Very Very Important !!

Book

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# BIOCHEMICAL PHARMACOLOGY OF ETHANOL

abstract pp 131

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Methanol
Withdrawl
Ethanol
Alcoholic
Woman
Formaldehyde

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first of the two structurally-identical sites of the dimer, affects conformation of the other site (93), thus showing that the two sites become non-equivalent as soon as the first ligand is bound and, therefore, not independent.

f. Ternary Complexes: Significance and Use. Pyrazole (94), isobutyramide (82,95,96) and *o*-phenanthroline (97,98) are well known inhibitors of ADH. Pyrazole forms a ternary complex with horse ADH and NAD (85) but not with NADH; a similar complex appears to be formed with the human enzyme (11). Isobutyramide is competitive with aldehyde and ketone but not with alcohol substrates (95, 96) and forms ternary complexes with reduced but not oxidized nucleotides. These differences between pyrazole and isobutyramide indicate that the alcohol and aldehyde binding sites of ADH are not the same. To explain this inhibitor differentiation between substrate and product, Theorell (6) proposed a mechanism involving alternate points of attachment for oxidized and reduced forms of pyridine nucleotides. How this is brought about in the process of catalysis is not yet understood but a change in the conformation of the active site during the hydride ion transfer is a possibility (126). Ternary complexes of ADH with pyrazole and isobutyramide serve as models of the catalytic ternary complexes; and because dissociation constants of these complexes are low, they are useful for the determination by titration of ADH normality. *o*-phenanthroline inhibits ADH by complexing $Zn^{++}$ at the active site (96) and is a competitive inhibitor with respect to coenzyme; this inhibitor competes with the nicotinamide (97) but not with the adenosine-diphosphate-ribose moiety (98) of the coenzyme.

g. Substrate Specificity. ADH has a broad substrate specificity, including primary and secondary (but not tertiary) saturated aliphatic and aromatic alcohols and corresponding aldehydes and ketones. The substrate specificity of horse (79,80,101,102,103) and human liver ADH (37,104), with respect to the above compounds, have been adequately reviewed (6,9). Substrate specificity of rat liver ADH resembles that of horse and human enzymes (41,42).

In 1965 (37), it was first reported that methanol was a substrate for the human ADH. It is now established that methanol is also a substrate for the horse ADH (105) as well as for the rat ADH (42). The maximum velocity $V_{max}$ with methanol as substrate for the human and horse liver ADH is similar (105). 2,3-unsaturated alcohols (17,12) and polyenoic alcohols such as farnesol (106) or retinol (13,16) are substrates for all three enzymes. Cyclic secondary alcohols such as cyclohexanols and cycloheptanols, but not cyclopentanols, are reversibly oxidized by liver ADH (9,107). Other substrates for ADH include nitro-substituted benzyl alcohols (108), N-alkylated aminoalcohols such as 2-dimethylaminoethanol at pH 9.5 (109) but not at pH 7.0, glycerol (14), pantothenyl alcohol (20),

as would be the case with the closed-circuit perfusion system employed in our experiments (Table 3). For example, lactate concentrations reach 5 mM and the albumin employed contains significant amounts of fatty acids. Oshino *et al.* (38) showed that the rates of hydrogen peroxide production were 10.2 μmoles/g/h at 30° in the presence of lactate and fatty acids between the ethanol concentration range of 0.05 and 5.0 mM. Higher rates would be predicted at the temperatures employed (37°) in our perfusions and if any hydrogen peroxide-generating substrates (such as glycolate or urate) were generated metabolically during the perfusion (rates up to 45 μmoles/g/h at 30° with 1 mM urate (36)), and at higher ethanol concentrations (39). Also, the possibility that xanthine oxidase generates hydrogen peroxide from acetaldehyde at higher ethanol concentrations cannot be ruled out at this time. Until exact rates of hydrogen peroxide production can be determined at high ethanol concentrations, this problem of stoichiometry will remain unresolved.

While these data clearly indicate that catalase is important in ethanol metabolism in perfused rat liver at high ethanol concentrations, one must be cautious in extrapolating these findings to other species. For example, methanol oxidation in monkeys was shown to be much more pyrazole sensitive than in the rat (60), suggesting that catalase may be more important in rat than in other species. However, before precise quantitation of the role of ethanol metabolizing systems in different species can be made, titrations of the type shown in Table 3 need to be performed in several species, especially man.

a. Activation of Ethanol Utilization by Hydrogen Peroxide-Generating Substrates. Boveris, Oshino and Chance (37) have shown that various compounds were effective activators of hydrogen peroxide generation in various compartments of the cell. For example, antimycin A was shown to activate hydrogen peroxide production in mitochondria, menadione activates hydrogen peroxide production by the endoplasmic reticulum (43), and hypoxanthine, a substrate for xanthine oxidase, should generate hydrogen peroxide in the cytosol. The addition of these agents to perfused liver, as well as the addition of hydrogen peroxide, did not significantly alter the rate of ethanol utilization in the presence of 4-methylpyrazole (Table 1). However, the addition of substrates for the peroxisomal hydrogen peroxide-generating flavoproteins glycolate oxidase, urate oxidase (40), and D-amino acid oxidase (41) significantly activated the rate of ethanol utilization at high concentrations of ethanol (25 to 50 mM; Table 2). This activation of ethanol metabolism could prove significant clinically. As glycolate and urate are toxic compounds, D-amino acids show the most promise as potential activators of hepatic ethanol metabolism.

3. Competitive Inhibitions of Enzyme Catalyzed Reactions

A number of metabolic effects produced by ethanol and its metabolic derivatives stem from their competition with endogenous substrates for the enzyme active sites. Typical examples of such competitive inhibition will be briefly discussed.

a. Accumulation of Methanol in Ethanol-Drinking Subjects. As will be discussed in greater detail later in this chapter, the accumulation of methanol in blood and urine has been demonstrated to occur in all human subjects and nonhuman primates studied during prolonged periods of consumption of alcoholic (ethanolic) beverages (10,11,12,13). Although traces of methanol are found in breath and urine of healthy human subjects (14), methanol does not accumulate normally because it is metabolized rapidly. Moreover, although both methanol and ethanol can be metabolized by the same enzyme systems, the kinetics of the reactions differ. The studies of Mani, Pietruszko and Theorell indicate that alcohol dehydrogenase oxidizes methanol at approximately one-tenth of the rate of ethanol (15). With $K_m$ values of $6.9 \times 10^{-3} M$ and $1.5 \times 10^{-3} M$, for methanol and ethanol respectively, as found for isolated human liver alcohol dehydrogenase, concentrations of ethanol above these amounts would severely inhibit methanol oxidation. This, in fact, corresponds with that found in the blood of alcoholic persons ($1.5 \times 10^{-2} M$ to $4.3 \times 10^{-3} M$) (10). Thus, during prolonged periods of drinking of large quantities of alcoholic beverages, alcohol dehydrogenase and other contributing enzyme systems are primarily utilized in the metabolism of ethanol resulting in the competitive inhibition of the oxidation of methanol. In view of the above observation, it is postulated that one of the physiological functions of alcohol dehydrogenase under normal conditions is the oxidative destruction of endogenously formed methanol.

b. Biogenic Amine Condensation Products. It is well known that aldehydes react with biogenic amines to form Schiff's base (17) intermediates which, in turn may give rise to such condensation products as tetrahydroisoquinolines, tetrahydro-β-carbolines and alkaloid precursors such as tetrahydropapaverolines (18,19,20). Thus, it has been suggested that the addictive properties of ethanol may be related in part to the concentration of acetaldehyde generated during the metabolism of ethanol and to subsequent formation of various compounds, some of them similar to opium alkaloids (18,20, 21,96). To fulfill the requirement for the formation of such alkaloids, it would be essential that the proper reactants, i.e., aldehydes derived from biogenic amines, would accumulate in the body in the presence of pharmacological concentrations of ethanol or acetaldehyde. Kinetic studies with rat brain mitochondrial aldehyde dehydrogenase revealed that $2.6 \times 10^{-6} M$ acetaldehyde competitively

Furthermore, since ethanol and other short chain aliphatic alcohols do not interfere with the oxidative system of brain mitochondria (54,98) but do suppress the respiratory activities of brain slices to the same extent at physiologically compatible concentrations (38,40), it was concluded by several authors (38,40,55,44) that the site of ethanol inhibition of metabolism is probably associated with that part of the metabolic system that is dependent on the normal functioning of neuronal cell membranes. Thus, ethanol's inhibition of cerebral metabolism probably has little direct relation to the peripheral or central metabolism of ethanol. The effects of ethanol on neuronal membrane and CNS functions have been extensively discussed by Kalant (99) and Grenell (55) and the reader is referred to these excellent reviews (Also see Hunt; this volume).

Finally, it may be inferred that the inhibition of various pathways of metabolism in the liver is directly related to the rates of the metabolism of ethanol. The more rapid the rate, the greater the restriction on the availability of coenzymes (NAD,CoA) for general metabolism. In the brain, however, where ADH activity is extremely low, the access to NAD by endogenous substrates is not much impeded and does not inhibit the cerebral metabolism in this manner.

## D. BLOOD CONCENTRATIONS OF ETHANOL, ACETALDEHYDE, ACETATE AND METHANOL DURING ACUTE AND CHRONIC ADMINISTRATION OF ALCOHOLIC BEVERAGES IN HUMANS AND ANIMALS

Alcohol consumed *via* the gastrointestinal tract is mostly absorbed into the blood and is distributed throughout the body depending upon the water content of particular tissues. About 90 to 95% of the administered ethanol passes through the liver where it is metabolized to acetaldehyde and, subsequently, to acetic acid. As was indicated earlier (Sections B and C), the inhibitory effects of ethanol on liver metabolism are the consequence of its own metabolism in the liver. Since the metabolism of ethanol in the brain is insignificant (53,38,44), any effects on brain metabolism due to peripheral metabolism of ethanol would be mediated through the metabolites of ethanol released from the liver and transported into the brain *via* the blood stream. Furthermore, the concentrations of ethanol in the brain and in other organs of the body depend primarily on the concentration of ethanol in the blood. The kinetics of ethanol absorption, distribution and elimination have been discussed thoroughly in an excellent review by Kalant (56); therefore, this section will be directed towards the concentrations of ethanol and related substances in the blood of alcoholic subjects during long-term alcohol intake which has been recently studied in this laboratory (10,13,41).

1. Subjects and Methods

In these studies (10,13,41) adult male alcoholic volunteers were admitted to a research ward in groups of four to six patients and were placed on a standardized 2000 calorie diet and given multivitamin supplements daily. Following the period of acclimation to the research ward, a 10 to 15 day drinking period was initiated. After cessation of the drinking period, the subjects remained on the research ward for seven to ten days. At the time of discharge they showed no evidence of withdrawal signs or intercurrent illness (10,41).

Daily determinations of blood ethanol and either blood acetaldehyde or methanol or acetate were carried out throughout the course of the studies. The determinations were done using fingertip blood. The blood samples were treated with zinc sulfate and barium hydroxide and the gas chromatographic analyses of the supernatant fractions were carried out by using an automated modification (57) of the method originally described by Roach and Creaven (58). The blood acetaldehyde concentrations were determined using the manual setting of the machine.

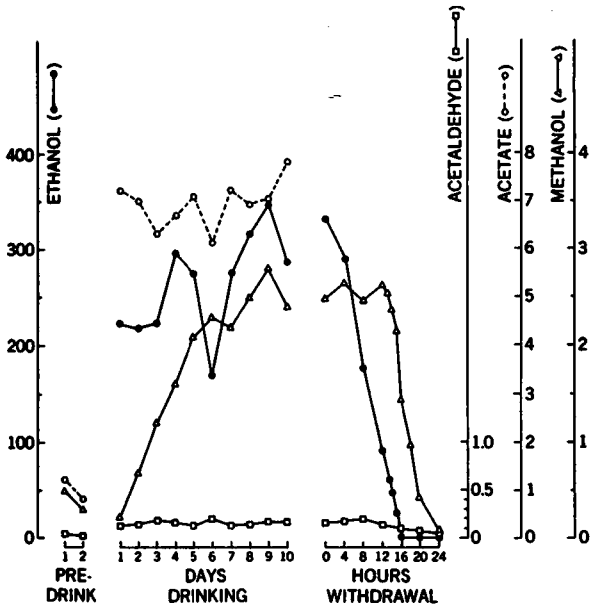


*FIGURE 1:* The concentrations of ethanol, acetaldehyde, acetate and methanol in the blood of an alcoholic subject consuming bourbon throughout the drinking period. All concentrations are expressed in milligrams per deciliter (mg/dl) of blood.

2. Blood Ethanol Levels

Throughout the entire period of drinking, the subjects were permitted to consume up to 1 liter of beverage alcohol (bourbon) or 50% grain alcohol daily on a free choice basis. During the continuous drinking periods lasting from 7 to 15 days, the daily blood ethanol levels fluctuated between 50 and 500 mg/dl and usually were high enough to sustain a significant degree of clinical intoxication (10,41).

3. Acetaldehyde

a. Methodological Considerations. The first step in the metabolism of ethanol is the formation of acetaldehyde. Since acetaldehyde suppresses a number of metabolic processes in isolated body organs and tissue preparations *in vitro* it has been suggested that a number of effects usually associated with either acute or chronic administration of alcoholic beverages are mediated by acetaldehyde generated during ethanol metabolism (54,98,40,87,60). It is well known that acetaldehyde metabolism is quite rapid and occurs at a faster rate than the oxidation of ethanol to acetaldehyde (See B.V. Plapp, This volume). Thus, it could be predicted that very little acetaldehyde would be detected in blood following ingestion of alcoholic beverages. Although it has been shown that blood acetaldehyde levels are relatively low following acute administration of ethanol (59,74, for review see Truitt, 60), historically two major problems related to the methods of study render the older data difficult to interpret in the light of recent findings (60,41,64,61).

First, most studies which assessed acetaldehyde levels in man have been carried out following acute administration of ethanol. No serial determinations of acetaldehyde levels have been reported following chronic high dosage ethanol administration. Moreover, relatively few studies have been carried out following chronic administration of ethanol to alcoholic as contrasted to non-alcoholic subjects in whom the blood ethanol levels have usually been lower than those which obtain in chronic alcoholic subjects during long-term drinking episodes. Thus, no dose response data for acetaldehyde were available which were relevant to the sustained high blood ethanol levels that exist in an alcoholic subject through continuous or intermittent spree drinking. The investigation of acetaldehyde levels in alcoholic subjects studied in a chronic drinking paradigm is especially important since it has been reported that alcoholic subjects may develop an induced increase in their rate of ethanol metabolism (62,63,77) which may be reflected in the higher levels of acetaldehyde in the blood.

Secondly, the chemical and enzymatic techniques employed for measurement of acetaldehyde in biological fluids, particularly blood, have been confounded by a number of serious artifacts. Chemical as well as chromatographic methods are subject to interference by acetone or by "bound" acetaldehyde which is released by ethanol or protein precipitating agents (e.g., tungstic acid, trichloracetic acid) (64,65). The enzymatic methods, although sensitive, may be affected by other aliphatic aldehydes present in the biological fluids. The most recent sensitive and specific techniques are various methods employing gas chromatography (66,67,58,68,64), which permit a simultaneous determination of ethanol, acetaldehyde and other compounds usually found in blood during ethanol consumption. However, the gas chromatographic methods are also subject to various experimental difficulties which may produce serious artifacts (64,65). Furthermore, it has been found that direct injection of a single sample of whole blood into the chromatograph will lead to permanent contamination of the column. The organic deposit remaining after whole blood injection appears to induce dry distillation effects which subsequently produce a large acetaldehyde memory trace for each subsequent injection (41).

A typical example of artifactually high blood acetaldehyde levels associated with the method of determination is shown in two reports by the same authors (69,42). In the earlier report (69) when acetaldehyde was determined using the microdiffusion method of Burbridge (70) the apparent blood acetaldehyde levels ranged between 0.7 and 2.2 mg/dl. However, when the studies were repeated and acetaldehyde was determined using the gas chromatographic technique of Roach and Creaven (58) the blood acetaldehyde levels ranged between 0.1 and 0.3 mg/dl (42). The latter results are in the range reported by others using the same methods for the determination of acetaldehyde.

b. Nonenzymatic Formation of Acetaldehyde. The original observation by Truitt (64) that acetaldehyde is released in the presence of protein precipitating agents or ethanol has been studied recently by Sippel (61,65). He reported that this acetaldehyde is formed by a nonenzymatic oxidation of ethanol by means of a semidehydroascorbate peroxy radical chain derived from the oxidation of ascorbic acid. It should be also noted that this nonenzymatic formation of acetaldehyde is dependent on temperature (64) and may be an important concern for some methods that measure acetaldehyde. For example, if the head space method is used, the perchloric acid (PCA) tissue extracts are incubated at 55° or 65°C in the presence of ethanol (67,64,61,65) increasing the likelihood of measuring artifactually high concentrations of acetaldehyde. These artifacts associated with the temperature increase during the incubation of the samples can be overcome by handling the samples at low temperatures (ice cold) as described in the method by Roach and Creaven (58).

The nonenzymatic formation of acetaldehyde was particularly pronounced with the PCA extracts of fresh liver homogenates (61,65, 71) and was insignificant in the blood plasma (61,65,71,72). It has been reported by Sippel (61,65) that this nonenzymatic formation of acetaldehyde in perchloric acid extracts is completely inhibited by thiourea, which prevents the autoxidation of ascorbic acid (73,65).

4. Acetaldehyde: Human Investigations

a. Chronic Studies. Studies of blood acetaldehyde levels and blood ethanol levels in alcoholic subjects during long-term, free choice drinking of either bourbon or grain alcohol revealed that blood ethanol levels peaked above 300 mg/dl and were sustained above 300 mg/dl for long periods of time (41). The blood acetaldehyde levels remained relatively steady throughout the entire course of 10 to 13 consecutive days of drinking of either alcoholic beverage. During the first eight hours of drinking of grain alcohol, the blood ethanol levels increased to 360 mg/dl and during the following 10 day drinking period blood ethanol concentrations were maintained above 300 mg/dl. Upon withdrawal, blood ethanol concentrations fell to zero within 14 hours. There was no significant relationship between blood acetaldehyde concentrations and blood ethanol concentrations during the ten day period of drinking when blood ethanol concentrations were sustained above 300 mg/dl and the blood acetaldehyde concentrations averaged between 0.1 and 0.2 mg/dl ($2.3 \times 10^{-5} M$ and $4.5 \times 10^{-5} M$). Although slightly higher levels of acetaldehyde in the blood were found at the end of the drinking period than on the first day of drinking, the differences were within the limits of experimental variation (41). It should also be noted that in these studies (41) the earliest blood samples were usually taken several hours after the initiation of the alcohol drinking period when blood ethanol levels were sufficient to saturate the ADH's activity. This is in contrast to most other studies, both in humans and in the experimental animals, where the determinations of blood and breath acetaldehyde levels were done during the first few hours after administration of ethanol.

b. Acute Studies. After acute administration of relatively low doses of ethanol (0.5 and 0.75 g/kg) both to alcoholic and nonalcoholic subjects, the blood acetaldehyde levels were quite variable between the subjects (74). These concentrations did not correlate well individually with the time curve of ethanol, but there was a rough parallelism between the mean values (74). There were considerable differences between those of alcoholic and nonalcoholic subjects. The mean values of the blood acetaldehyde

levels averaged 108 and 73 ng/ml 60 minutes after administration of ethanol to alcoholic and nonalcoholic subjects, respectively (74).

Freund and O'Hollaren (75) found that after single doses of 0.5 ml of ethanol per lb. body weight (0.73 g/kg) the acetaldehyde concentrations in alveolar air increased rapidly and reached a plateau within 1 or 2 hours. After 6 to 7 hours when the concentrations of ethanol declined from 40-60 to 15-20 µg/dl of air, the acetaldehyde concentrations declined rapidly from 700 to 200 ng/dl. This correspondence between the concentrations of acetaldehyde and ethanol in the alveolar air was even more pronounced in fasted subjects. Similar relation between ethanol and acetaldehyde concentrations in blood and expired air were observed at relatively low ethanol concentrations in rats (72,76) and mice (78,79) and in perfused liver (80). Thus, it is apparent that the concentrations of acetaldehyde in the blood and in the alveolar air are reflections of the rates of ethanol metabolism in the liver. During continuous drinking periods associated with high intake of alcoholic beverages or after large single doses of ethanol, the ethanol metabolizing enzymes become saturated in the presence of high ethanol levels. At this point, the metabolism of ethanol conforms to zero order kinetics resulting in the establishment of a plateau in both, blood and alveolar air acetaldehyde concentrations. No apparent dose or dose-time relation response is observed. After low single doses of ethanol, when ethanol metabolism conforms to first order kinetics throughout the entire period of the experiment, the blood acetaldehyde levels parallel the blood ethanol levels.

## 5. Acetaldehyde: Animal Studies

The studies of acetaldehyde formation in animals revealed several new developments both in the methodology and in the scope of the findings in relation to sex, species and alcohol preference. Also, equally interesting were the findings on acetaldehyde concentrations in brain.

a. Methodology. In the area of methodology, Redmond and Cohen (78) introduced a technique of measuring acetaldehyde in the expired air of rodents. This technique consists of placing an animal 15 minutes after administration of ethanol in a glass rebreathing chamber which was flushed with oxygen prior to the initiation of the experiment. The samples of chamber atmosphere were drawn for the gas chromatographic determination of ethanol and acetaldehyde 17 minutes after placing the animals in the chamber.

Another technique for trapping acetaldehyde in expired air was reported by Forsander and Sekki (72) who placed the animal in a horizontal cylinder through which compressed air was passed. The gas was bubbled through an absorption tube containing ice-cold water in which ethanol and acetaldehyde contained in the expired air were extracted. The efficiency of this extraction after passing through a single absorption tube was 84% to 88% for acetaldehyde and 99% for ethanol. The overall efficiency for acetaldehyde absorption was further improved by passing the gas through a second absorption tube. This latter technique is reminescent of that used for absorbing the respiratory $C^{14}O_2$ after the administration of labeled ethanol and other metabolic substrates to rats (69). Carbon dioxide was trapped in two Kiefer absorption columns connected in series containing potassium hydroxide (69). However, none of these methods for the determination of acetaldehyde in the expiratory air takes into account possible contamination of the chamber atmosphere by acetaldehyde released from urine voided by animals during the period of enclosure in the chamber.

b. Sex, Strain and Alcohol Preference. Redmond and Cohen (78) determined acetaldehyde in expired air after intraperitoneal administration of ethanol (4 g/kg) and found that the mean acetaldehyde level for male mice were about five times higher than those for female mice. These authors also found that the levels of acetaldehyde formed a plateau within about 20 minutes after administration of ethanol, which corresponds to the time that ethanol levels reached a maximum. The comparison of castrated mice of both sexes revealed that acetaldehyde in males was lower by about 85% but no changes were observed in female mice. No significant differences were observed in the mean ethanol levels between males and females either castrated or normal at any time period. Redmond and Cohen suggest that these differences in acetaldehyde concentrations may be the consequence of the effects of testicular hormones on tissue enzymes or due to differences in the activity on the ethanol metabolizing enzymes. They point to the fact that the microsomal ethanol oxidizing system (MEOS) activity (81) in male rats is greater than that found in females and that similar sex differences in drug metabolizing enzymes have also been reported (Conney) (82).

A recent study by Eriksson (71) involved the metabolism of ethanol and acetaldehyde in both sexes of rats outbred and selected for their ethanol preference (84). He used the AA strain which prefers a 10% ethanol solution to water and the ANA strain which rejects ethanol solution as a drinking fluid. It was calculated from extrapolated elimination rates, that female rats of both groups eliminated ethanol 13 and 28% faster than the males in the ANA and

AA groups, respectively. Furthermore, blood acetaldehyde concentrations were about two to four times lower in the ethanol preferring rats than those in the non-preferring group. Significant differences were observed between males and females of the ANA strain in the blood acetaldehyde concentrations. During the first two hours after administration of ethanol (1.5 g/kg) to females, higher blood acetaldehyde concentrations were observed in the nonpreferring strain as compared to the preferring strain (71).

c. Acetaldehyde in Brain. Although the differences in the concentrations of acetaldehyde in blood and in the expired air of animals are of interest, the studies in brain draw particular attention since such studies are not done in humans. In Table 1 are shown the ranges of acetaldehyde and ethanol concentration in blood and brain after single doses of ethanol and in rats rendered physically dependent upon ethanol. It can be seen that the results from three laboratories (86,42,88) for brain acetaldehyde levels are in good agreement despite the differences in the methods used for the determination of acetaldehyde, the doses of ethanol given to rats or the sex of the animals. Only the results reported by Kiessling (85) are significantly different from those reported by others. An interesting observation has been reported by Sippel (88) that 30 minutes after a single intraperitoneal dose of ethanol (65 mmoles/kg (3g/kg)) to female rats, acetaldehyde concentrations in cerebral blood ranged between 100 and 360 nmoles/ml (0.4 and 1.6 mg/dl). No significant amounts of acetaldehyde were found in brain samples when the cerebral blood concentrations were less than 250 nmoles per ml. It is possible that these variations may be related to the estrus cycle of the female animals. However, the author makes no mention of what point during the estrus cycle the experiments were done or whether this parameter was controlled. These findings suggest that the levels and distribution of acetaldehyde between blood and brain may be significantly related to the sex of the animals.

Preliminary studies in male rats (42) revealed that the blood acetaldehyde concentrations after single intragastric doses of ethanol (g/kg) were similar to those in animals rendered physically dependent upon ethanol. After the termination of ethanol administration, rats had typical withdrawal signs and reactions (rigidity, tremors and convulsive seizures (89), comparable to those observed in alcoholic subjects. At the time of decapitation, blood ethanol and blood acetaldehyde usually ranged between 50 and 400 mg/dl and 0.1 and 0.3 mg/dl, respectively. At high blood ethanol levels the concentrations of ethanol and acetaldehyde in the brain were not significantly different from those found in the blood during the induction of ethanol dependence and subsequent withdrawal period (42). Similar to earlier results from alcoholic subjects (41), no significant correlation was found between the length of ethanol

TABLE 1

BRAIN AND BLOOD LEVELS OF ETHANOL AND ACETALDEHYDE IN RATS

| *Treatment* | *Dose of ethanol (g/kg)* | *Sample (time after ethanol) (hr)* | *Method for AcCHO determination* | *Ethanol* | | *Acetaldehyde* | | *Reference* |
|---|---|---|---|---|---|---|---|---|
| | | | | *Blood (mg/ml)* | *Brain (mg/g)* | *Blood (µg/ml)* | *Brain (µg/g)* | |
| Acute i.p. | 1- 3 | 1.0 | Stotz(83) ($H_2SO_4$-$Na_2WO_4$) | - | - | - | 4.2-7.7 | Kiessling 1962(85) |
| Acute i.g. | 4.7 | 1.0 | Stotz(83) (PCA) | - | - | 2.0 | 2.9 | Ridge 1963(86) |
| Acute TETD* i.p. | 2 | 1.5 | G.C.H.Sp. ($H_2O$-$ZnSO_4$ -Ba $(OH)_2$) | - | 1.22 | - | 9.5 | Duritz & Truitt 1966(87) |
| Acute TETD i.p. | 2 | 1.5 | " | 1.7 | - | 20.0 | - | " |
| Acute i.p. | 2 | 1.5 | " | 1.5 | - | 4.0 | - | " |
| Acute i.g. | 5 | 1-8 | G.C.S. ($ZnSO_4$- -Ba $(OH)_2$) | 1.0-5.0 | 1.0-5.0 | 1.0-3.0 | 1.0-3.0 | Majchrowicz 1973(42) |
| Chronic i.g. | 10-15** | 10-15 | " | 0.5-3.0 | 0.5-3.0 | 1.0-3.0 | 1.0-3.0 | " |
| Acute*** i.p. | 3 | 0.5 | G.C.H.Sp. (PCA) | 3.45 | 3.45 | 4.4-15.8 | 0.5-2.7 | Sippel 1974(88) |

**TETD: disulfiram pretreated; **g/kg/day for 4 days; ***female rats; i.g.: intragastric; i.p.: intraperitoneal. G.C.H.Sp.: gas chromatography, head space; G.C.S.: gas chromatography of supernatant. In parenthesis are shown the blood protein precipitating agents.*

administration period, blood ethanol and blood acetaldehyde concentrations (Figure 1).

Although there are some differences in the findings discussed above, it is apparent that the concentrations of acetaldehyde found in blood and brain are of the same order of magnitude in both ethanol-dependent and acutely treated animals. Therefore, these findings suggest that the observed concentrations of acetaldehyde, derived most likely from the peripheral metabolism of ethanol are not sufficient to produce physiologically significant alterations of the major metabolic pathways in the brain. However, the sustained presence of even low concentrations of acetaldehyde in the brain may have a number of possible toxic effects on brain metabolism and poses a question which should be further investigated.

## 6. Acetate

About 40 years ago, Lundsgard (90) and Leloir and Munoz (33) and others established that the oxidation of ethanol results in the formation of acetaldehyde which is oxidized virtually instantaneously to acetate. The appearance of acetate in animal and human blood following short term administration of ethanol was reported by Forsander and Raiha (35) and by Lundquist (34) who concluded that most of the acetate formed in the liver is released into the blood stream and distributed throughout various organs where it is metabolized to carbon dioxide and water. Recent studies of blood acetate and ethanol concentrations were conducted in alcoholic subjects during free choice drinking periods lasting up to 14 days (91). Upon commencement of drinking, the blood acetate concentrations increased up to 7 mg/dl and remained at approximately this level for the entire drinking period, ranging between 7 and 9 mg/dl. The mean blood ethanol concentrations averaged between 50 and 400 mg/dl. The blood acetate levels were on a plateau and there was no significant dose-response relationship apparent between the blood ethanol and blood acetate concentrations except at very low concentrations of ethanol (Figure 1).

## 7. Methanol Accumulation

The occurrence of endogenous methanol and a variety of other alcohols and aldehydes has been suggested since the turn of the century (92,93). Eriksen and Kulkarni (14) only recently reported that trace amounts of methanol can be identified and accurately determined in human breath samples. Furthermore, an enzymatic formation of methanol from S-adenosylmethionine in animal and human pituitary has been reported by Axelrod and Daly (94). About 20 years ago, it was established (95) that the rationale for treatment of methanol poisoning with ethanol depended on the ability of etha-

nol to competitively inhibit the oxidation of methanol, thus preventing the formation of highly toxic formaldehyde and formic acid. These observations suggested that during long-term consumption of alcoholic beverages, ethanol might competitively inhibit the metabolism of endogenously derived methanol, resulting in the progressive accumulation of methanol in body fluids and tissues. Accordingly, a systematic study of blood methanol levels was undertaken during long-term consumption of alcoholic beverages (bourbon) and grain alcohol.

Consumption of alcoholic beverages was associated with a progressive accumulation of methanol in the blood and urine (10,11), of all subjects. By the end of the first day of drinking, the subjects' blood ethanol levels had risen to 200 to 400 mg/dl. Afterwards, their blood ethanol levels fluctuated daily, but remained high enough to induce and to sustain an observable degree of intoxication until the end of the experiment. Changes in blood methanol levels followed a different pattern. At the initiation of drinking, the blood methanol levels never exceeded 0.1 mg/dl. A pronounced increase in blood methanol to 0.2 mg/dl was recorded at 4 to 8 hours of drinking. After that, the blood methanol level increased progressively to 2 to 4 mg/dl at the end of the experiment lasting up to 14 days (10) (Figure 1).

After the subjects stopped drinking on the withdrawal day, blood ethanol clearance was complete within 10 to 18 hours, depending upon the existing blood ethanol levels at the cessation of alcohol intake. The highest levels of blood methanol were found at the termination of drinking period. After the initiation of the alcohol withdrawal period, blood methanol levels remained relatively stable for about 10 to 18 hours, but when blood ethanol levels decreased to approximately 70 to 20 mg/dl, methanol levels began to decline coincident with the emergence of the withdrawal signs and symptoms. The blood methanol clearance lagged behind the linear disappearance of ethanol by approximately 6 to 8 hours. Complete clearance of the accumulated methanol in grain alcohol drinkers was similar to that in the bourbon drinkers (Figure 1).

The most severe signs and symptoms of the alcohol withdrawal syndrome were observed in those subjects whose blood methanol concentrations were highest and blood ethanol concentrations were approaching zero level. The temporal correlation between the withdrawal signs and symptoms corresponded more closely to methanol rather than to ethanol clearance from the blood (13).

These findings suggest that methanol may accentuate the severity of the alcohol withdrawal syndrome after the termination of long-term consumption of alcoholic beverages. The recent demonstration that long-term administration of ethanol to rats enhances an increased activity of alcohol metabolizing enzymes in the brain (53), sug-

gests that alcohol dehydrogenase may become accessible for the oxidation of methanol during the withdrawal period when blood ethanol has been cleared from the circulation and alcohol dehydrogenase is released from the oxidation of ethanol. This event may result in the formation of formaldehyde, which may in turn react with various biogenic amines in the brain, resulting in the formation of aberrant neurotransmitters. Although the formation of aberrant neurotransmitters has been demonstrated in perfused bovine adrenals in the presence of relatively high concentrations of formaldehyde (96), the final verification of this hypothesis will depend upon the demonstration of formaldehyde formation in the brain of alcohol addicted animals or the isolation of the putative aberrant neurotransmitters in the central nervous system. (For discussion of false neurotransmitters see Chapters by Dr. Alivisatos and by Dr. Smith in this volume).

## SUMMARY

Following the administration of alcoholic beverages, ethanol exerts a number of direct and indirect effects on the body and in turn, ethanol is itself metabolized. Liver and brain are two major organs which are immediately concerned with the effects of ethanol. Ethanol acts as a CNS depressant and as a source of energy. Since the metabolism of ethanol in the liver proceeds at a constant rate until completion, acetate is produced regardless of energy requirements of the body. Thus, ethanol plays the role of an aberrant nutrient.

Although ethanol has no effect on oxygen consumption in the liver, it severely suppresses the production of carbon dioxide in the Krebs cycle resulting in the corresponding suppression of respiratory quotient. This indicates that ethanol diverts the utilization of oxygen for the oxidation of reducing equivalents which accumulate as a consequence of increased formation of NADH. This is reflected in the shift from the oxidative to reductive components of a number of oxido-reductive couples, e.g.: pyruvate-lactate, oxaloacetate-malate and acetoacetate-β-hydroxybutyrate. These actions of ethanol are exarcerbated by the fact that the metabolism of ethanol is also associated with the diversion of the availability of a number of enzymes and coenzymes from the metabolism of endogenous substrates towards the metabolism of metabolites of ethanol, thus resulting in the competitive inhibitions of a number of enzyme catalyzed reactions, e.g., inhibition of methanol metabolism during long-term ethanol consumption resulting in the accumulation of methanol in body fluids; shift in the peripheral metabolism of biogenic amines from oxidative to reductive pathways; and formation of aberrant neurotransmitters (*in vitro*); inhibition of the oxidation of fatty acids in the liver.

Since ethanol inhibits acetate metabolism, it appears that ethanol inhibits its own intermediary metabolism in the liver. The metabolism of glucose carbon *via* the hexosemonophosphate shunt is not significantly affected by ethanol. Only the portion of glucose metabolized through the citric acid cycle is inhibited similarly to that of acetate.

Among the most characteristic biological properties of ethanol are two diverse mechanisms subserving the inhibitions produced in the liver and in the brain. The inhibitory effects of ethanol on liver metabolism are not caused by ethanol *per se* but are the results of its metabolism in the hepatocyte cytoplasm. The inhibitions observed in the brain appear to result from the direct interferance by ethanol with some, as yet not clearly identified, biophysical and/or biochemical interactions with the neuronal plasma membranes.

Acute and chronic administration of alcoholic beverages results in the elevation of blood acetaldehyde and acetate concentrations in human and nonhuman primates and rodents. Accumulation of blood methanol has been observed in primates and to a lesser extent in rodents. At high blood ethanol levels sustained during long term drinking periods no significant dose or dose-time response was observed between blood ethanol levels and blood acetaldehyde levels.

Since ethanol competitively inhibits the oxidation of methanol by alcohol dehydrogenase it is postulated that one of the physiological functions of alcohol dehydrogenase under normal conditions is the oxidative destruction of endogenously formed methanol.

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to be the *sinequanon* requirement for storage. This structural non-specificity of the storage mechanism permits catechol or β-hydroxylated analogues of norepinephrine (even tetrahydroisoquinoline derivatives) (7) to displace the catecholamine (4). Even more drastic distortions of the π-system of phenylethylamine, as possibly occurring in tetrahydroisocarbolines, formed either *in vitro* (8) or *in vivo* (9) are, probably, compatible with the requirements for storage and release.

Among the most convincing evidence of the possibility of a "physiological" exchange of transmitters is that reported by Murphy (10). The experiments were performed in platelets obtained from mentally depressed and normal humans. A comparison of platelets and brain showed that the mechanisms of storage and release of serotonin, as well as the presence of monoamine oxidase (MAO) in the mitochondria, the effects of monoamine oxidase inhibitors (MAOI), reserpine, imipramine, cocaine and lithium are shared by both tissues. A major difference is that platelets do not biosynthesize the amines, which are exclusively taken up from the environment. Platelets, then, offer an ideal system for studies of storage and of the exchange of one type of amine (i.e., serotonin) for another (e.g., catecholamines) after exogenous *in vivo* administration of the $C^{14}$-labeled precursor amino acids (e.g., of L-dopa). In the experiments of Murphy (10), the platelet serotonin content increased as expected on administration of clinically used dosages of L-tryptophan, while it was decreased with L-dopa treatment, suggesting that both of these amine precursors are effectively metabolized to their respective amines by both normal and depressed patients and that both precursors produce effects on cellular serotonin. The critical evaluation of these results by Murphy (10) is that there is a need for combined studies of different neurotransmitters. Administration of one type of neurotransmitter may alter conditions in various aminergic systems, rendering interpretations difficult.

## 2. False Neurotransmitters and Alcoholism

More relevant to this survey are studies related to a possible role of false neurotransmitters in the physical dependence upon alcohol. The ideas for such a correlation stem from Davis and Walsh (11,12) and from the concurrent work of Cohen and Collins (13).

Briefly, these ideas may be summarized as follows: Alcohol, upon injection, is primarily metabolized to acetaldehyde. The latter is further catabolized *via* NAD-linked aldehyde dehydrogenases. Saturation of this system leads to excessive accumulation of aromatic aldehydes produced from endogenous catecholamines or indolamines (i.e., dopamine or serotonin). These "biogenic aldehydes" (11,14) or, acetaldehyde *per se* (15) condense with intact catecholamines or indolamines leading to production of variously substituted alkaloids,

mostly of the tetrahydropapaveroline or tetrahydrocarboline type, or simple tetrahydroisoquinoline derivatives (the methyl-derivative being known as salsolinol) (see *Fig. 1*, Section 2b).

## 3. Formation and Inhibition of Tetrahydroisoquinoline Derivatives

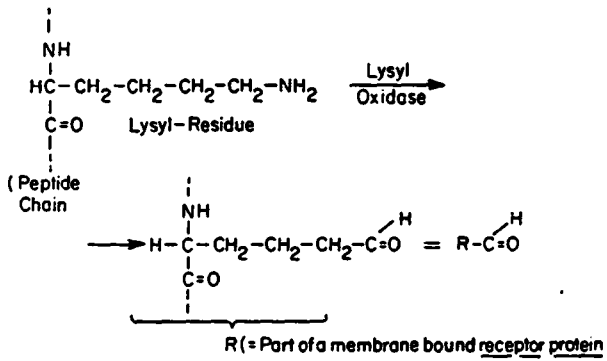
According to these ideas, the problem of physical addiction to alcohol is transposed to addiction to alkaloids - similar to those present in plants (e.g., *Papaver somniferum* [16,17,18]). It is evident, though, that the sequence of reactions described above, if occurring in animals *in vivo* (see below) would not explain the molecular basis of the effect of these or other alkaloids, since the actual mechanism of addiction to morphine or its derivatives is not well understood. Furthermore, reversible or practically irreversible reactions of "biogenic aldehydes", or acetaldehyde, could occur with a number of cellular nucleophils (in the chemical sence), like amines, sulfhydryl groups, quinones or existing imines (substitution) (19).

Similar ideas led one of us (Alivisatos) in 1971 to propose the administration of excess quantities of trapping agents (14,20) which in theory would prevent the Pictet-Spengler condensation. Indeed, it was shown that in the presence of rat brain homogenates, cysteine, at moderate concentrations, may completely arrest the condensation leading to tetrahydroisoquinoline derivatives (14). Trapping of acetaldehyde or other aldehydes occurs, in this instance, through thiazolidine-formation (14,21). Other agents, like ascorbate, may trap the aldehydes through complexing, while penicilamin is expected to substitute other less reactive amines (e.g., bioamines) (14).

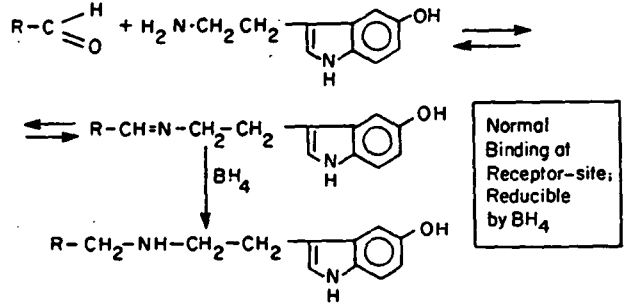
In their recent work, Cohen (7) and Dajani and Saheb (9) demonstrated that salsolinol or tetrahydrocarboline derivatives, respectively, may act as false transmitters and may be released upon stimulation. These findings are interesting and may be relevant to the physical basis of alcoholism, provided that the action of tetrahydroisoquinoline or tetrahydrocarboline alkaloids, as neurotransmitters (?) is quantitatively (4) and qualitatively sufficiently different and of sufficient long duration to impart relatively long-term changes of the synaptosomal membranes (22, see also, 24).

Other pertinent observations are those of Majchrowicz and Mendelson (23,33) who demonstrated ethanol-induced accumulation of methanol in the blood and urine of humans and primates. According to this author, production of methanol is endogenous and its accumulation is due to the fact that both ethanol and methanol are metabolized by the same enzyme system, which, after ingestion of alcoholic beverages is saturated by exogenous ethanol (see also, above, theories of Davis and Cohen). Methanol, after metabolism,

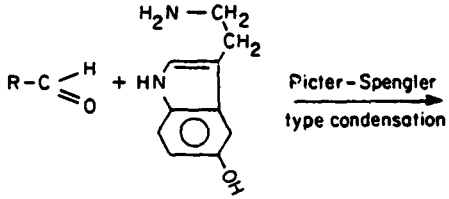
**Reaction 1**



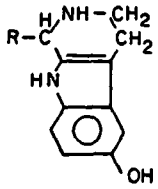

**Reaction 2a**




**Reaction 2b.**

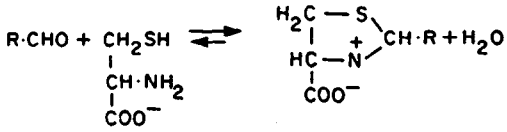



Aberrant (alternative) attachment, leading to membrane-bound tetrahydrocarboline derivatives (No boro-hydride effect)




**Reaction 3**

Trapping of the aldehyde (Canad. J. Biochem. 51, 28, 1973), e g, with cysteine, would prevent (both in vitro and in vivo· Biochem. Pharmacol 22, 1905, 1973) Both reactions (2a and (or) 2b):




(i.e., formation of a thiazolidine derivative of the membrane bound receptor protein).

reacts more readily with properly activated (hydroxylated) aromatic amines in a Pictet-Spengler condensation type of reaction and leads to tetrahydroisoquinoline (13).

In our laboratory, we previously observed that biogenic aldehydes bind to rat brain mitochondria *in vitro* (24). We later demonstrated similar binding to end-membranes obtained by differential centrifugation procedures in sucrose gradients (25). We also observed that binding of biogenic aldehydes or the bioamines *per se* may be prevented by the same trapping agents (e.g., cysteine) as those preventing the Pictet-Spengler condensation (26). Finally, it was clearly demonstrated that inhibition of an NADPH-linked aldehyde reductase by barbiturates (27), together with the ingestion of alcohol (28), leads to extensive binding onto membranous lipoproteins (synaptosomal membranes).

## 4. Newer Concepts Related to the Involvement of Biogenic Amines in Alcoholism

We recently established the existence of lysyl oxidase-like enzymes in 105,000 x g supernatants of beef and mouse brain. Such enzymes, acting in specific sites at the polypeptide level, and converting the ε-amino group of the lysyl-residue to allysine (α-amino adipic δ-semialdehyde, see *Fig. 1*, Reaction 1), has been described by Tanzer (29). This enzyme would be also capable of oxidizing lysyl residues within the specific context of a polypeptide chain, i.e., at the receptor sites, to the corresponding aldehyde or semialdehyde derivative (Alivisatos, Ungar and Arora, Unpublished results).

The binding of serotonin at its binding sites involves Schiff's base formation with its amino group and an carbonyl residue at the receptor sites (30). The formation of aldehyde or semialdehyde by the enzyme lysyl oxidase at the receptor (acceptor) proteins may

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*FIGURE 1:* Reactions demonstrating the possibility of formation of "bound" or "free" tetrahydrocarboline derivatives (R = a specific peptide sequence containing lysyl residues in a way suitable to serve as substrate to α-lysyl-oxidase like enzyme, or a free 5-OH-indole-3-acetaldehyde generated by the action of MAO upon 5HT). *Similar reactions would, obviously, occur with catecholamine-derivatives. 1, the lysyl-oxidase reaction, as it would occur in connective tissue (29); 2a, participation of imines at the level of receptor-binding; 2b, Pictet-Spengler type of condensation; 3, prevention of the Pictet-Spengler condensation through "trapping" of the aldehydes (free or protein bound) by thiazolidine formation.*

account for the carbonyl residues previously postulated at the receptor sites (30). The gross nature of the binding areas are shown to be a combination of protein and lipids. These sites consist of protein core, embodied in the membranous lipid layer, which serves not only as a supporting base, but also as a constraining agent shaping the protein at the tertiary configurational level and thus imparting its specificities (see *Fig. 1*, Section 2a).

At an early stage the binding of intact amines with their receptors is completely reversible, confirming with the basic requirement for a neurotransmitter. Later, however, *in vitro* studies, changes may ensue at the receptor sites similar to those occuring *in vivo* at the onset with endogenously formed biogenic aldehydes. The biogenic aldehydes, however, bind irreversibly from the onset, and as shown in *Fig. 1* (Reactions 2a and 2b) they may lead to *in situ* (i.e., at the end-synaptosomal membranous level) Pictet-Spengler type of condensations, with local alkaloid formation. Recent work involving $C^{14}$-labeled cysteine and monoamine oxidase inhibitors of the hydrazide type (31) (i.e., Iproniazid or Catron) confirmed this possibility of endogenous *in situ* alkaloid formation, e.g., of specific (receptor) areas of synaptosomal membranes. In this instance, interaction takes place between biogenic aldehyde formed by the action of monoamine oxidase on bioamines and ε-amino group of lysyl residues at specific receptor sites (*Fig. 1*, Section 2b). The prevention of binding of biogenic amines or their derivatives, i.e., biogenic aldehydes, by cysteine (*Fig. 1*, Section 3) leading to thiazolidine formation of the protein under consideration or by hydrazides (Catron, Iseniazid), strongly supports this view (Alivisatos, Arora and Ungar, Unpublished results). It was also shown that various inhibitors of the attachment of aldehydes on to the receptor protein compete among each other, e.g., it is possible to suppress thiazolidine formation by hydrazine derivatives leading to corresponding hydrazones.

This *in situ* (i.e., at the end-membranous level) condensation will not only explain previously experienced difficulties in detecting free alkaloid in the cerebrospinal fluid or in excreta, but it will also throw some light on the molecular mechanism of addiction. This "weeding", so to say of the synaptic membranes, is expected to have far reaching repercussions upon the permeability (to ions) and electrical properties of membranes.

The relevance of such changes to physical dependence upon alcohol may be obvious. As a final word of precaution, we should always keep in mind the admonitions of M. Victor (32) who suggests that the majority of the workers in this field often forget that the symptomatology and etiology of alcohol intoxication is different from that of addiction and from that of withdrawal with its multiple symptomatology.

SUMMARY

The possible involvement of false neurotransmitters in the biological aspects of addiction to alcohol has been reviewed and discussed. Current evidence is somewhat ambiguous, although suggestive, of a cause-effect relationship between possible metabolic products of biogenic amines (i.e., tetrahydroisoquinoline derivatives etc.) and addiction. A novel hypothesis of the mode of action of these derivatives developed on the basis of experiments in the reviewer's laboratory is also discussed. According to the latter hypothesis, alkaloid formation may occur *in vivo* at the membranous level *in situ*, by interaction of indoleamines and (or) catecholamines with the products of polypeptide chains and thereby modifying the properties of plasmalemmal membranes.

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# INTERACTION OF BIOGENIC AMINES WITH ETHANOL

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Ethanol ingestion produces physiological responses which suggest increased sympathetic activity. Heart rate and systolic blood pressure usually rise while sweating and flushing often occur. The euphoria and garrulousness also suggest central sympathetic activation. Experiments in volunteers treated with a depletor of norepinephrine, α-methyltyrosine, and then ethanol, show a lessening of these commonly observed effects of the drug.

Serotonin, a putative neurotransmitter thought to regulate sleep function, has also been implicated as a mediator of some effects produced by ethanol. Notable is the fragmentation of sleep patterns during ethanol ingestion. Furthermore, volitional drinking of ethanol solutions is greatly modified by drugs which reduce serotonin levels. Research on the pharmacology and biochemistry of ethanol, therefore, includes the influence of this drug on biogenic amine activity and metabolism.

This article reviews some changes in monoamine catabolism induced by ethanol and its catabolite, acetaldehyde. These changes encouraged much speculation about the pharmacological effect of such

altered catabolism. More recent and provocative studies have claimed an important role in alcoholism for alkaloids arising from the condensation of monoamines with an aldehyde derived catabolically from the parent amine or by cyclization with acetaldehyde produced from ethanol. Methanol has also been found in blood of subjects consuming large doses of beverage alcohol or ethanol for prolonged periods of time. When oxidized to formaldehyde it can also react with monoamines to produce isoquinoline or carboline derivatives. An attempt will be made to consider the merits of some of these hypotheses relating the etiology of alcohol abuse to alkaloid formation.

Some new data supports the view that ethanol may have a mode of action quite distinct from other narcotic drugs such as methadone or pentobarbital. These data indicate that the respiratory depression induced in the mouse by a moderate single dosage of ethanol is mediated primarily by serotonergic pathways. In contrast, serotonin plays no apparent role in the respiratory depression induced by methadone or pentobarbital whereas increased noradrenergic activity deepens the respiratory depression induced by pharmacological dosages of methadone. These significant differences strongly suggest a unique mode of action for one of the depressive functions of ethanol. Serotonergic mechanisms may also operate in volitional drinking of ethanol solution by inbred strains of rodents and perhaps in the lethal effect of ethanol. These findings by others will be discussed in the light of data to be presented.

## 1. Effects of Ethanol on Monamine Metabolism

Large amounts of ethanol have been reported (Gursey, and Olson, 1960) to diminish the serotonin and norepinephrine levels in brain stem of rabbit. This reserpine-like effect of ethanol could not be confirmed by others (Effron and Gessa, 1961). Many subsequent investigations of monoamine metabolism and turnover have been completed since publication of these earlier works. Much of this newer material has been reviewed recently (Feldstein, 1973, Majchrowicz, 1973). Only those reports which are relevant to the issues raised in this and other sections of the article will therefore be discussed.

Monoamine catabolism has been under intensive study for perhaps the last 25 years. In the early part of this century, chemists such as F. Ehrlich were already aware that tyramine was converted to the corresponding alcohol, tyrosol, in yeast culture. Oxidation of tyrosol to phenylacetic acid was thought to be the mechanism by which tyramine was ultimately catabolized to the acid. The correct sequence was described subsequently by Blaschko: deamination of tyramine by the enzyme, monoamine oxidase to yield the intermediary aldehyde. This compound was then either oxidized by aldehyde dehydrogenase to the acid or reduced to the corresponding alcohol by a

reductase. For most β-substituted ethylamines, oxidation to the acid appears to be the preferred route. However, β-hydroxylated ethylamines such as norepinephrine are almost equally metabolized into the acidic and corresponding alcohol forms (Smith and Gitlow, 1967). The glycol, 3-methoxy-4-hydroxyphenylglycol, is the major catabolic product of norepinephrine in the central nervous system of man and in the peripheral tissues of the rat.

Disulfiram (Antabuse$^{R}$) inhibits aldehyde dehydrogenase, an enzyme responsible for oxidation of intermediary aldehydes derived from the alcohols and from the biogenic amines. That this compound can alter the metabolism of tryptamine was first demonstrated in 1960 (Smith and Wortis, 1960a). Rats treated orally with disulfiram and then injected with tryptamine produced significant amounts of typtophol whereas control rats excreted only indoleacetic acid. In the control guinea pig, O-methylated norepinephrine was catabolized equally to vanillylmandelic acid (VMA) or to 3-methoxy-4-hydroxphenylglycol (MHPG). However, disulfiram shifted the catabolic sequence towards a preponderance of the glycol (Smith and Wortis, 1960b). Human subjects treated with disulfiram, 0.5 g daily, for control of alcoholism, excreted markedly increased labeled MHPG after infusion with dl-norepinephrine-7-$H^3$ (Smith and Gitlow, 1967). The ingestion of alcohol by healthy volunteers produced identical shifts in oxidative catabolism of the infused norepinephrine (Smith and Gitlow, 1967). In this study, the duration of shift in oxidative catabolism corresponded closely to the dose of alcohol ingested. Since acetaldehyde is produced at a constant rate regardless of the dose of alcohol, (Majchrowicz and Mendelson, 1970) the competitive inhibition by acetaldehyde of aldehyde dehydrogenase (Majchrowicz and Lahti, 1969) is likely to be the mechanism responsible for the shift. Once the ethanol was metabolized the ratio of the acidic to reduced forms returned to normal. Aspects of this work have been reported independently by others (Davis, *et al.* 1967a; Ogata, *et al.* 1971).

Ethanol affects the metabolism of serotonin in a qualitatively similar way (Davis, *et al.* 1967b). Serotonin is converted to 5-hydroxytryptophol instead of the corresponding acid in keeping with the observation that simple ethylamines are primarily oxidized to their corresponding acids.

Dopamine, a substrate of monoamine oxidase is initially catabolized to 3,4-dihydroxyphenylacetaldehyde. In animals treated with ethanol, the intermediary aldehyde derived from dopamine did not form increased amounts of the corresponding alcohol despite the finding of reduced oxidative catabolism. These investigators (Davis and Walsh, 1970) have suggested that the alkaloid tetrahydropapaveroline may have been produced. Disulfiram treatment causes qualitatively similar changes in monoamine catabolism. In animals treated with disulfiram, MHPG has been demonstrated to increase (Smith and Gitlow, 1967) in amounts equal to the loss determined for the

oxidized acidic form. Perhaps cyclization of dopamine with acetaldehyde to produce salsolinol accounts for the absence of the reduced form in ethanol-treated animals.

## 2. Aberrant Neurotransmitters: Hypothetical Role in Alcoholism

Morphine and related alkaloids form in the poppy plant, *Papaver somniferum*, from condensation reactions of dihydroxyphenylalanine or dopamine. The formation of opium alkaloids by this route had been predicted more than a half century earlier by Robinson (1955) and confirmed by Leete (1959) using labeled compounds.

It is interesting that the pressor catecholamines when given in relatively large dosages produce a morphine-like narcosis in animals (Rothballer, 1959) and analgesia in man. Tolerance quickly develops to the narcotic effect. Both morphine and epinephrine also produce transient cataracts in the lenses of mice (Smith, 1963). Prior ethanol injection strongly potentiated the cataractogenic effect of epinephrine. Because acetaldehyde shifted norepinephrine catabolism to the reductive pathway it was considered that ethanol might also permit the intermediary aldehyde to enter alternate catabolic pathways, notably, condensation with the unaltered norepinephrine to produce a tetrahydropapaveroline. This compound might be responsible for the somnolence produced in the rabbit by relatively large intravenous dosages of norepinephrine, (2 mg/kg). The dosage of morphine required for a similar response is about 10 mg/kg.

In order to demonstrate some pharmacological identity between the catecholamines and morphine, studies were undertaken of cross-tolerance to the cataractogenic effect of two drugs, epinephrine or levorphanol, the latter a strong opioid. Mice made tolerant to cataractogenic effect of levorphanol were found not to be tolerant to the same effect produced by epinephrine (Smith, 1963). Conversely, epinephrine-tolerant mice were not cross-tolerant to the cataractogenic effect of levorphanol. Clearly, the two drugs shared no common cataractogenic mechanism. Further study showed that phenoxybenzamine, the α-adrenergic antagonist, blocked the lenticular effect of epinephrine but not of levorphanol. Similarly levallorphan, the narcotic antagonist, inhibited only the cataractogenic effect of levorphanol and not that of epinephrine.

These early studies in the mouse revealed significant differences in the mechanisms responsible for epinephrine or levorphanol-induced cataracts and for tolerance development. But it may be argued that isoquinoline condensation products from epinephrine do not bear sufficient structural resemblance to opioids to act at identical receptors. In an attempt to identify aberrant distribution, uptake or catabolism of the catecholamine, a metabolic study (Kaplan, *et al.* 1963) was undertaken in rabbits made tolerant to large

intravenous dosages of norepinephrine. Tolerance developed after two weeks. The rabbits were then infused with dl-norepinephrine-7-$H^3$ for a one hour period. At the end of this time blood samples were taken at frequent intervals. No differences in rates of norepinephrine disappearance in tolerant as compared to control rabbits were observed. Uptake into the peripheral tissues also did not vary except in a group treated with reserpine, a drug known for its ability to profoundly diminish uptake.

Catabolites of the infused dl-norepinephrine-$^3H$ were determined in urines obtained one hour after start of the infusion. Scans of the paper chromatograms revealed all of the known catabolites but with significant increase in the percentages of unknown catabolites in the urines of norepinephrine-tolerant animals. Since the rate of metabolism and tissue uptake of unchanged dl-norepinephrine-7-$^3H$ was not different in the tolerant animal than in control, the finding of additional unknown compounds was attributed to nonspecific reactions. These new compounds may have formed from the stores of unlabeled norepinephrine taken up from the large dosages of previously injected norepinephrine. Because of seemingly slight peripheral changes in norepinephrine metabolism, tolerance development was attributed to some change in the central rather than peripheral nervous system. What this change might be has not been adduced but the mechanism is unrelated to morphine tolerance since puromycin treatment did not prevent tolerance development to epinephrine.

Incubation of dopamine (1.25 mg/ml) in rat brain homogenate, in the presence of acetaldehyde (0.5 to 2 mM) was reported (Davis and Walsh, 1970), to yield tetrahydropapaveroline (THP). This compound is credited with playing a possibly important etiological role in alcoholism since tetrahydropapaveroline is a precursor of morphine and morphine is an addictive drug. Barbiturates and chloral hydrate also show some pharmacological similarity to ethanol. Interestingly, barbiturates inhibit a NADPH-dependent aldehyde reductase of brain (Tabakoff and Erwin, 1970). Although norepinephrine catabolism would theoretically be shifted by barbiturates towards oxidative pathways it has been reported (Davis, 1971) that phenobarbital actually enhances formation of THP-type alkaloids. Thus, the cross-tolerance exhibited by ethanol, chloralhydrate and barbiturate may be explained by a common biochemical denominator, THP.

Simple aldehyde derivatives of catecholamines are also said to play a causal role in the induction and maintenance of alcoholism (Cohen, 1973). Such compounds include salsolinol, an isoquinoline (TIQ) derived by non-enzymatic reaction of acetaldehyde with dopamine *via* a Schiff base formation with spontaneous ring closure. Synthesis of such compounds was demonstrated in the isolated cow adrenal perfused with formaldehyde or acetaldehyde. At so-called

physiological levels of acetaldehyde (100 µg/dl) (Cohen, 1971) TIQ was found in chromaffin granules using $C^{14}$ labeled acetaldehyde. The TIQ was released in the same fashion as the endogenous catecholamines (Greenberg and Cohen, 1972).

Labeled TIQ was subsequently found to be taken up and concentrated in synaptosomes isolated from rat brain. In addition, uptake of norepinephrine was inhibited by the TIQ salsolinol. Uptake of ($H^3$-TIQ) into sympathetically innervated tissues was also recently reported (Cohen, *et al.* 1972). These studies were done in rodents treated with 6-hydroxydopamine in order to pharmacologically destroy much of the peripheral sympathetic system. Other studies were performed in partially denervated rats or in mice treated with such inhibitors of monoamine uptake mechanisms as desipramine or cocaine.

The formation, uptake and release of TIQ compounds *in vivo* indicate that such substances can act as false transmitters in place of norepinephrine. The first question that may be asked is whether these compounds affect sympathetic nerve function in a quantitatively meaningful way. The second question relates to the postulated role of TIQ substances in the development of alcoholism or to their involvement in the autonomic disturbance that accompanies the withdrawal state. It is known that large doses of drugs capable of acting as false transmitters can have adverse effects on blood pressure regulation. These include metaraminol, a potent sympathomimetic agonist and octopamine, a naturally occuring phenylethanolamine whose concentration in tissues may be increased with the use of monoamine oxidase inhibitors. Neither of these drugs when given acutely or chronically produce symptoms associated with ethanol withdrawal. Such treatment has not been reported to initiate a withdrawal syndrome or a sudden urge to consume beverage alcohol.

## 3. Serotonergic Regulation of Respiratory Depression Induced by Ethanol

Most central nervous system depressants inhibit respiratory function by diminishing sensitivity of the central carbon dioxide chemoreceptor. As a consequence, $pCO_2$ in blood rises with a concomitant fall in pH. Ethanol or sodium pentobarbital also increase blood $pCO_2$ when given in dosages substantially less than the dosage required for anesthesia. We have measured the respiratory depression caused by these drugs in mice during depletion and repletion of one or more of the neurotransmitters. We found (Smith, *et al.* 1974) that drugs which depleted serotonin, blocked the rise in blood $pCO_2$ normally produced by ethanol whereas respiratory depression produced by methadone or by pentobarbital remained unchanged. Ethanol was further distinguished; the intracerebral injection of norepinephrine decreased the ethanol-induced rise in $pCO_2$ while increasing blood $pCO_2$ in mice treated with methadone. The elevated $pCO_2$

found in mice treated with pentobarbital was unaffected by intracerebral norepinephrine injection.

## 4. Discussion and Critique

According to Davis and Walsh, (1970) catecholamine condensation products may play a role in etiology of alcoholism. The acetaldehyde derived from ethanol enhances formation of THP which is a precursor of morphine. Morphine is addictive. Therefore, alcoholism can be explained in terms of opiate addiction.

Most authorities as well as heroin addicts I have interviewed agree that no cross-dependency can be shown between morphine and ethanol. Furthermore, the withdrawal syndrome from ethanol differs radically from that seen in morphine dependency. On a biochemical level, it is possible *in vitro* to promote THP formation by disulfiram treatment. Yet, neither laboratory animals nor man show the least sign of alcoholic withdrawal nor opiate dependency while treated with doses of disulfiram sufficient to substantially block aldehyde dehydrogenase (Smith and Gitlow, 1967). Acceptable evidence that THP is addictive or plays a role in alcoholism should demonstrate that administration of these compounds causes behavioral tolerance to ethanol and also requires that discontinuance of the drug is followed by the signs of alcohol withdrawal.

It has been suggested (Davis, 1973) that barbiturates show cross-tolerance with ethanol because they block aldehyde reductase and by so doing enhance THP formation. Since THP is a precursor of morphine it is difficult to understand why is there no cross-tolerance of morphine with either ethanol or barbiturates. Perhaps cross-tolerance between ethanol and barbiturates arises from a diffuse cortical latent hyperexcitability of the cortex which may arise from several brain sites, whereas subsequent latent hyperexcitability focuses on the final common pathway. This would explain the similarity between abstinence syndromes of ethanol and barbiturates. Respiratory depression induced by ethanol seems specifically dependent on the presence of serotonin. Neither methadone nor pentobarbital require this neurotransmitter. Ethanol preference also appears to require the presence of this neurotransmitter (Myers and Martin, 1973). While THP and morphine alkaloids bear a close biosynthetic relationship no clear pharmacological relationship exists between ethanol and morphine.

Isoquinoline compounds (TIQ) readily form *in vitro* from catecholamines and the acetaldehyde derived from oxidation of ethanol. Such compounds have been demonstrated in chromaffin granules after perfusion of the isolated cow adrenal (Cohen, 1973) with high concentrations of acetaldehyde (100 mg/dl). These substances may be

incorporated into the synaptosomes and released upon nerve stimulation. As such they qualify as "false transmitters". Such compounds have been considered as etiological agents in alcoholism. As previously discussed, TIQ's have pharmacological activity, and full replacement of norepinephrine in sympathetic stores by TIQ's would no doubt seriously alter the function of the sympathetic nerves. It seems quite a large step, however, to consider that such a replacement and subsequent release of the TIQ's would result in an acute abstinence syndrome. Recent studies in mice (Goldstein, 1973) and rats (Majchrowicz, 1973, 1974) suggest that the withdrawal syndrome is dependent entirely on the previous maintenance of high blood-alcohol levels (BAL). Acetaldehyde is not produced more rapidly with high rather than with low blood ethanol levels. (Majchrowicz and Mendelson, 1970). It seems, therefore, that ethanol itself is the drug which causes physiological dependency and not acetaldehyde.

The high incidence of hepatic cirrhosis in some Europeans indicates a large ethanol intake. However, chronic consumption of dilute ethanol, as in wine, does not inevitably lead to obvious dependency and withdrawal despite the concomitant presence of significant amounts of acetaldehyde derived from the beverage alcohol. While it is clearly impossible to dismiss biogenic amine catabolites as etiological factors in alcoholism, abuse of ethanol appears to be basically a complex social disorder with serious medical consequences. To attribute the origins of this disorder to aberrant catabolism of biogenic amines has an instant appeal because of its simplicity and novelty. Let us hope that a more complete understanding of the mode of action of ethanol on the central nervous system, as compared with other depressants, will provide some additional insight into the etiology of alcohol abuse and alcoholism.

## SUMMARY

Ethanol through its primary catabolite, acetaldehyde, competitively inhibits oxidation of aldehyde dehydrogenase substrates. As a consequence biogenic amines form increased quantities of alcohols rather than the corresponding acids. During this biotransformation, condensation reactions between deaminated and intact amines may occur which can yield tetrahydropapaverolines. These compounds are closely related to precursors of opioids which is cause to link ethanol abuse to morphine addiction. There is, however, no pharmacological or clinical evidence suggesting similarities between ethanol dependency or opioid addiction.

Acetaldehyde plays an additional role in alkaloidal formation *in vitro*. Biogenic amines may react with acetaldehyde to form isoquinoline or carboline compounds. Some of these substances have significant pharmacological activity. Furthermore, they may enter

neural stores and displace the natural neurotransmitter. Thus, they can act as false neurotransmitters. Some investigators believe that chronic ethanol ingestion leads to significant formation of such aberrant compounds which may then upset autonomic nervous system balance. This disturbance may explain the abnormal sympathetic activity seen in withdrawal.

While these ideas about the etiology of alcohol abuse have a definite appeal, they are naturally based on *in vitro* preliminary work. Much study of the quantitative pharmacology of these compounds in animals is required before judgement can be made as to the merits of the proposed hypotheses.

In the meantime, pharmacological studies on the ability of ethanol to depress respiration in the mouse has revealed that unlike opioids or barbiturates, respiratory depression induced by ethanol requires the presence in brain of serotonin. This neurotransmitter also mediates the respiratory effects of several other alcohols but curiously, not chloral hydrate, yet this compound is purported to alter biogenic amine metabolism much like ethanol.

Thus, the response to ethanol can be pharmacologically separated from other major narcotic classes such as opioids and barbiturates by respiratory depression effects. The specific requirement for serotonin mediation exhibited by ethanol and several other alcohols opens the door for a rational therapeutic approach to the treatment of alcohol abuse. At the same time, this finding tends to lessen the probability that alcoholism is in some way connected with the formation of addictive alkaloids.

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