and porphyrin synthesis. The oxidation of

alcohol produces relatively large amounts of the reduced form of nicotinamide adenine dinucleotide (NADH) resulting in the increase of the ratio of NADH/NAD in the soluble fraction (Sardesai, 1969).

Several investigators have tried to determine whether alcohol is normally formed in the animal body and whether it may be formed during the course of metabolism of other substances or as a result of bacterial fermentation in the intestinal tract (Sardesai, 1969). Lester, 1962, using gas-liquid chromatographic technique, concluded that normal human blood may contain alcohol in concentration of up to 1.5 ug/liter (Lester, 1962). McManus et al. have found that ( $^{14}\text{C}$ )-labeled pyruvate, when incubated with liver slices, was converted into alcohol (McManus et al., 1960; McManus et al., 1966). They postulated that alcohol formation might result from the action of alcohol dehydrogenase or, under strongly reducing conditions, upon acetaldehyde arising from the action of pyruvic oxidase upon pyruvic acid. At any rate, it seems clear that alcohol is, in fact, a trace Physiological constituent of mammalian tissues.

## CHAPTER III

### EPIDEMIOLOGICAL STUDIES

#### Introduction

Perhaps the earliest clinical observation associating chronic alcohol consumption with cancer was that of J.C. Warren, noted Boston surgeon, who in 1836 described a case of lingual cancer in a tobacco chewer by saying, "predisposition...was generated by the long use of ardent spirits..." (Warren, 1976). Since then, large-scale case-control studies conducted in the United States have firmly established that chronic alcohol consumption markedly increases the risk for some cancers.

Epidemiological principles, which have done so much to promote the understanding of and prevention of infectious diseases, are now applied with equal vigor and hope to the understanding of and prevention of chronic illnesses. In this field, the ultimate goal of epidemiology is to establish ways by which a given disease process can be avoided. In searching for environmental and etiological factors, one must study all possible factors in order to determine the relationship of any of these factors to the disease process (Wynder et al., 1957).

To present the statistics supporting the relationship between alcohol consumption and human cancer, it must be explained how these figures were gathered. The epidemiological reports were formulated from case studies. Even though several different studies were reviewed for this report, they were performed in a similar way. The researchers would begin by interviewing and/or giving a questionnaire to a large number of cancer patients (800 to over 1,000) and controls of similar age, race, sex, and from the general population represented by the patients. These interviews or questionnaires were administered by trained interviewers to solicit information concerning tobacco use, alcohol use, diet, occupation, oral health and other characteristics of the participants. The information gathered would then be statistically analyzed and adjusted for many factors to try to isolate certain parameters.

The breakdown of the amount of ethanol per drink, and what was considered a serving size in the studies documented can be found in Table I (Harvey et al., 1987):

### Epidemiological Statistics

#### Smoking and Drinking Interrelations

From calculations of attributable risk, it is estimated that tobacco smoking and alcohol drinking combine to account for approximately three-fourths of all oral and pharyngeal cancers in the United States (Blot et al., 1988). A case-control study of oral and pharyngeal cancer



TABLE I  
 AMOUNTS OF PURE ETHANOL PER SERVING  
 BY TYPE OF ALCOHOLIC BEVERAGE

Type of Beverage	Ethanol per oz.(g)	Serving size (oz)	Ethanol per serving (g)
beer	1.1	12.0	13.2
wine	2.9	4.0	11.6
hard liquor	9.4	1.5	14.1

(Harvey et al., 1987)

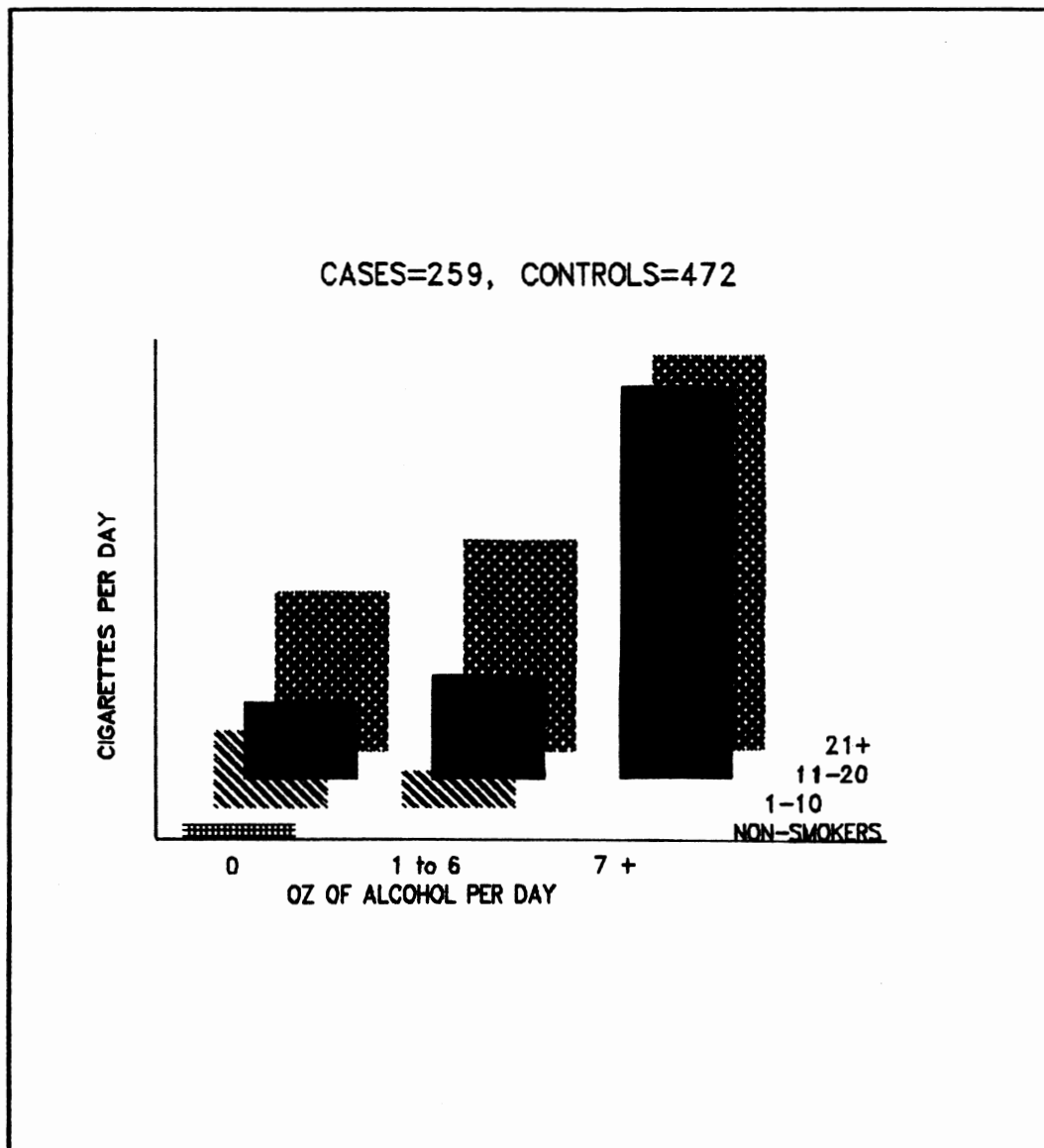
conducted by William J. Blot and his research team provided information on the tobacco and alcohol use of 1114 patients and 1268 population-based controls in four areas of the United States (Blot et al., 1988).

Because of the large study size, it could be shown that the risks of these cancers among nondrinkers increased with the amount smoked, and conversely that the risks among nonsmokers increased with the level of alcohol intake. Among consumers of both products, risks of oropharyngeal cancer tended to combine more in a multiplicative than additive fashion and were increased more than 35-fold among those who consumed two or more packs of cigarettes and more than four alcoholic drinks/day (Blot et al., 1988). This shows the effect of alcohol as a promoting agent.

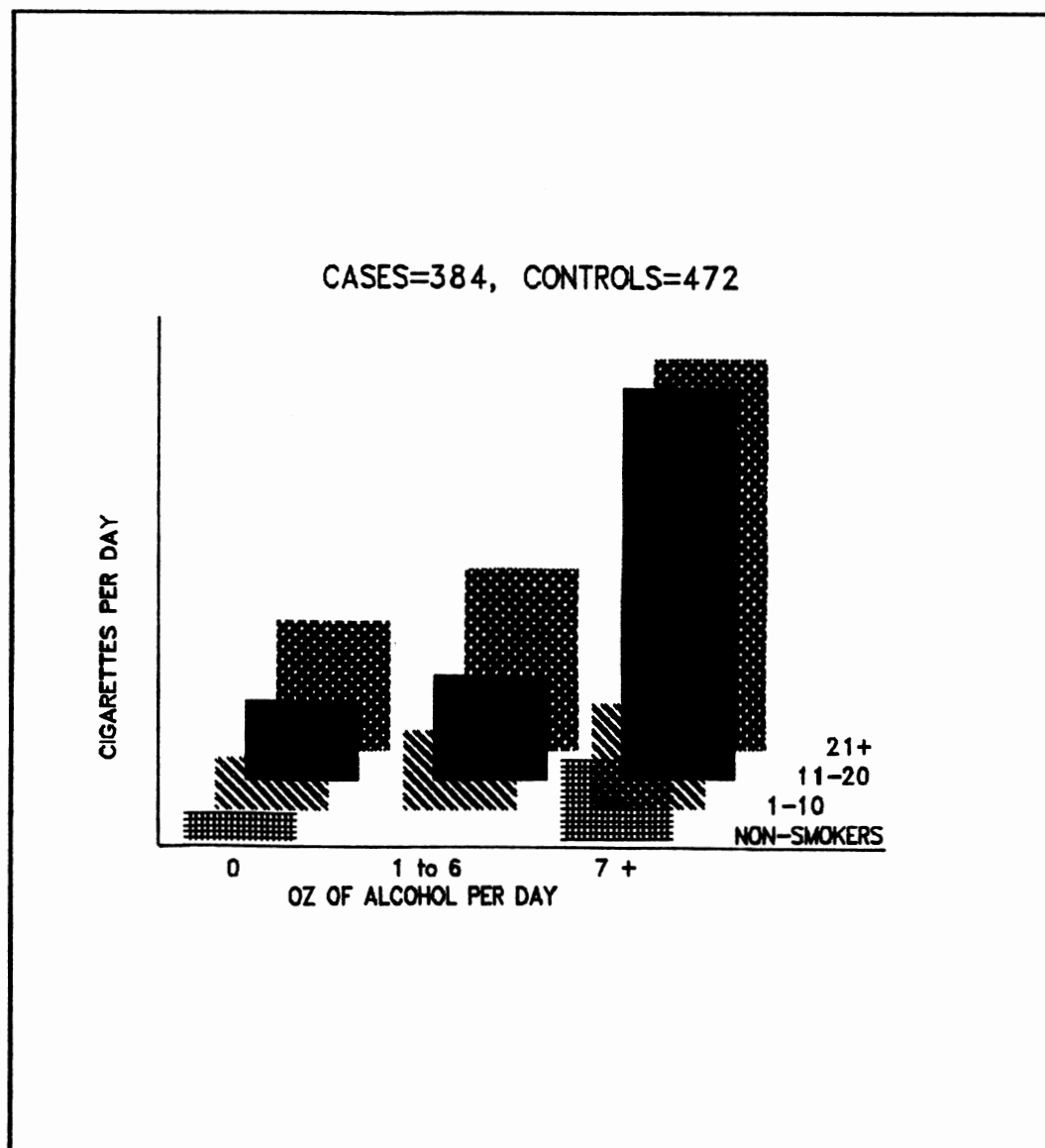
As shown in Figures 1 and 2, as alcohol and cigarette use increases, so does the risk of oral and larynx cancer (McCoy and Wynder, 1979). This evidence that increased risk among smokers was associated with the amount and duration of alcohol consumption, also lead to the conclusion that alcohol acts primarily as a cocarcinogen or promoter (McCoy and Wynder, 1979).

#### The Effects of Different Types of Alcoholic Beverages

A variety of carcinogens including nitrosamines, polycyclic hydrocarbons, fuel oils, asbestos fibers, and aflatoxins have been found in different types of alcoholic drinks (McGlashan et al., 1968; Biles and Emerson, 1968). However, with the possible exception of regions such as Brittany and Normandy in France, several areas in Africa, Puerto Rico, and the Southern United States, 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 (Tuyns, 1970; Cook, 1971; and Brown et al., 1988). The regional beverage question has been examined in greatest detail in Brittany and Normandy where the incidence of esophageal cancer is particularly high. Walker and his colleagues have demonstrated that the local, widely consumed apple ciders and apple brandies not only contain dimethylnitrosamine (DMN), which is also found in a variety of beers,



**Figure 1. Relative risks of larynx cancer by daily consumption of alcohol and cigarettes for males ( $P < 0.05$ ) (McCoy and Wynder, 1979).**



**Figure 2. Relative risks of oral cavity cancer by daily consumption of alcohol and cigarettes for males ( $P < 0.05$ ) (McCoy and Wynder, 1979).**

wines, and liquors, but also contain relatively high concentrations of diethylnitrosamine (DEN) (Walker et al, 1979). 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 in the Department of Calvados, Normandy (Tuyns et al, 1979). 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.

William J. Blot and his workers showed that the risks of cancer incidence varied by type of alcoholic beverage, being higher among those consuming hard liquor or beer than wine. The data obtained through this study are shown in Tables II and III (Blot et al, 1988).

The measure of association between oropharyngeal cancer risk and tobacco and/or alcohol intake used in this analysis is the odds ratio. Point estimates and 95% confidence intervals of summary (adjusted) OR were calculated using logistic regression analyses (Blot et al., 1988). Blot and his workers used logistic models to conduct formal tests of interaction between tobacco and alcohol. To determine whether the two agents combined more in an additive or multiplicative fashion, they fit models containing a parameter representing a transition from a subadditive to

TABLE II

ODDS RATIOS (OR) FOR OROPHARYNGEAL  
CANCER ASSOCIATED WITH  
DRINKING FOR MALES

Type of	No. of alcohol	No. of drinks/ week	No. of cases	OR*	95% CI
All	40	139	1.0		
	1-4	71	206	1.2	0.7-2.0
	5-14	99	219	1.7	1.0-2.7
	15-29	154	150	3.3	2.0-5.4
	30+	389	118	8.8	5.4-14.3
Hard liquor	173	337	1.0		
	1-4	134	240	1.0	0.7-1.3
	5-14	138	165	1.3	0.9-1.8
	15-29	118	56	2.6	1.7-3.9
	30+	179	31	5.5	3.4-9.1
Beer	146	333	1.0		
	1-4	130	231	1.2	0.8-1.7
	5-14	141	161	1.7	1.2-2.4
	15-29	134	62	3.4	2.7-5.1
	30+	195	44	4.7	3.0-7.3
Wine	497	490	1.0		
	1-4	114	205	0.7	0.5-1.0
	5-14	70	110	0.7	0.4-1.0
	15-29	31	21	0.9	0.5-1.8
	30+	35	6	2.5	0.9-6.5

\* OR adjusted for smoking, age, race, study location, and respondent status.

(Blot et al., 1988)

TABLE III

ODDS RATIOS (OR) FOR OROPHARYNGEAL CANCER  
ASSOCIATED WITH DRINKING  
FOR WOMEN

Type of	No. of alcohol	No. of drinks/ week	No. of cases	OR* controls	95% CI
All	63	171	1.0		
	1-4	75	129	1.2	7-1.9
	14	72	93	1.3	0.8-2.1
	15-29	55	29	2.3	1.2-4.5
	30+	87	9	9.1	3.9-21.0
Hard liquor	135	278	1.0		
	1-4	78	95	1.3	0.9-2.1
	-14	65	50	1.5	0.9-2.5
	15-29	32	5	4.9	1.6-14.3
	30+	41	3	7.8	2.1-29.2
Beer		180	343	1.0	
	-4	73	60	2.2	1.4-3.6
	-14	48	20	2.9	1.5-5.6
	15-29	24	7	2.3	0.9-6.5
	30+	27	1	18.0	2.1-15.9
Wine	230	273	1.0		
	-4	60	109	0.6	0.4-1.0
	-14	41	41	0.8	0.4-1.4
	15-29	7	7	0.5	0.1-2.3
	30+	13	1	1.6	0.2-13.6

\* OR adjusted for smoking, age, race, study location, and respondent status.

(Blot et al., 1988)

a supramultiplicative combination of risks. All analyses were adjusted for age, race, study location, and respondent status (self versus next-of-kin interview) (Blot et al., 1988). A high odds ratio would indicate a higher risk for cancer incidence than would a low odds ratio.

Table II and III show that the odds ratios for oropharyngeal cancer, controlled for smoking, rose with increasing total alcohol consumption and with increasing intake of hard liquor and beer. Over one-half of the male cancer cases were heavy drinkers (30 or more drinks/week), compared to only 14% of the noncancerous male controls who were heavy drinkers. Approximately one-fourth of the female patients were heavy drinkers, where only 2% of the female controls were heavy drinkers. The smoking-adjusted excess risk associated with high alcohol consumption among both men and women was approximately 9-fold. The risk of cancer incidence was relatively small among both men and women when alcohol intake was moderate (1-14 drinks/week) (Blot et al., 1988).

The data above shows the risk of oropharyngeal cancer incidence in humans differed according to the type of alcoholic beverage consumed. The risks were highest for beer and hard liquor consumption. There was little or no excess risk for wine drinking, reported by about one-third of both male and female patients, after adjusting for liquor and beer intake, except when consumption exceeded 4 drinks/day.



Relative-Risk Estimates According to the  
Level of Alcohol Intake

Schatzkin and colleagues (1987) investigated the relationship between alcohol consumption and breast cancer in the Epidemiological Follow-up Study of the first National Health and Nutrition Examination Survey, a cohort study based on a sample of the U.S. population. A total of 7188 women 25 to 74 years of age who were examined during the period 1971 through 1975 were included in the analysis. Information about alcohol consumption was obtained during the base-line interview. The median follow-up period for this cohort was 10 years. One hundred twenty-one cases of breast cancer that developed after the baseline examination were identified through hospital records or death certificates (Schatzkin et al., 1987).

Schatzkin and his colleagues used Cox's proportional-hazards regression technique to analyze the simultaneous relationship of alcohol, age, and other variables to the incidence of breast cancer in the cohort. The analyses were performed with the PROC PHGLM procedure available in the SAS statistical package (Schatzkin et al., 1987).

The results of this study, consistent with those of two other cohort studies and a number of case-control studies, suggest that moderate alcohol consumption is associated with an elevation in the risk of breast cancer of 50 to 100 percent (Willett, 1987).

Relative-risk estimates from proportional-hazards regression models that include variables for age and alcohol consumption are shown in Table IV (Schatzin et al., 1987). The study showed a 40 to 50 percent increase in risk among the women who drank less than 5g of alcoholic beverages per day (equivalent to about three drinks per week). The greatest risk in the study was seen among women who drank 5 grams of alcohol or more per day (Schatzkin et al., 1987).

TABLE IV

RELATIVE-RISK ESTIMATES FOR BREAST CANCER ACCORDING  
TO THE LEVEL OF ALCOHOL INTAKE

Relative-risk estimate	Alcohol Intake Level (g/day)				
	NONE	ANY	0-1.2	1.3-14.9	5
Age-adjusted*	1.0	1.5	1.4	1.5	1.6
Multivariate**	1.0	1.6	1.4	1.6	2.0

\* Based on age-adjusted regression coefficients from the proportional-hazards models (121 cases).

\*\* Based on 88 cases with complete covariate information, including age (years); education (12 years); body-mass index (combined second through fourth quintiles, 21 to 29; fifth quintile, =30); total dietary fat (grams per day) (separate second through fifth quintiles; 34.2 through 47.5; 47.6 through 61.4; 61.5 through 80.6; =80.7); age at first parturition (19 through 20, 21 through 22, 23 through 24, and =25), age at menarche (<M=>12), parity (nulliparity, 1 or 2 births), positive family history, and premenopausal status (Schatzkin et al., 1987).

### Problems With Case Studies

There are some problems involved with collecting data through case studies that might result in inaccurate conclusions. Some of these problems include the difficulty in studying the differences between beverages. For example, 92% of the people may drink wine, but only 10% drink wine exclusively. It is also difficult to measure the amount consumed accurately and consumption may be misreported.

Errors of data collection may also occur. Errors of data collection may be categorized as classification errors and selection errors (Yuspa and Harris, 1982). These two types of errors may be contrasted with the problem of confounding. Confounding involves variables other than the principal study variables, and given information about these other variables, analytical procedures are available to control for confounding. Classification errors occur when individuals included in the study are classified incorrectly as to the principal exposure or disease variables. Selection errors are a problem of the choice of study subjects and result from the inclusion of groups that are to be compared (Yuspa and Harris, 1982).

The epidemiologic statistics presented in this paper were not gathered through scientific experiments, but rather from comparing existing data. The scientists conducting these case-studies made correlations between cancer patients and control groups (non-cancerous individuals) to try to establish a connection between alcohol consumption

and cancer incidence. Some of these statistics may not be significant, but they may serve as a guide leading to further scientific experiments.

## CHAPTER IV

### ALCOHOL AS A PROMOTER

The events involved in cancer induction are classified into two distinct stages, initiation and promotion. Alcohol has been classified as a promoting agent. Tumor promotion requires repeated exposure to promoting agents at frequent intervals, and if the interval between exposures is prolonged, the effects are reversible. Promoters induce tumor development only after initiation (cellular changes caused by a carcinogen) by another agent occurs. Even if a long interval separates the two events promotion in the absence of, or prior to, initiation will not result in tumor formation (Yuspa and Harris, 1982). Promoting agents usually induce proliferation in target tissue and induced changes are progressive (Yuspa and Harris, 1982). The characteristics of promoting and initiating agents are outlined in Table V (Yuspa and Harris, 1982). The information in Table V is defined in a broad sense as agents that can both initiate cancer in limited dosages and induce cancer in higher dosages or in states of increased host susceptibility.

Although there is no experimental evidence that ethanol per se is carcinogenic, numerous studies conducted

TABLE V

GENERAL PROPERTIES OF INITIATING AGENTS  
AND PROMOTING AGENTS

Initiating Agents	Promoting Agents
1. Carcinogenic by themselves	1. Not carcinogenic alone; must be given after initiating agent to exert effect.
2. Activity strictly determined by molecular structure.	2. Activity strictly determined by molecular structure.
3. Generally active in more than one tissue.	3. Action of individual exposure is reversible and not cumulative. Repeated exposures are required.
4. No detectable threshold dose; action is cumulative and irreversible.	4. Metabolism or macromolecular binding may not be required.
5. Most require metabolic activation and covalently bind to macromolecules.	5. Not mutagenic but may enhance the expression of induced mutations.
6. Most are mutagens	6. Usually induce proliferation in target tissue.
7. More active in proliferating tissues.	7. Induced changes are progressive; stable inter-mediate stages may be observed prior to overt malignancy.

(Yuspa and Harris, 1982)

over the last 25 years have shown that ethanol is capable of acting as a cocarcinogen, or promoter, 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 constituents of tobacco smoke, as the inducing carcinogens. In one of the first such studies, Protzel and colleagues, 1964, 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) (Protzel et al., 1964). 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 (Elzay, 1966). 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 and in a similar application was reported to enhance the induction of esophageal tumors in mice (Horie et al., 1965). 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 (Capel et al., 1978).

In more recent studies of tobacco-associated carcinogens, McCoy and colleagues, 1986, 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) (McCoy et al., 1986). Similarly, Griciute and his researchers, 1986, 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 (Griciute et al., 1986). 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 (Gastonguay et al., 1984). Isocaloric pair-feeding of ethanol also has been shown to promote the progression of esophageal tumors initiated by N-nitrosomethylbenzylamine (MBN) in rats (Mufti et al., 1989). 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 in combination with a zinc-deficient diet enhanced the induction of MBN-induced esophageal tumors (Gabrial et al., 1982). 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-amyl nitrosamine (Garro and Lieber, 1990).

## CHAPTER V

### POSSIBLE MECHANISMS FOR THE ASSOCIATION BETWEEN ALCOHOL AND CANCER

#### The Mechanisms

There are several possible mechanisms that can be envisioned for the association between alcohol and cancer.

These mechanisms are the following:

- 1) alcohol as a solvent
- 2) alcohol-induced increases in carcinogen metabolism
- 3) alcohol-induced decreases in liver metabolism
- 4) alcohol-induced cytotoxic and mitogenic effects
- 5) alcohol-suppression on retinol (Vitamin A) metabolism
- 6) effect of alcohol on lipid peroxidation
- 7) alcohol-inhibition of DNA-alkylation repair
- 8) alcohol-induced sister chromatid exchange (SCE)
- 9) alcohol-suppression of immune responses

The evidence for these mechanisms is discussed in this chapter. This evidence has been shown through experimental data and shows a strong relationship between alcohol consumption and the incidence of human cancer.

#### Alcohol as a Solvent

This mechanism assumes that entry of tobacco-related carcinogens into target tissues is facilitated because of enhanced solubility of the carcinogen and easier passage through cellular membranes (McCoy and Wynder, 1979).

Ethanol can alter membrane fluidity or composition, or both. Ethanol molecules can enter cell membranes by intercalating between the lipids of the bilayer membranes. This expands membranes and increases their fluidity, which in turn affects cell agglutination, phagocytosis, membrane transport, membrane enzyme activities and many other membrane functions (McCoy et al., 1978).

Membranes are largely composed of lipids and proteins. Thus, membrane organization results from the interaction of these molecules with each other and potentially with elements of the cytoskeleton and extracellular matrix (Tank et al., 1981). There is recent data indicating that ethanol and other alcohols have a specific effect on the structural properties of different membrane domains. Treistman and colleagues (1987) examined the effects of alcohol upon lipid lateral diffusion in the plasma membrane of Aplysia neurons. The technique of fluorescence photobleaching recovery was used to measure the lateral diffusibility of fluorescent lipid analogues in neurons. The purpose of this study was to determine the extent to which the plasma membrane can be treated as having a bulk membrane fluidity. The overall fluorescence measured in membranes, indicated by counts per second, did differ in different regions of the neuron (Treistman et al., 1987). This suggests that all regions were not identical with respect to either incorporation of probe, and/or environment surrounding the probe. It has been suggested that alcohols may selectively

partition into specific domains within the plasma membrane, and therefore affect the bulk membrane fluidity of these local environments. Similarly, it has been suggested that lipid analogues selectively partition into specific domains within the membrane and therefore report not on the bulk membrane fluidity, but on the fluidity of local environments (Treistman et al., 1987). These results indicated a complexity of organization with the Aplysia neuronal plasma membrane. Treistman's alcohol data suggest that the membrane is submicroscopically heterogeneous (Treistman et al., 1987).

Several different domains in cellular membranes have been described, e.g., hydrophilic, hydrophobic, lateral, exofacial and cytofacial leaflets (Treistman et al., 1987) (Hitzemann et al., 1986). The surface of the membrane is more hydrophilic as compared to the interior, which is more hydrophobic. Fluidity is greater in the hydrophobic core of the membrane and ethanol has a greater effect in that region. These differences between the hydrophilic and hydrophobic domains of the membrane have been observed using probes that report at different depths within the bulk membrane (Wood and Schroeder, 1988).

Alcohols, including ethanol, have a specific in vitro effect on the fluidity of different membrane domains. However, the question still remains as to whether changes in fluidity in turn affect the functional activity of the membrane such as transport, signal transmission and ultimately

sensitivity and tolerance (Wood and Schroeder, 1988). It has been proposed that there is an optimal fluidity required of specific membrane domains, in relation to each other, for membrane function (Sanderman, 1978). Changes in the homeostasis of the physical properties of membrane domains may be involved in sensitivity to ethanol. For example, alcohols have a differential effect on one membrane leaflet as compared to the other. The more fluid leaflet is most affected by the alcohols (Wood and Schroeder, 1988).

Most studies examining effects of chronic ethanol consumption on membranes have found that membranes of ethanol-tolerant animals are less fluid and/or less perturbed by ethanol in vitro (Hunt, 1985). The change in fluidity of membranes from ethanol-tolerant groups has been attributed to changes in the lipid composition of the membrane. There is a potential relationship between cancer and ethanol at the membrane because ethanol rapidly enters, expands, and fluidizes all biological membranes. These changes in membrane properties could suppress or enhance the induction, growth, spread, or treatment of cancers (McCoy and Wynder, 1979). It has been demonstrated that administration of the carcinogen DMBA dissolved in ethanol resulted in a reduced latent period and increased skin tumor formation compared to mice treated with DMBA dissolved in acetone (McCoy and Wynder, 1979).

This mechanism is an attractive one for oral cavity and esophageal cancer but is not sufficient to explain the association between alcohol and glottic cancer, since this area does not come into direct contact with alcohol.

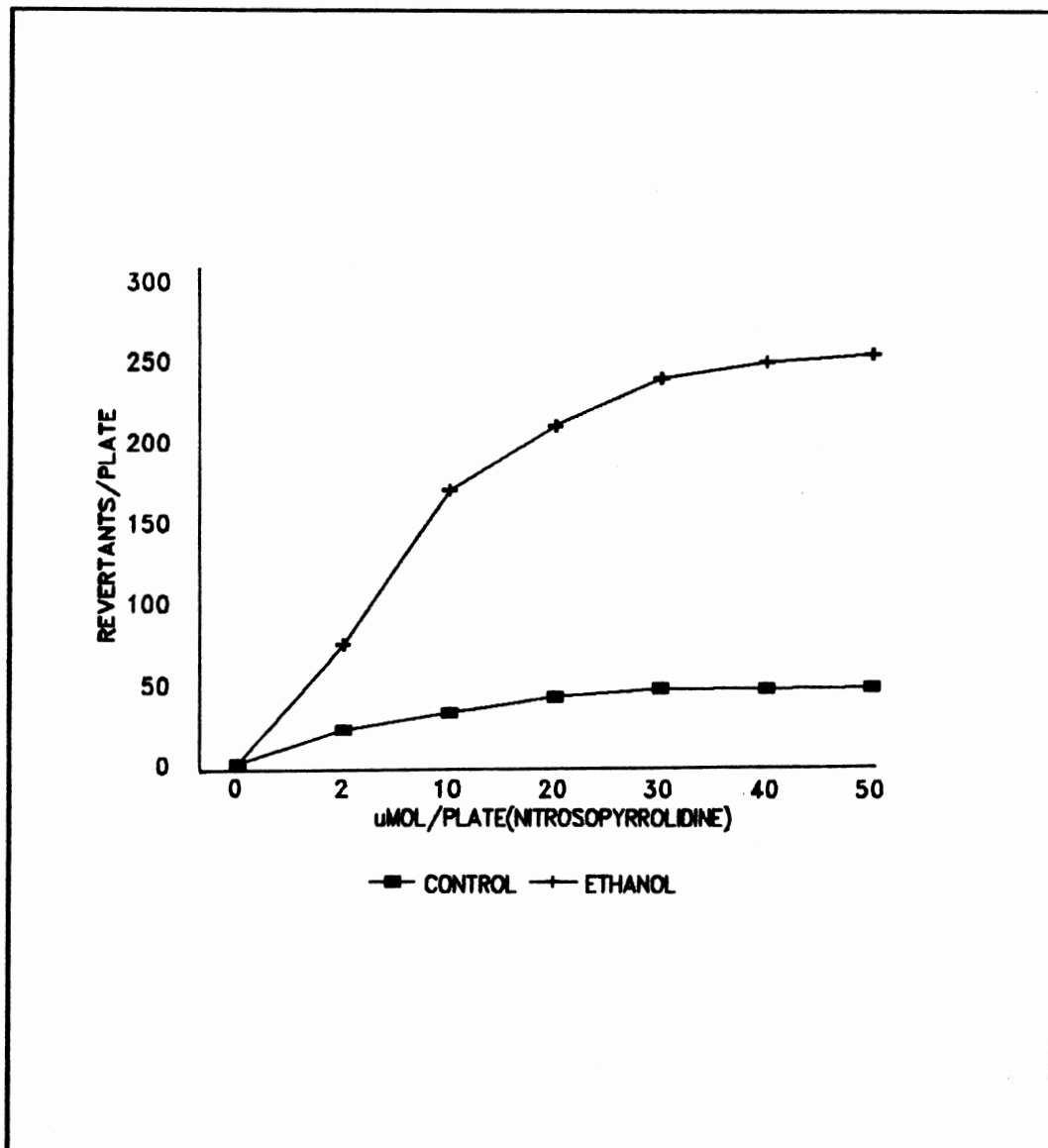
#### Enhanced Carcinogen Metabolism

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 (Lieber, 1973). 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 (Miller, 1970). An association has been suggested between the levels and distributions of various types of P-450 isozymes and susceptibility to some cancers. The general approach used in these studies has been to prepare microsomal fractions 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 or to other detectable end products (Garro and Lieber, 1990).

Work conducted by Garro and Lieber (1990) 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 (Garro and Lieber, 1990). These tissues include the lungs and intestines, which are major portals of entry for tobacco smoke and dietary carcinogens, and the esophagus. 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 (Farinati et al., 1989).

Chronic ethanol consumption leads to an enhancement in the liver microsomal drug-metabolizing capabilities of both humans and experimental animals (Misra et al., 1971). It has been demonstrated that the in vitro metabolism of the hepatocarcinogen N-nitrosopyrrolidine (NPY) is increased in microsomal fractions isolated from ethanol-consuming animals. Postmitochondrial supernatants isolated from ethanol-consuming animals are capable of much greater conversion of NPY to a mutagen than are control preparations as shown in Figure 3 (McCoy and Wynder, 1979). It is concluded that alpha-hydroxylation of NPY is probably the mechanism by which NPY is converted to a mutagen and that this pathway can be induced by ethanol (McCoy and Wynder, 1979). In addition it was postulated that in the absence of extensive destruction of liver tissue, increased production of a proximate carcinogen occurred which was then delivered to the target tissues and further metabo



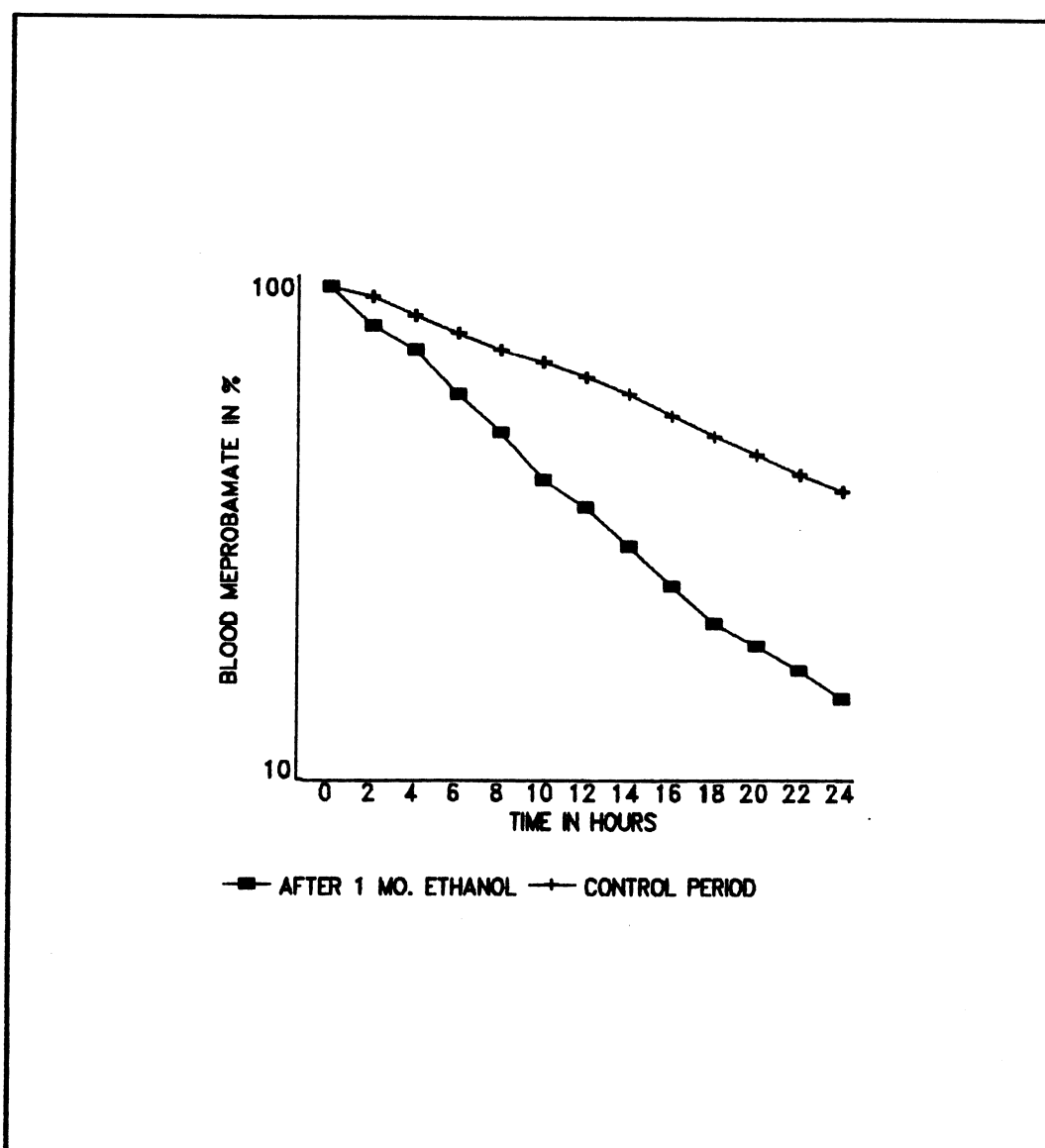
**Figure 3. Conversion of NPY to a mutagen (McCoy and Wynder, 1979).**



lized to its ultimate carcinogenic form (McCoy and Wynder, 1979).

Because enhanced hepatic microsomal drug metabolism may lead to tumor induction by increasing the production of carcinogens, the following experiments are of importance in the understanding of this process. It has been demonstrated that chronic ethanol ingestion results in accelerated drug clearance from the blood caused, at least in part, by enhanced hepatic microsomal drug metabolism (Misra et al., 1971). Alcoholics admitted to a hospital and tested in the absence of blood ethanol levels have an accelerated rate of clearance of ethanol and other drugs (Kater et al., 1969b; 1969a). The changes in drug metabolism in the alcoholic can be due to a large number of factors other than ethanol itself. These include malnutrition, congeners of alcohol and an associated high intake of other drugs (Pirola, 1978). However, the intake of ethanol alone with a nutritionally adequate diet under metabolic ward conditions resulted in a striking increase in the blood clearance of meprobamate and of pentobarbital (Misra et al., 1971). This was demonstrated in both alcoholic and non-alcoholic subjects and similar findings were obtained in rats. These findings are shown in Figures 4, 5, and 6.

The principal enzyme system involved in the metabolism of electrophilic derivatives is the microsomal cytochrome P-450-dependent mixed function oxidases. The inductive



**Figure 4. Clearance of meprobamate from the blood of alcoholics (McCoy and Wynder, 1979).**

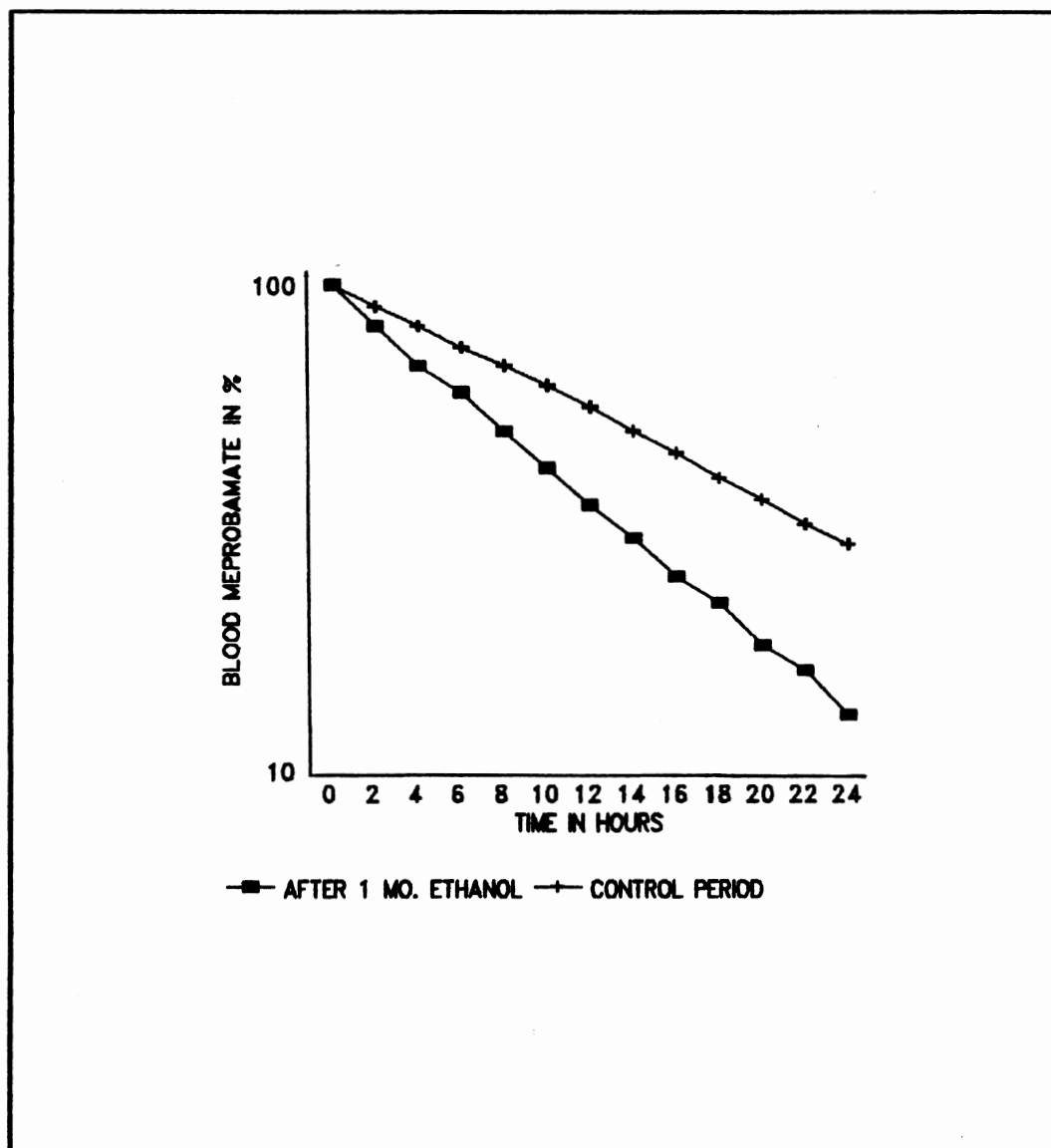
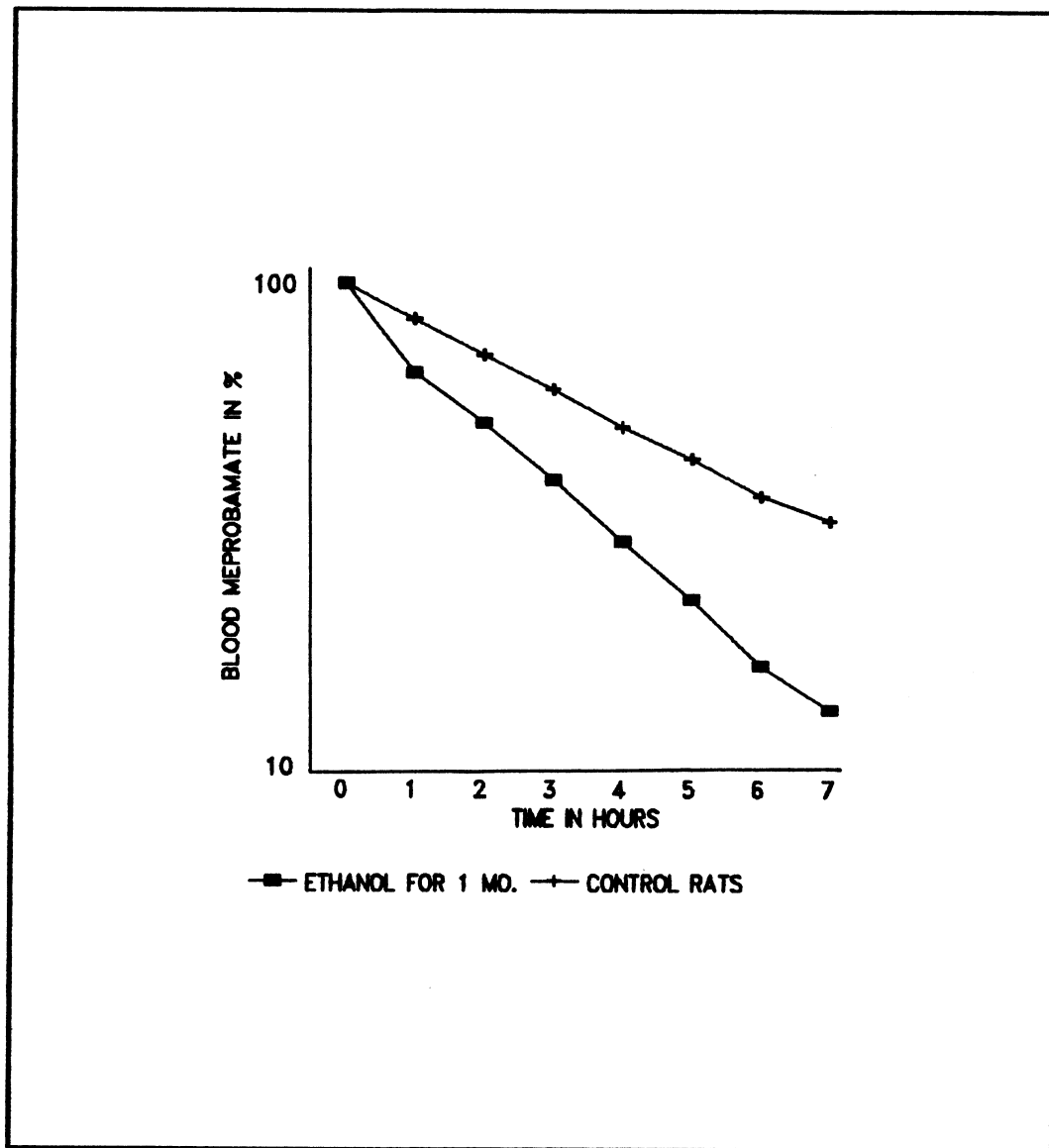


Figure 5. Clearance of meprobamate from the blood of nonalcoholics (McCoy and Wynder, 1979).



**Figure 6. Clearance of meprobamate from the blood of a rat litter (McCoy and Wynder, 1979).**

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. In rats, ethanol has been reported to increase cytochromes P450IIB1, P450IIIA, and P450IIE. Cytochrome P450IIE is a form which activates the hepatotoxin acetaminophen and the potent carcinogen dimethylnitrosamine. Cytochromes P450IIB1 is involved in the metabolism of several lipophilic drugs (Sinclair et al., 1991).

#### Decreased First Pass Liver Metabolism

It has been found that ethanol will alter profoundly the distribution of two carcinogenic nitrosamines in the rat (Swann et al., 1984). Small oral doses of dimethylnitrosamine (NDMA) are absorbed from the portal blood as it passes through the liver and eliminated, and therefore do not reach the extrahepatic organs; this is known as first pass clearance. First pass clearance is extremely important because it prevents exposure of extrahepatic organs to nitrosamines entering the body from the gastrointestinal tract (Swann et al., 1984).

Ethanol consumption has been shown to inhibit first pass clearance (Swann et al., 1984). For example, methylation of kidney DNA occurs when NDMA reaches the kidney, resulting from its inability to be completely metabolized by the liver. When oral doses of NDMA are given in water,

the methylation of kidney DNA ranged from no methylation at low doses to a dose-response relationship at higher doses. This shows that the ability of the liver to metabolize all of the NDMA decreased as the doses increased. When oral doses of NDMA was given in ethanol, the methylation of kidney DNA was continuously high, even at low doses of NDMA that had previously shown first pass clearance. This shows the inability of the liver to metabolize NDMA in the presence of ethanol, thus resulting in a loss of first pass clearance.

The most important consequence of this change in clearance is the increased exposure of extrahepatic organs. This increased exposure of extrahepatic organs to carcinogens could promote tumor formation. It is therefore, tentatively proposed that the effect of ethanol on human cancer incidence is mediated through similar influences on the metabolism and disposition of the nitrosamines to which man is exposed.

#### Cytotoxic and Mitogenic Effects

Replicating DNA, partially because of its single-stranded nature, is more reactive with many chemical carcinogens than resting DNA (Garro and Lieber, 1990). 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 (Garro and Lieber, 1990).

Chronic ethanol consumption is proposed to alter intracellular metabolism of epithelial cells at the target sites in mice, resulting in enhanced metabolic activation of tobacco-associated carcinogens (McCoy and Wynder, 1979). Some of the more attractive features of this proposal are that the cancers associated with the upper alimentary tract (UAT) and the upper respiratory tract (URT) are epithelial in origin. Secondly, these surface epithelial cells are most exposed to tobacco-associated carcinogens. Thirdly, site specificity of the carcinogen need not be invoked since all sites, by virtue of their anatomical location, will be exposed to tobacco smoke (McCoy and Wynder, 1979). This is an important point since no class of carcinogen need be initially excluded from consideration because it cannot be said with any degree of certainty which of the many potential carcinogens in tobacco smoke are actually involved in the initiation of carcinogenesis in the head and neck area (McCoy and Wynder, 1979).

William and Horn (1979) noted that the Third National Cancer Survey data gradient of decreasing cancer risk paralleled the successive dilution of alcohol in the alimentary tract and portal circulation. Therefore, cancer risk was 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 (1972) found that dyskaryotic cells (preinvasive malignant cells) occurred with higher frequency in the esophagus of heavy drinkers relative to other patients, and Winship and colleagues (1968) noted the occurrence of alcohol-associated functional abnormalities in the esophagus, which may represent either direct myopathic or neurophathic effects of ethanol (Anderson, 1972).

In the liver, large numbers of autopsy studies have shown that the occurrence of hepatoma is closely associated with cirrhoses, which are generally related to alcohol consumption. The incidence of cirrhosis in patients with hepatoma varies from 60 to 90% in different studies (Lieber et al., 1979). Furthermore, a few studies have indicated that in alcoholics, hepatomas may occur even in the absence of cirrhosis (Lieber et al., 1979). 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 (Garro and Lieber, 1990). Chronic ethanol consumption has been reported to stimulate rectal cell proliferation in the rat, possibly as a consequence of acetaldehyde exposure (Seitz et al., 1984). The ethanol-associated increase in dimethylhydrazine-induced cancer in this species may be related to this cell-proliferative effect (Seitz et al.,



1984). This proposal is further supported by the observation that chronic ethanol consumption is also cocarcinogenic in the rectum with the direct-acting carcinogen azoxymethyl-methylnitrosamine (AMMN) (Garzon et al., 1986). Ethanol also has been shown to be mitogenic for esophageal epithelium and to potentiate tracheal squamous metaplasia caused by vitamin A deficiency in rats (Garro and Lieber, 1990).

#### Alcohol Suppression on Retinol (Vitamin A) Metabolism

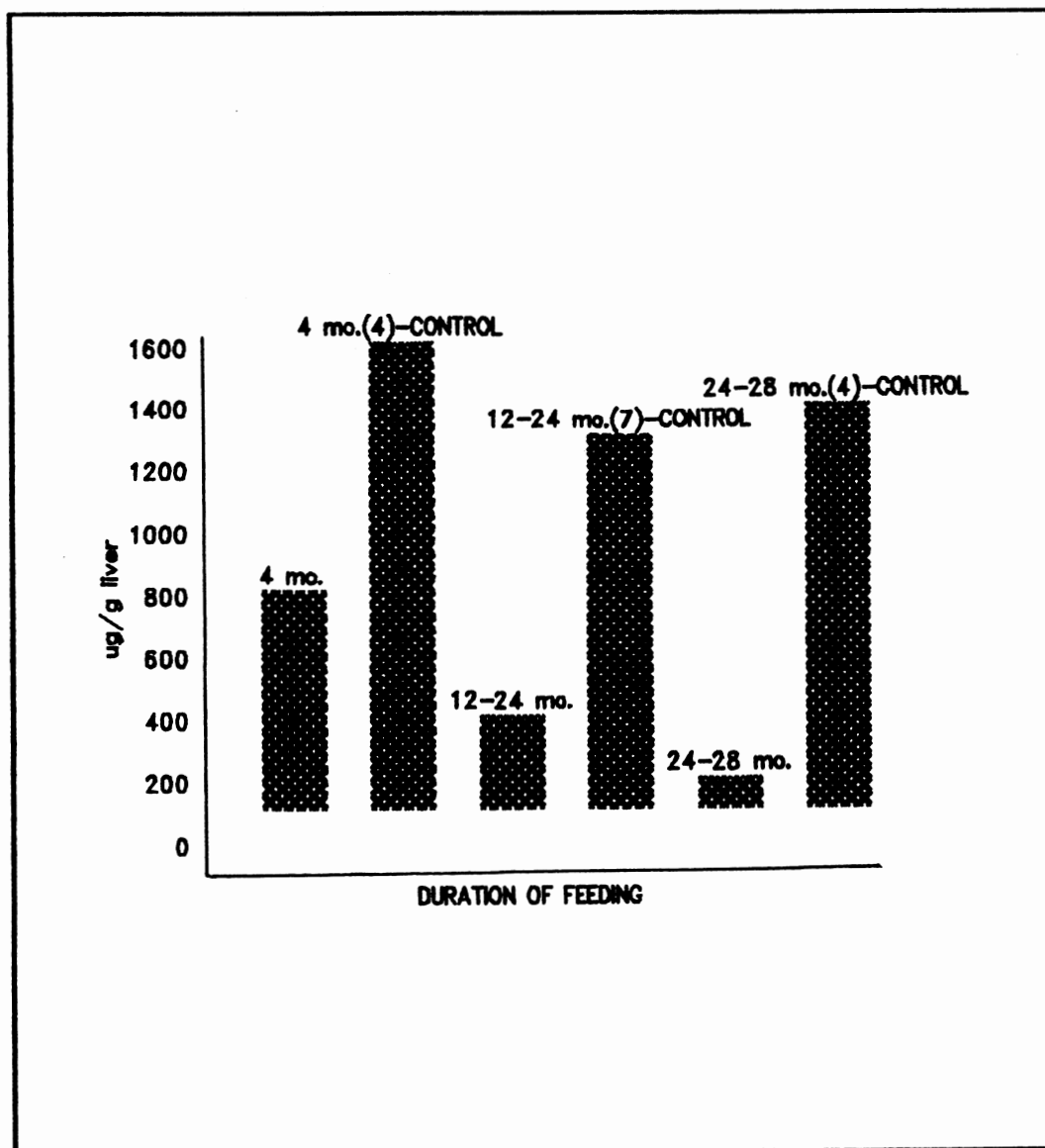
Ethanol consumption has been shown to depress hepatic vitamin A levels (Garro and Lieber, 1990). This effect of ethanol may be of importance in carcinogenesis as vitamin A plays an essential role in the maintenance of normal growth and control of cell differentiation in a variety of epithelial and mesenchymal tissues (Sporn and Roberts, 1983). Ethanol consumption results in a severe depression in hepatic vitamin A levels through at least two mechanisms: (a) increased mobilization of vitamin A from the liver to other organs; (b) by inducing a cytochrome P-450-mediated breakdown of both retinol and retinoic acid (Sato and Lieber, 1981).

To evaluate the possible effects of alcohol consumption on vitamin A status, Sato and Lieber (1981) pair-fed baboons a nutritionally adequate liquid diet containing 50% of total calories either as ethanol or isocaloric carbohydrate. Fatty liver developed after 4

months of ethanol feeding with a 59% decrease (P) in hepatic vitamin A levels (Figure 7). Fibrosis or cirrhosis developed after 24-84 months with a 95% decrease (P) in hepatic vitamin A levels (Sato and Lieber, 1981).

Similarly, hepatic vitamin A levels of rats fed ethanol (36% of total calories) were decreased after 3 weeks (42%, P) and continued to decrease up to 9 weeks. When dietary vitamin A was increased 5-fold, hepatic vitamin A was again decreased in ethanol-fed rats. When dietary vitamin A was virtually eliminated, depletion rate of vitamin A from endogenous hepatic storage was 2.5 times faster in ethanol-fed rats than in controls. It is concluded that chronic ethanol consumption decreases hepatic vitamin A, and that some mechanisms other than malnutrition and malabsorption may be involved in this process (Sato and Lieber, 1981).

Dietary carotenoids and retinyl esters are the major sources of vitamin A, which is stored in the liver in the form of retinyl esters. It has been known for more than 50 years that retinoids, the family of molecules comprising both the natural and synthetic analogues of retinol, are potent agents for control of both cellular differentiation and cellular proliferation (Sporn and Roberts, 1983). It was inevitable that the basic role of retinoids in control of cell differentiation and proliferation would eventually find practical application in the cancer field, and there have been great advances in this area, particularly for prevention of cancer. Many studies have shown that



**Figure 7. Effect of chronic ethanol feeding on hepatic vitamin A levels in baboons (Sato and Lieber, 1981).**

Baboons were pair-fed ethanol or control diet and fasted for 12 hours before sampling. Ethanol was withdrawn 20 hours before sampling. The number of pairs is shown in parenthesis (Sato and Lieber, 1981).

retinoids can suppress the process of carcinogenesis in vivo in experimental animals, and these results are now the basis of current attempts to use retinoids for cancer prevention in humans. Furthermore, there is now an extensive literature on the ability of retinoids to suppress the development of the malignant phenotype in vitro, and these studies corroborate the use of retinoids for cancer prevention. Finally, it has been shown that retinoids can exert effects on certain fully transformed, invasive, neoplastic cells, leading in certain instances to a suppression of proliferation and in other instances to terminal differentiation of these cells, resulting in a more benign, nonneoplastic phenotype (Sporn and Roberts, 1983).

In animals, retinoic acid is just as effective as retinal as a dietary supplement and retinoic acid is more effective than either retinol or retinal as an anticarcinogen or inducer of cellular differentiation in vitro (Lotan, 1980). Epidemiological studies involving different geographic locales have associated dietary-retinoid deficiency and low-serum vitamin A levels with increased cancer risk, particularly cancers of the esophagus and lung (Moon et al., 1977). These epidemiologic studies have been supported by animal studies that have demonstrated the efficacy of retinoids in prevention of cancers at different body sites (Peto et al., 1981).

Ethanol consumption interacted synergistically with vitamin A deficiency in increasing the incidence of tra-

cheal squamous metaplasia in the rat (Mak et al., 1987). These results are 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. 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 (Mak et al., 1987). Increased numbers of lysosomes also have been observed on ciliated tracheal cells following exposure to carcinogens and compound cilia have been observed in animals exposed to carcinogens and in humans with bronchial cancer (Harris et al., 1971).

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.

#### Effect of Alcohol on Lipid Peroxidation

Lipid peroxidation has been implicated in promoting the carcinogenic process. 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. This is associated with increased lipid peroxidation in ethanol-fed animals.

Several studies indicate that the susceptibility of subcellular membranes to lipid peroxidation is decreased or lost in tumor cells (Szebeni et al., 1986). It was therefore of interest to establish the effect of a potent carcinogenic treatment on the ethanol-induced lipid peroxidation in vivo. In a study performed by Szebeni and colleagues (1986) the exhalation of ethane was used as a measure of in vivo lipid peroxidation in rats (Szebeni et al., 1986). These study rats were treated with methylbenzyl nitrosamine (MBN) three times a week for three weeks. One week after the treatment, the animals were pair-fed an isocaloric liquid diet for 18 months. The experimental diet contained 7% ethanol such that 36% of the total caloric intake was due to ethanol. In the control diet, the ethanol calories were replaced by dextrin-maltose (Szebeni et al., 1986). Ethane exhalation was increased in MBN-treated plus ethanol fed rats in comparison to the control rats (Figure 8) (Szebeni et al., 1986). These results confirm the debated concept that there is increased lipid peroxidation following ethanol consumption (Szebeni et al., 1986).

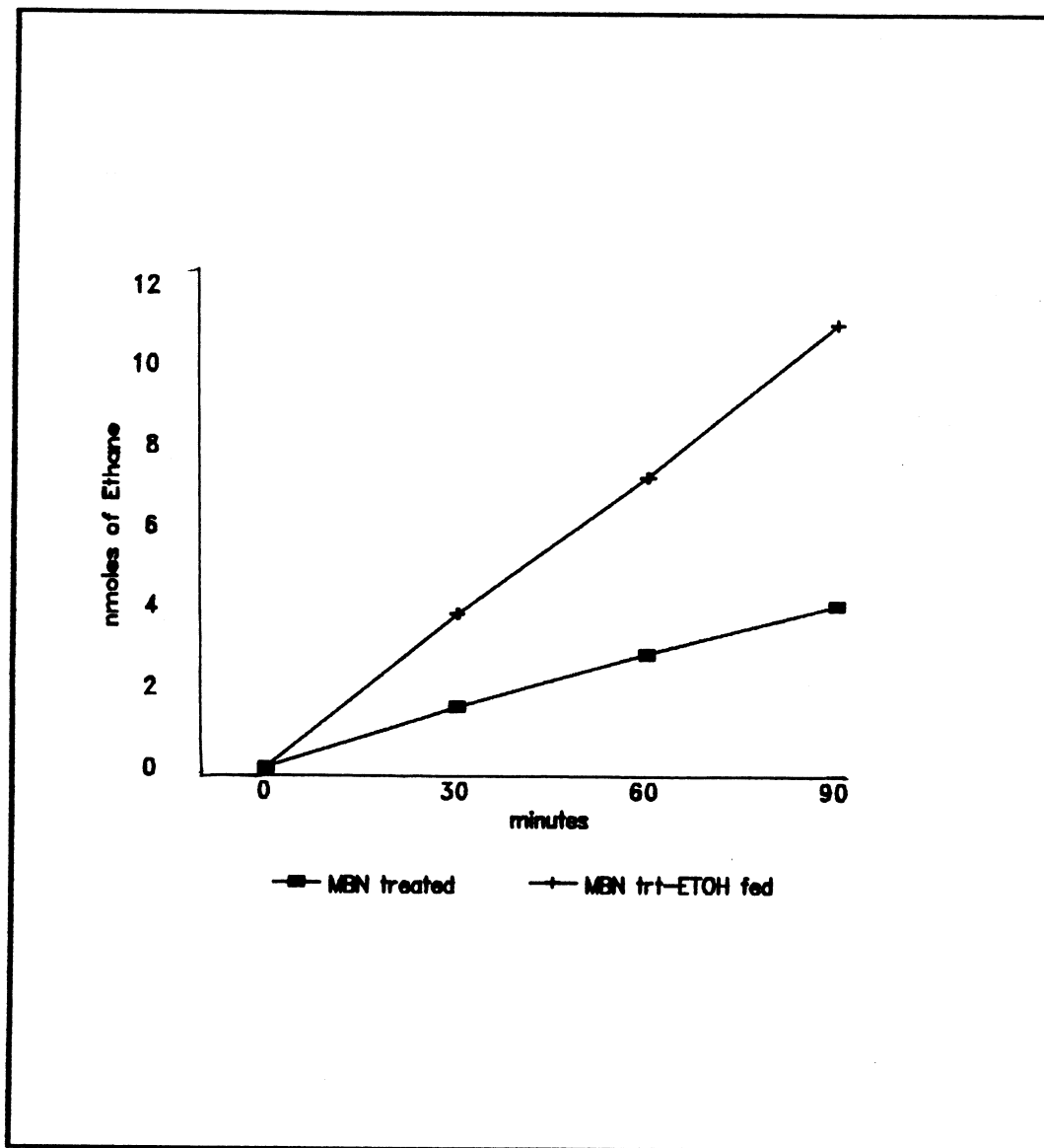


Figure 8. Exhalation of Ethanol (Szebeni et al., 1986).

The ethanol fed group was treated with methylbenzylnitrosamine (MBN) 18 months before ethane measurements. The difference at 90 minutes is significant at the 5% level (Szebeni et al., 1986).

### Alcohol 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 (Garro and Lieber, 1990). 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 (Becker, 1986). Chronic alcohol consumption may increase cancer risk by inhibition of the DNA-repair enzyme, O<sup>6</sup>-methylguanine transferase (O<sup>6</sup>-MeGT), which removes alkyl groups (methyl and ethyl) from the O<sup>6</sup> position of guanine (Lemaitre et al., 1982).

Craddock and colleagues (1982) showed that removal of O<sup>6</sup>-methylguanine from DNA occurs by transmethylation, the methyl group giving rise to acid-stable S-methylcysteine residues in protein (Craddock et al., 1982). The induced enzyme, like the constitutive enzyme, appears to be a transmethylase. Acid hydrolysis of the reaction product followed by the determination of the amount of S-methylcysteine formed provides a rapid simple method for assay of the O<sup>6</sup>-methylguanine DNA transmethylase in small samples of normal and pathological cells and tissues (Craddock et al., 1982).

In rats, chronic and acute alcohol consumption causes an increased persistence of diethylnitrosamine (DMN)-



induced hepatic O<sup>6</sup>-MeG DNA adducts and acetaldehyde has been shown to inhibit both rat and human O<sup>6</sup>-MeGT enzyme activity (Mufti et al., 1988).

The major DNA base alkylation products generated by exposure to alkylating nitroso compounds such as DMN, in order of frequency of occurrence, are N<sup>7</sup>-MeG, O<sup>6</sup>-MeG, and O<sup>4</sup>-Methylthymine. Persistence of O<sup>6</sup>-MeG in DNA of various organs has been associated with carcinogenicity of several alkylating agents and alkylation of the O<sup>4</sup> position of thymine may also be significant. O<sup>6</sup>-MeG is an alkyl transferase which transfers methyl or ethyl groups from the O<sup>6</sup> position of guanine to a cysteine residue located in the enzyme that in turn inactivates the transferase (Pegg and Perry, 1981).

The first major indication that chronic alcohol consumption interfered with the repair of O<sup>6</sup>-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<sup>6</sup>-MeG, but not N<sup>7</sup>-MeG, adducts persisted for longer periods in ethanol-fed rats relative to controls (Mufti et al., 1988). Moreover, this effect appeared to be specific for O<sup>6</sup>-MeG repair as removal of acetylaminofluorene adducts, which are repaired by a separate excision pathway, was not affected. Isolation of O<sup>6</sup>-MeGT from ethanol-fed and control-diet animals showed a loss of hepatic O<sup>6</sup>-MeGT activity following ethanol consumption. This in vivo decrease in O<sup>6</sup>-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<sup>6</sup>-MeGT activity following ethanol administration (Espina et al., 1988). Furthermore, both rat and human O<sup>6</sup>-MeGT were shown to be significantly inhibited *in vitro* by acetaldehyde concentrations as low as 0.1  $\mu$ M. Ethanol also was observed to inhibit O<sup>6</sup>-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<sup>6</sup>-MeGT preparations (Espina et al., 1988). However, some studies have failed to detect an effect of ethanol on the repair of DMN-induced O<sup>6</sup>-MeG adducts or an inhibition of O<sup>6</sup>-MeGT activity by acetaldehyde at concentrations up to 300  $\mu$ M. The reasons for these conflicting results are not known at present but may be due to methodological differences in the various studies (Garro and Lieber, 1990).

#### Alcohol Induced Sister Chromatid Exchange (SCE)

Lymphocytes of alcoholics exhibit higher incidences of exchange-type aberrations of the chromosome including dicentric and ring chromosomes and chromatid translocations, as compared with controls or nonalcoholics (Obe and

Ristow, 1977). The frequency of sister chromatid exchanges are examined by the bromodeoxyuridine method (Obe and Ristow, 1977). Acetaldehyde, the first metabolite of ethanol, was found to induce SCEs in cells grown in tissue culture. Daily treatment of Chinese hamster cell cultures with concentrations of acetaldehyde ranging from  $0.25 \times 10^{-3}$  to  $1.5 \times 10^{-3}$ % v/v produced a dose-dependent increase in SCEs (Obe and Ristow, 1977). Acetaldehyde also has been shown to induce SCEs in human lymphocytes exposed in vitro (He and Lambert, 1985). Along these same lines, Obe and Ristow (1979) 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. 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 benzo(a) pyrene in hamster lung and itself induces laryngeal tumors in hamsters and nasal tumors in rats (Feron et al., 1982). Consistent with these observations, acetaldehyde, in the presence of ethanol, has been shown to form mixed acetal-

nucleoside DNA adducts and to be mutagenic in a number of test systems (Dellarco, 1988).

#### Alcohol-Suppression of Immune Responses

Many tumor cells display novel surface antigens that, theoretically, should lead to the recognition and elimination of these cells by the immune system (Garro and Lieber, 1990). As there is both epidemiological and experimental evidence linking alcohol abuse and ethanol or its metabolites to the suppression of immune responses, alcohol-associated immunosuppression has been considered for some time as a possible contributing factor in the increased cancer incidence seen in alcohol abusers.

Animal and in vitro studies involving isolating 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 and Mendenhall (1984) reported a significant decrease in lymphocyte response to mitogens after chronic ethanol treatment in guinea pigs. Jerrells and his colleagues (1986) demonstrated that acute ethanol administration in rats resulted in a rapid loss of lymphocytes from spleen and thymus (Jerrells, 1986). Mufti and his researchers, 1988, showed a similar depletion of splenic lymphocytes following chronic ethanol consumption in rats and a change in T-helper to T-suppressor cell ratios (Mufti et al., 1988).

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 (Frizzera et al., 1980). 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 (Stutman, 1979). Nevertheless, the immune system may play a vital role in the defense against virally induced tumors.

## CHAPTER VI

### CONCLUSION

The health hazards of alcohol consumption are a concern and warrant the attention they are receiving. The majority of the public consumes alcoholic beverages, if not regularly, then at least occasionally. Alcoholic beverages are mainly known for their effect on the central nervous system and liver, rather than for the role they play in human cancer. Epidemiologists began increasing the awareness of this latter role by performing case-studies on cancer patients to determine their health-related habits. An association between alcohol consumption and cancer incidence has been consistently established through these case studies.

Given the awareness of this association between ethanol and cancer, scientists began conducting studies to try to find the mechanisms involved in the tumor induction process promoted by alcoholic beverages. Several studies have shown strong evidence to support the opinion that ethanol acts as a promoter in cancer incidence. Other studies dealing with alcoholic beverages effects have shown probable mechanisms for its promotion of cancer in the study subject.

Alcohol acting as a solvent to facilitate the entry of tobacco-related carcinogens into target tissues has been shown to be a possible mechanism. This idea focuses on the action of ethanol on lipid membranes and the possible loss of functionality by the membrane. Several studies have shown that the lipid bilayer's fluidity is changed when it contacts ethanol. This is an attractive mechanism to explain cancers of the oral cavity and esophagus.

Enhanced carcinogen metabolism and activation may explain ethanol's ability to act as a cocarcinogen at remote body sites as well as at ethanol-contact sites. This mechanism relates to ethanol's capacity to act as an inducer of the microsomal cytochrome P-450-dependent bio-transformation system. Several investigators have shown increased metabolic activity, due to ethanol exposure, to convert procarcinogens to mutagens.

Decreased liver metabolism, although contradicting the above mechanism, has also been shown to be a plausible mechanism for alcohol-induced cancer. This mechanism explains ethanol's ability to inhibit the first pass clearance of potential carcinogenic agents. These agents are then allowed to reach sensitive extrahepatic organs and could possibly initiate cancer in these areas.

Cytotoxic and mitogenic effects caused by ethanol could result in cancer. Several scientists have shown chronic ethanol consumption to alter intracellular metabolism, affect DNA expression in cells, and to stimu-

late cell proliferation. These alterations in normal cellular functions could result in cancer incidence.

Alcohol suppression on vitamin A metabolism may act as a promoting affect in cancer. 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. Researchers have shown that alcohol consumption depresses hepatic vitamin A levels therefore interfering with the normal functioning of vitamin A by reducing its abundancy.

Lipid peroxidation has been implicated in promoting the carcinogenic process. Another mechanism shown through experimental data is the effect of alcoholic beverage consumption on lipid peroxidation. Alcohol consumption has been shown to increase lipid peroxidation hence increasing the carcinogenic process.

Alcohol inhibition of DNA-alkylation repair could result in tumor formation. DNA-repair processes are important in protecting cells from chemical carcinogens that alter DNA structure and sequences. Experimenters have shown that alcohol consumption can inhibit the DNA-alkylation repair process, thus making the cell sensitive to carcinogens.

Alcohol has been shown to induce sister chromatid exchange (SCE). Compounds with SCE-inducing activity could theoretically act as promoters by increasing the frequency of SCEs and enhancing the possibility that recessive muta-



tions are expressed.

Finally, alcohol has been shown to suppress immune responses. Studies have shown that immunosuppressed patients or animals exhibit increased cancer incidence.

These mechanisms are all probable designs as to ethanol's role as a promoter in cancer incidence, but much more investigation into these underlying mechanisms must be done. Concentrated efforts in this direction would contribute much to our understanding of the etiology of cancer in humans and could ultimately lead to the prevention of alcohol-related cancer.

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