Very Very Important !!

Book

BIOCHEMICAL PHARMACOLOGY OF ETHANOL

abstract pp 131

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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.

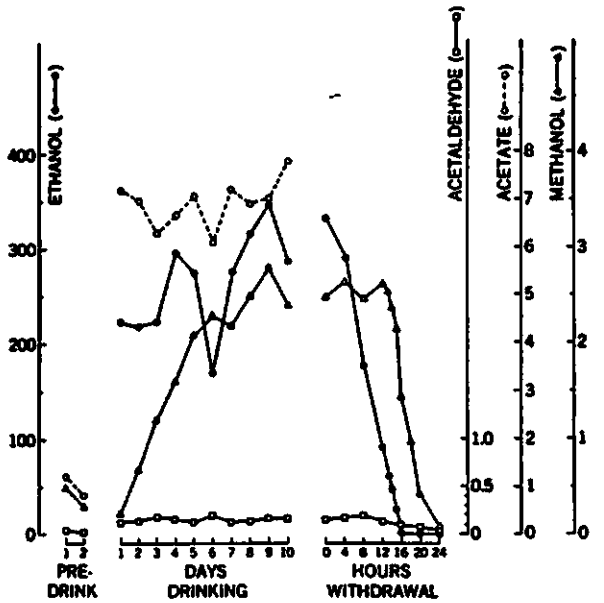


FIGURE 1: The concentrations of ethanol, acetaldehyde, acetate and methanol in the blood of an alcoholic subject consuming bourbon throughout the drinking period. All concentrations are expressed in milligrams per deciliter (mg/dl) of blood.

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.

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

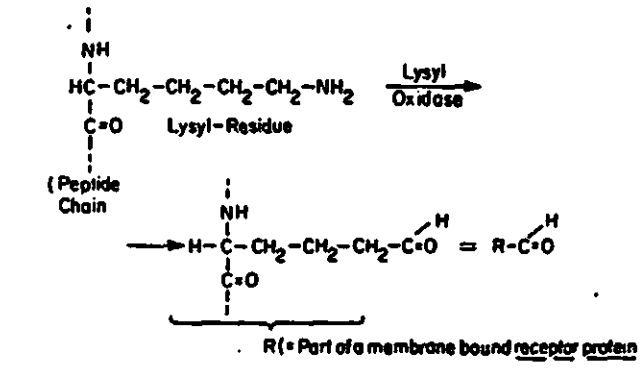
According to these ideas, the problem of physical addiction to alcohol is transposed to addiction to alkaloids - similar to those present in plants (e.g., *Papaver sominiferum* [16,17,18]). It is evident, though, that the sequence of reactions described above, if occurring in animals *in vivo* (see below) 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).

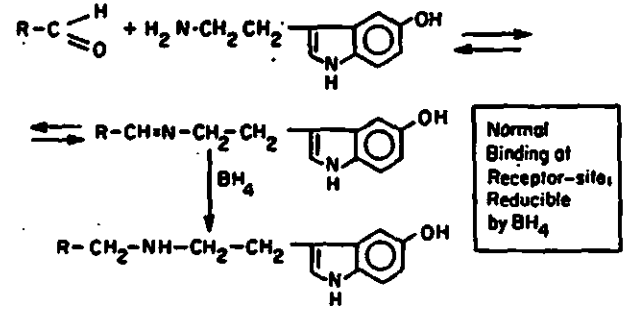
In their recent work, Cohen (7) and Dajani and Saheb (9) demonstrated that salsolinol or tetrahydrocarboline derivatives, respectively, may act as false transmitters and may be released upon stimulation. These findings are interesting and may be relevant to the physical basis of alcoholism, provided 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,

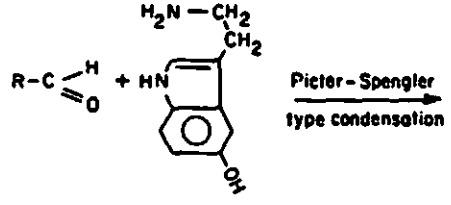
Reaction 1



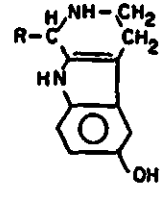
Reaction 2a



Reaction 2b.

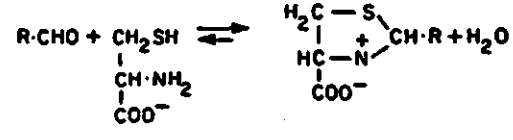


Aberrant (alternative) attachment, leading to membrane-bound tetrahydrocarboline derivatives (No boro-hydride effect)



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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REFERENCES

1. Carlsson, A. and Lindqvist, M.: *In vivo* decarboxylation of alpha-methyl-dopa and alpha-methyl tyrosine. *Acta Physiol. Scand.*, 54: 87-94, 1962.
2. Day, M.D. and Rand, M.J.: Awakening from reserpine sedation by alpha-methyl-dopa. *J. Pharm. Pharmacol.*, 15: 631-632, 1963.
3. Kopin, I.J.: False adrenergic transmitters. *Ann. Rev. Pharmacol.*, 10: 377-394, 1968.
4. Kopin, I.J.: Unnatural amino acids as precursors of false transmitters. *Fed. Proc.*, 30: 904-907, 1971.
5. Alivisatos, S.G.A. and Seth, P.K.: Current approaches in the study of receptors in the CNS. In: Methods in Neurochemistry, R. Fried, (ed.), *Marcel Dekker, Inc.*, 2: 205-273, 1971.
6. Muscholl, E. and Maitre, L.: Release by sympathetic stimulation of α-methyl noradrenaline stored in heart after administration of α-methyldopa. *Experientia*, 19: 658-659, 1963.
7. Cohen, G.: Tetrahydroisoquinoline alkaloids, uptake, storage and secretion by the adrenal medulla and by adrenergic nerves. In: Alcoholism and the Central Nervous System. F.A. Seixas and Suzie Eggleston, (eds.), *Ann. N.Y. Acad. Sci.*, 215: 116-119, 1973.

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

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Biochemical Pharmacology of Ethanol. 1973;1-275.

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 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 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.