murine leukemia viruses fail to cross species barriers and seldom infect human cells (*15*). However, recent studies indicate that some laboratory-adapted strains of murine leukemia and sarcoma viruses may infect and propagate in human cells (*15, 16*).

Our studies and others (*6, 7*) indicate that newly isolated field strains of feline leukemia and sarcoma viruses readily infect and propagate in human cells. In addition, we have observed that FSV induces morphological transformation in WI-38 diploid human embryonic lung cultures, similar to that observed in WHE cultures. Antigenic and host-range modifications of FSV do not occur in one passage in WHE cells. The fact that FSV transforms human cells and the accompanying FeLV replicates to demonstrable levels within 7 days, even when diluted to 10^{-6}, demonstrates the extreme susceptibility of human cells to the naturally occurring tumor viruses of the domestic cat.

Dog cells are extremely susceptible to the cell-transforming effects of FSV. They support the growth of feline leukemia and sarcoma virus as well as cat and human cells do (*5–7*). It is noteworthy that the feline C-type viruses are oncogenic in vivo in other hosts; for example, Rickard (*17*) found that FeLV produces lymphosarcoma in dogs and Dienhardt and Thielen (*17*) found that their strain of FSV produces sarcoma in the marmoset. Feline leukemia virus has been found in salivary glands of leukemic cats (*18*). It is therefore conceivable that human cells that are quite susceptible to infection in vitro may have some degree of susceptibility in vivo, such as when the virus is introduced parenterally through a bite or scratch by a viremic cat with or without clinical symptoms of leukemia. Although there is no evidence to implicate feline leukemia and sarcoma viruses in human cancer, further studies are necessary to determine the possible occurrence of some horizontal spread of cancer by this mode.

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10. Whole human embryo cultures were purchased as primary cultures from Microbiological Associates, Inc., Bethesda, Maryland. The karyotype was determined to confirm that they were of human origin. The cells were propagated for two passages and stored in liquid nitrogen in a storage medium containing dimethylsulfoxide, as described by P. S. Sarma and F. Edwards [*Proc. Soc. Exp. Biol. Med.* **125**, 92 (1967)].
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13. The cells were pelleted and fixed in 2 percent glutaraldehyde in cacodylate buffer, *p*H 7.2. They were then postfixed in Dalton's chrome-osmium, dehydrated in methanol and propylene oxide, and embedded in Epon-Araldite. Ultrathin sections, stained with uranyl acetate and lead citrate, were examined with a Hitachi HU 11-E electron microscope.
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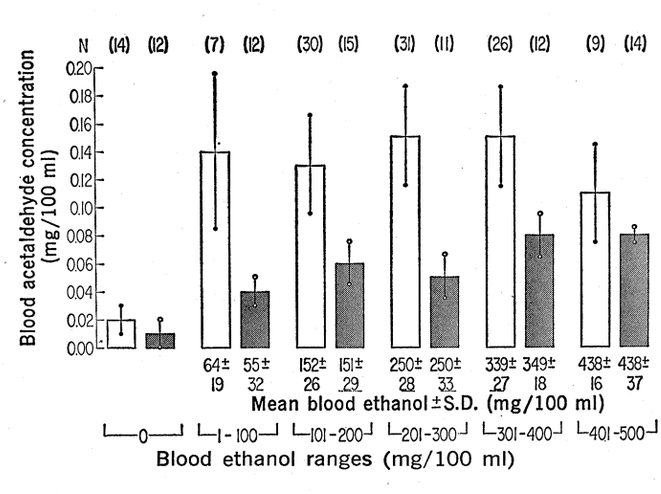
Blood Concentrations of Acetaldehyde and Ethanol in Chronic Alcoholics

Abstract. *Fifteen adult male alcoholic volunteers were studied before, during, and after a 10- to 15-day period of experimentally induced intoxication. Blood acetaldehyde concentrations ranged from 0.11 to 0.15 and from 0.04 to 0.08 milligrams per 100 milliliters when blood ethanol concentrations ranged from 1 to 400 milligrams per 100 milliliters after consumption of bourbon or grain ethanol, respectively. No dose or dose-time relationships were found between blood ethanol concentrations and blood acetaldehyde concentrations during any phase of this study.*

The biological concomitants of acetaldehyde metabolism have been stressed as potentially important factors in the determination of many pathophysiological processes associated with alcohol addiction (*1*). It has been demonstrated that a number of unique effects induced by acetaldehyde are not caused by ethanol alone (*2*), and that acetaldehyde causes significant effects on biotransformation and metabolism of catechol and indole amines (*3–5*). Demonstration that acetaldehyde competitively inhibits 5-hydroxyindoleacetaldehyde oxidation to 5-hydroxyindoleacetic acid (*4*) explains, in part, the mechanism of the increased formation of 5-hydroxytryptophol and methoxyhydroxyphenylglycol (*5*) in man during alcohol ingestion. Another consequence of competitive inhibition may be an accumulation of intermediary aldehydes derived from biogenic amines that would react with the intact amines to form Schiff bases which, in turn, may give rise to various alkaloids such as tetrahydroisoquinoline and tetrahydropapaveroline. Thus it has been suggested that the addictive properties of ethanol may be related, in part, to the concentration of acetaldehyde generated during ethanol metabolism and to the subsequent formation of tetrahydroisoquinolines or tetrahydropapaverolines (*6*).

The rate of acetaldehyde metabolism is quite rapid (*7*), and blood acetaldehyde concentrations are relatively low after short-term administration of ethanol to man (*8*). However, no serial determinations of acetaldehyde concentrations have been reported after long-term administration of high-dosage eth-



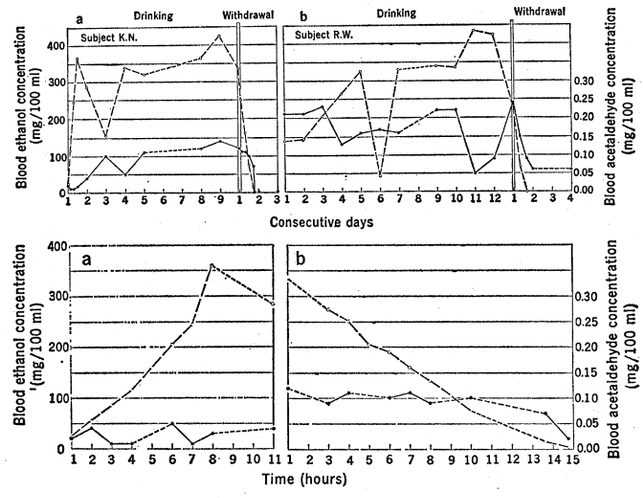


Fig. 1 (left). Blood acetaldehyde concentrations as a function of blood ethanol values. (White areas) Bourbon, 100 proof; (hatched areas) 50 percent grain ethanol (U.S.P.). *N*, number of blood samples. Fig. 2 (upper right). Blood acetaldehyde (——●——) and blood ethanol (------○------) concentrations (a) in subject (K.N.) who consumed 50 percent grain ethanol and (b) in subject (R.W.) who consumed bourbon throughout the drinking period. Fig. 3 (lower right). Blood ethanol (------○------) and blood acetaldehyde (——●——) concentrations in a subject (K.N.) who consumed grain ethanol during (a) the first day of drinking and (b) the first day of withdrawal.

anol. Moreover, relatively few studies have been carried out after long-term administration of ethanol to alcoholic as contrasted to nonalcoholic individuals, especially at the high dose levels found in alcoholic subjects during drinking. The investigation of acetaldehyde concentrations in alcoholic subjects studied in a long-term drinking paradigm is especially important since alcoholics may develop an induced increase in their rate of ethanol metabolism (*9*). We have therefore studied blood ethanol and acetaldehyde concentrations after free-choice drinking in alcoholic subjects.

Blood ethanol and blood acetaldehyde concentrations were determined each day of the study in finger-tip blood samples (0.1 ml). Because chemical and enzymatic methods for the determination of blood acetaldehyde are liable to serious artifacts, we used a modification of the gas chromatographic method of Roach and Creaven (*10*). Modification was necessary because repetitive injections of the supernatant from the $ZnSO_4$-$Ba(OH)_2$ deproteinization, as described by Roach and Creaven, produced gradual accumulation of a solid deposit at the entrance of the column which, in turn, induced an acetaldehyde memory trace in the column. In the standard gas chromatographic injection system we used an injection receptacle 1.6 by 11.2 cm with a perforated interchangeable injection port which was changed after every six to ten injections.

Fifteen adult male volunteers were selected from a group of inmates in an alcohol rehabilitation facility. The volunteers had been in the institution for at least 1 week before the study and had ingested no alcoholic beverages during that period of time. The subjects ranged in age from 26 to 49 and had a history of alcoholism of 12 to 36 years' duration. Most subjects were spree drinkers accustomed to consuming about 1 quart (0.946 liter) of whiskey per day. All subjects were free from detectable disease. The subjects were admitted in groups of four to six patients to a research ward and were placed on a standardized 2000-calorie-a-day diet and given multivitamin supplements daily. After 7 days of acclimation to the research ward a 10- to 15-day drinking period was initiated during which subjects were permitted to consume each day up to 32 ounces of a 100-proof beverage alcohol (bourbon) or 50 percent grain ethyl alcohol on a free-choice basis. After cessation of the drinking period the subjects remained in the research ward for 7 to 10 days. At the time of discharge they showed no evidence of withdrawal signs or of intercurrent illness.

Blood acetaldehyde concentrations of the subjects as a function of blood ethanol concentrations after consumption of 50 percent grain ethanol or bourbon are shown in Fig. 1. Mean acetaldehyde concentrations before the onset of drinking were 0.02 and 0.01 mg per 100 ml for subjects who consumed bourbon or grain ethanol, respectively. For blood ethanol concentrations that ranged between 1 and 400 mg per 100 ml after consumption of bourbon, blood acetaldehyde concentrations averaged 0.11 to 0.15 mg per 100 ml. During grain ethanol consumption, blood acetaldehyde concentrations for the subjects were approximately one-half these values for similar ranges of blood ethanol concentrations. No significant dose-response relationship was observed between ascending blood ethanol concentrations and blood acetaldehyde concentrations, and mean blood acetaldehyde concentrations were relatively constant through all dose ranges of ethanol.

The difference between blood acetaldehyde concentrations observed when subjects were ingesting bourbon as contrasted with grain ethanol cannot be accounted for in terms of different mean blood ethanol concentrations. Mean blood ethanol concentrations for subjects who consumed grain ethanol and for those who consumed bourbon were either identical or extremely similar throughout all dosage ranges. The difference between the blood acetaldehyde concentrations of the subjects who consumed bourbon and of those who consumed grain ethanol could be accounted for by the presence of acetaldehyde and other congeners in bourbon. Indeed, the acetaldehyde concentration in bourbon, as determined by gas chromatographic techniques, was 53 mg/liter. Higher blood acetaldehyde concentrations in bourbon drinkers may also be due to competitive inhibition of aldehyde dehydrogenase by alde-

hydes derived from alcohols of higher molecular weight which are present in bourbon. After such inhibition the resultant delay in the oxidation of acetaldehyde would be reflected by its accumulation in blood. The average daily consumption of bourbon per drinker was 649 ml which contained 34 mg of acetaldehyde. If we assume that bourbon was consumed only during a 12-hour period per day, the dose of administered acetaldehyde would be 0.041 mg per kilogram per hour (0.49 mg per kilogram per 12 hours). However, in previous studies on humans (*11*) 124 to 164 mg of acetaldehyde was infused during 6 to 8 minutes with no significant effects.

Figure 2 presents representative data for two subjects who consumed grain ethanol and bourbon for 10 and 13 consecutive days, respectively. Although blood ethanol concentrations peaked above 400 mg per 100 ml and were sustained above 300 mg per 100 ml for long periods of time, blood acetaldehyde concentrations remained relatively stable throughout the course of long-term drinking.

Figure 3 presents consecutive hourly blood ethanol and blood acetaldehyde concentrations for a subject who consumed grain ethanol. On initiation of grain ethanol intake, blood ethanol concentrations rose to 360 mg per 100 ml after 8 consecutive hours of drinking. However, blood acetaldehyde concentrations remained relatively low with no dose or dose-time relationship to ascending blood ethanol concentrations. On the first day of withdrawal from alcohol, blood ethanol concentrations fell from 335 mg per 100 ml to almost 0 within 14½ hours. No descending dose or dose-time relationship was found between blood ethanol and blood acetaldehyde concentrations immediately after cessation of drinking.

The findings of this study indicate that some effects of the presence of acetaldehyde observed in animal and in in vitro experiments may also occur in alcoholics during long-term drinking. For example, in vitro kinetic studies (*4*) with rat brain mitochondrial aldehyde dehydrogenase revealed an inhibition constant K_I for acetaldehyde of $2.62 \times 10^{-6} M$, whereas the Michaelis constant K_m for 5-hydroxyindoleacetaldehyde was $5.44 \times 10^{-6} M$. These data suggest that a similar type of inhibition may occur in the human brain during long-term alcohol intake with the blood acetaldehyde concentrations ($5 \times 10^{-5} M$) reported here. However, the concentrations of acetaldehyde reported here are relatively low in comparison to dose levels used by a number of other investigators in studies of intermediary metabolism and biotransformation of catecholamines (*2, 3*).

Both tolerance and physical dependence have dose and dose-time relationships to blood ethanol concentrations (*12*). Such dose-response relationships were not found for blood acetaldehyde concentrations reported here. However, it remains to be determined if sustained elevations in acetaldehyde concentrations at neural tissue sites may be related to the addictive process in alcoholism.

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Ochratoxin A: Inhibition of Mitochondrial Respiration

Abstract. *Ochratoxin A is a fungal metabolite which induces pathological changes in animals. The toxin was isolated from cultures of* Aspergillus ochraceus *and purified by thin-layer chromatography. Ochratoxin A and one of its hydrolysis products, dihydroisocoumarin, severely inhibited coupled respiration when applied at low concentration to rat liver mitochondria.*

Ochratoxin A is a fungal toxin produced primarily by certain isolates of *Aspergillus ochraceus* Wilh. (*1*), although its production by a species of *Penicillium* has also been reported (*2*). On acid hydrolysis, it yields L-phenylalanine and dihydroisocoumarin (*3*) (Fig. 1).

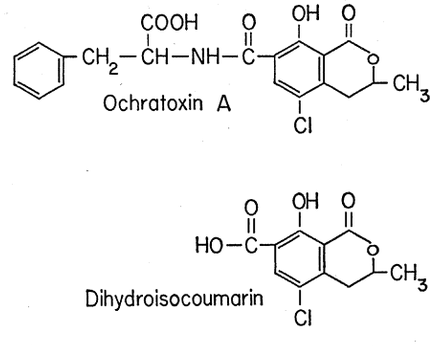

Fig. 1. Structure of ochratoxin A and the dihydroisocoumarin derivative (5-chloro-3,4-dihydro-8-hydroxy-3-methylisocoumarin-7-carboxylic acid) (*3*).

Aspergillus ochraceus is widely distributed in nature and has been isolated from soils, decaying vegetation, and animal feeds, and from foodstuffs intended for human consumption (*1, 4*). In culture, the fungus will synthesize the toxin in quantity (*5*). Ochratoxin A was reported to be a natural contaminant (110 to 150 ppb) of poor-grade corn in storage (*4*).

Both ochratoxin A and feeds infested with toxin-producing strains of the fungus are highly toxic to experimental animals (*6, 7*). The toxicity of a single dose of ochratoxin A toward ducklings is similar to the toxicity of aflatoxin B_1 (a liver poison produced by *A. flavus*) (*1*). Pathological changes were found in the liver cells of day-old ducklings (*6*) 4 hours after the ducklings were given 100 μg of crystalline ochratoxin A. Mild fatty infiltration of