BIOCHEMICAL ASPECTS OF ALCOHOLISM

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SUMMARY
(1) The peripheral metabolism of alcohol has implications for CNS function beyond removal of ethanol from the circulation. (2) The activity of many enzymes is altered because of the presence of ethanol and acetaldehyde or because of the increased NADH/NAD ratio. Examples of this are found in cholesterol, steroid hormone, and neurotransmitter metabolism. CNS metabolism and function may in turn be altered by these peripheral events. This concept is termed "amplification" since a relatively inactive compound, such as acetaldehyde, may give rise to much more potent endogenous aldehydes by virtue of inhibition of the metabolism of these aldehydes. (3) The chemistry of ethanol preference, CNS depression, and addiction are reviewed, with emphasis on the enzymology involved. The importance of establishing dose-response relationships in this area is stressed.

INTRODUCTION
The biochemistry of alcoholism is an elusive concept but, for purposes of definition, I will focus on those aspects of the acute or chronic effects of alcohol that involve some identifiable enzyme system. In order to limit this review, the discussion will be on new or particularly promising areas of research, and the other aspects will be mentioned only for sake of completeness and correlation.

The review will be broken into two parts, (1) peripheral metabolism of ethanol as related to the CNS and (2) more direct effects of ethanol in the CNS.

PERIPHERAL METABOLISM OF ETHANOL AS RELEVANT TO CNS EFFECTS
The 'Golden Fleece' for many investigators in this area has been the 'natural substrate' for alcohol and aldehyde dehydrogenases. Both of these enzymes are (1) essential to ethanol metabolism, (2) have a very broad substrate specificity and (3) are present in relatively large amounts in peripheral tissues. Faced with large amounts of ethanol and acetaldehyde, the normal functions of both alcohol and aldehyde dehydrogenase must be forfeited until the concentrations of ethanol and acetaldehyde decrease. In the meantime, however, endogenous alcohols and aldehydes must wait their turn and thus their concentrations increase. Here, at least two things can be envisioned: either the increased concentration of these endogenous alcohols and aldehydes allow for abnormal reactions, or the deficiency of their normal metabolic products leads to altered biochemical or physiological responses. The term 'amplification' has been applied to this situation since the presence of acetaldehyde, for example, may lead to an accumulation of an endogenous aldehyde of much greater potency than acetaldehyde itself (Deitrich & Erwin, 1975).
(a) **Metabolic pattern**

Ethanol is primarily metabolized in the liver by alcohol dehydrogenase, although this enzyme does exist in other tissues (von Wartburg & Eppenberger, 1961; Erwin & Deitrich, 1972). The amount of this enzyme in the brain is exceedingly small and no function has yet been proven for it (Raskin & Sokoloff, 1968). Liver alcohol dehydrogenase catalyzes the oxidation of ethanol to acetaldehyde and the simultaneous reduction of NAD to NADH (Fig. 1). The enzyme is localized in the cytosol and, because it has a relatively high $K_m$ (von Wartburg, 1971), it is functioning at its maximal capacity at relatively low blood alcohol concentrations. The rate limiting step in this process then becomes the rate at which the liver can regenerate NAD from NADH. In order to accomplish this, pyruvate is reduced to lactate by NADH and lactate is spilled into the blood. Lactate itself may have CNS and metabolic effects (Pitts, 1969).

Besides NADH, the other product of alcohol dehydrogenase action is acetaldehyde, and many investigators feel that this molecule may be more damaging than ethanol itself (Korsten, Matsuzaki, Feinman & Lieber, 1975; Deitrich & Erwin, 1975). The enzymes that oxidize acetaldehyde and other aldehydes are located in the mitochondrial, cytosolic and endoplasmic reticulum regions of the cell (Deitrich, 1966). Under normal circumstances, it is the enzyme in the mitochondrial matrix that is effective in oxidizing acetaldehyde because it is present in relatively large amounts and has a very low $K_m$ (Grunnet, Quistorff & Thieden, 1973; Tottmar, Pettersson & Kiessling, 1973; Deitrich & Siew, 1974). In spite of this, some acetaldehyde remains in the cytosol. The cytoplasmic aldehyde dehydrogenase is not as well suited to oxidize small amounts of acetaldehyde. It has a relatively high $K_m$ and is present in smaller amounts (Deitrich, Collins & Erwin, 1972). The acetaldehyde, which escapes oxidation, is free to diffuse into the blood or bring about its toxic effects.

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**Fig. 1.** Alcohol dehydrogenase reaction. Alcohol$_1$ is ethanol, aldehyde$_1$ is acetaldehyde and acid$_1$ is acetic acid. Aldehyde$_2$ is glyceraldehyde which can be generated from fructose, and alcohol$_2$ is glycerol. The darkness of the lines is roughly proportional to the rates of the various steps. Normally, dissociation of NADH from the enzyme is rate limiting.
while still in the liver (Korsten et al., 1975). A related group of enzymes are the aldehyde reductases (Erwin & Deitrich, 1972). These enzymes are alcohol dehydrogenases as well, but since they do not oxidize ethanol the name aldehyde reductase has been given to them to distinguish the two types of alcohol dehydrogenases. The relationship of these enzymes to metabolism of endogenous aldehydes and alcohols will be pointed out as the occasion warrants.

(b) **Relationship of liver enzymes to human alcohol metabolism**

Liver alcohol dehydrogenase in humans as well as other animals is a mixture of a number of isozymes (Smith, Hopkinson & Harris, 1973). Some of these isozymes metabolize steroidal alcohols preferentially and will be the subject of a later discussion. There is, however, one particular genetic variant of importance to humans. This is the ‘atypical’ enzyme originally discovered by von Wartburg, Papenberg & Aebi (1965). This enzyme has lower pH optimum, and much greater catalytic activity than the ‘typical’ enzyme. This is probably the result of the replacement of an alanine by a proline residue in the coenzyme binding site (Berger, Berger & von Wartburg, 1974). Strangely enough, these individuals having the genetic variant do not metabolize ethanol significantly faster than those with the ‘typical’ enzyme (Edwards & Evans, 1967; von Wartburg & Schürch, 1968). The explanation is that the rate of NAD regeneration from NADH is the rate-limiting step in the metabolism of alcohol and not the amount of alcohol dehydrogenase. In the Caucasian population only a few per cent of the people have this ‘atypical’ enzyme. In the Japanese population, the percentage is much higher (Fukui & Wakasugi, 1972). A unique twist can be introduced by using these findings. Orientals are known to tolerate alcohol poorly and have a facial flush when it is given (Ewing, Rouse & Pellizzari, 1974). Acetaldehyde is known to cause such a flush as best illustrated in the ‘Antabuse reaction’ during which acetaldehyde levels increase because of inhibition of aldehyde dehydrogenase. We can now postulate a mechanism for this finding. Initially, when alcohol is ingested, the liver NADH/NAD ratio is normal and, perhaps for only a few min, the amount of alcohol dehydrogenase will be rate limiting. If the amount of this enzyme is particularly high, the quantity of acetaldehyde formed will exceed the capacity of the liver to oxidize it. The result is that more acetaldehyde escapes into the blood producing the flush. Unfortunately, all studies carried out so far have examined acetaldehyde concentration in the blood 30–60 min after ethanol intake, missing any large spike of acetaldehyde in the first few min. If this postulate is true we have the unique situation of an *increased* enzyme content leading to a *decreased* ability of an individual to partake of the substrate.*

(c) **Techniques for altering the rate of metabolism of alcohol**

Techniques are now available both to speed ethanol oxidation to some degree and to slow it markedly. Whether or not either of the procedures should be used in alcohol intoxication or for treatment of alcoholism seems to be more a matter of philosophy than science. The arguments in favor of slowing alcohol metabolism with pyrazole or related compounds (Bloomstrand & Theorell, 1970) depend upon the fact that much of the peri-

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* Acknowledgment is due to Prof. J-P. von Wartburg for many useful discussions on this topic.
pheral tissue damage is due to the metabolic alterations accompanying ethanol oxidation. If the rate of ethanol oxidation is slowed sufficiently, this problem will be alleviated. As pointed out previously (Deitrich, 1975), this will result in prolonged and increased blood levels of ethanol, a situation which leads to addiction (Goldstein, 1975). It would be necessary to accompany such a treatment with re-education of the alcoholic patients regarding their drinking habits, a difficult if not impossible task. Another proposal is to accelerate the metabolism of ethanol by various means. Fructose has long been advocated for this purpose. The mechanism behind the effect of fructose is assumed to be production of glyceraldehyde which could function as the second aldehyde in Fig. 1. The short-circuiting of the alcohol dehydrogenase reaction results in the production of 1 molecule of glycerol for every ethanol molecule oxidized by the pathway. Glycerol, being an endogenous molecule, causes little harm. There is debate both as to the mechanism (Grunnet, Quistorff & Thieden, 1973; Lundquist, Damgaard & Sestoft, 1974) and usefulness of this procedure in humans (see Nantel, 1974).

(d) *Effect of ethanol on blood glucose*

Since the brain has unique requirements for carbohydrate as an energy source, the effect of alcohol on blood glucose is of considerable importance. Paradoxically both hypo- and hyperglycemia occur as a result of alcohol intake, depending on the circumstances.

The mechanism of ethanol-induced hypoglycemia revolves around the excess production of NADH as do so many peripheral effects of ethanol. This was reviewed several years ago (Madison, 1968) and the mechanism proposed appears to be well established. In starved animals, including humans, liver glycogen stores are low and maintenance of normal blood glucose depends upon gluconeogenesis from amino acids. Increased NADH/NAD ratios resulting from metabolism of ethanol leads to inhibition of gluconeogenesis at several points. Searle, Shames, Cavalieri, Bagdade & Porte (1974) have recently studied the inhibition of gluconeogenesis in more detail and confirm that all fasted individuals exhibit hypoglycemia after ethanol intake. In individuals of normal weight, the fall in blood sugar following ethanol intake is exaggerated by an increase in peripheral glucose utilization while obese individuals show a less marked increase in glucose metabolism. Thus, obesity provides some protection against alcohol induced hypoglycemia.

The hyperglycemia observed in fed individuals on taking alcohol has been studied for years and is usually attributed to catecholamine release by the adrenal medulla (Stokes, 1971; Arky, 1971). Recently, attention has focused on the insulin response. Dornhorst & Ouyang (1971) found glucose intolerance is produced by 0.5 g/kg of ethanol in normal women. They did not find reduced insulin output and attributed their results to reduced glucose utilization in the periphery. Phillips (1971) previously had observed similar responses, although he had seen a delayed but augmented peak in the insulin response. A decreased peripheral utilization of glucose was also proposed. These results have been confirmed by Sereny, Endrenyi & Devenyi (1975) and Joffe, Seftel & van As (1975). The latter workers, however, find a suppressed insulin response to glucose in alcoholics. Colwell, Feizimer, Cooper & Zuckerman (1973) have studied the effect of alcohol on the insulin response to intrapancreatic cAMP in dogs. Their results suggest that alcohol directly inhibits insulin release mediated by cAMP but that this normally applies only to the
glucagon-induced insulin release. The hyperglycemic response to alcohol has not usually been considered pathological but in view of this recent work, this view may have to be altered. They find that glucose-induced insulin release is \textit{potentiated} by alcohol and must be due to a different response.

\textbf{(e) Effect of ethanol on steroid metabolism}

Cholesterol, as the starting point for all steroid synthesis, is derived ultimately from acetate by a long series of complex reactions. Cholesterol is used to form bile acids in the liver, and in other tissues the steroid hormones are formed. While the interactions of ethanol with cholesterol and steroid hormone synthesis at the level of enzymatic reactions are numerous, the modulation of steroid hormone levels via effects on release of ACTH have received the bulk of the attention. It is worth reiterating, however, that the concept of amplification, defined above, is important here. The interference of ethanol and acetaldehyde with the normal synthesis of these steroids can occur at two levels. The most obvious is the function of these compounds as substrate competitive inhibitors. This type of inhibition will give rise to higher concentrations of endogenous alcohols or aldehydes and decreased concentrations of acids or aldehydes, depending on the point of interference. Inhibition may also occur by direct effect of ethanol, acetaldehyde, acetate or the increased concentrations of endogenous compounds on various enzymes. Presumably, but not necessarily, this inhibition will be noncompetitive. The altered ratio of NADH/NAD will also have an effect on some of these reactions (Table I).

The initial reaction in synthesis of cholesterol is the condensation of 3 molecules of acetyl CoA to produce $\beta$-hydroxy-$\beta$-methylglutaryl CoA (HMG CoA). Acetate molecules formed from ethanol can also contribute (Eskelson, Cazee, Towne & Walske, 1970). This is not surprising, since there is no reason to expect that acetate derived from ethanol will be different from acetate derived from any other source. The next reaction is probably the rate limiting reaction for cholesterol synthesis and also the point of feedback inhibition for regulation. HMG CoA reductase is a NADPH dependent reaction in which an acyl CoA is reduced completely to an alcohol without release of an intermediate aldehyde (Lehninger, 1970). A series of phosphorylations and condensations follow and several pyrophosphateal alcohol esters are formed. All of these compounds can be attacked by a microsomal pyrophosphatase resulting in liberation of the free alcohol. These alcohols serve as substrates for liver alcohol dehydrogenase, and the resultant aldehydes as substrates for at least a liver cytosolic aldehyde dehydrogenase (Christophe & Popjak, 1961; Popjak, 1971; Edmond & Popjak, 1974). The identity of these enzymes with the alcohol and aldehyde dehydrogenases oxidizing ethanol and acetaldehyde is not proven, however. These alcohols are all allyl alcohols, giving rise to the very reactive allyl aldehydes. In some cases such aldehydes have been found to be effective inhibitors of various enzymes, including alcohol dehydrogenase (Rando, 1972). It is likely that the pentenyl alcohols eventually give rise to HMG CoA again, forming a cycle (Popjak, 1971).

A series of condensations beginning with the pyrophosphate esters results in formation of lanosterol. Lanosterol has three methyl groups which are oxidized by microsomal enzymes sequentially to the alcohol, aldehyde and acid before an NAD dependent decarboxylation
TABLE I. POSSIBLE INFLUENCE OF ETHANOL ON STEROID METABOLISM

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Distribution</th>
<th>Expected effect of:</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allyl alcohols</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allyl aldehydes</td>
<td></td>
<td></td>
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<tr>
<td>Allyl acids</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Compounds</td>
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<tr>
<td>R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allyl alcohol</td>
<td>H</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farnesol</td>
<td>C₁₇H₃₀</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Geranil</td>
<td>C₁₅H₂₄</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reaction</td>
<td>Distribution</td>
<td>Expected effect of:</td>
<td>Reference</td>
</tr>
<tr>
<td>----------</td>
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</tr>
<tr>
<td>Lanosterol</td>
<td></td>
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<tr>
<td>Cholesterol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reaction</td>
<td>Distribution</td>
<td>Expected effect of:</td>
<td>Reference</td>
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</tr>
<tr>
<td>Cholesterol</td>
<td>5β Cholesterol 3α,7α,12α,26 Tetraol</td>
<td></td>
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<tr>
<td>5β Cholesterol 3α,7α,12α,26 Tetraol</td>
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<tr>
<td>5β Cholesterol 3α,7α,12α,26 Tetraol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corticosterone</td>
<td>Alcohol</td>
<td>Aldehyde</td>
<td>Acid</td>
</tr>
<tr>
<td>Monder and Wang (1973); Bradlow et al. (1973)</td>
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</tr>
</tbody>
</table>
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takes place (Gaylor, 1972). Apparently cytosolic enzymes do not participate in these reactions, and the relationship to the microsomal ethanol oxidizing system or its induction by ethanol and other drugs is not known.

In the metabolism of bile acids and other steroids, a number of secondary alcohol–ketone oxidoreductions occur. Some of these are catalyzed by alcohol dehydrogenase, which utilizes NADH and produces a 3-β-hydroxyl from a 3-keto compound (Waller, Theorell & Sjövall, 1965) as well as a 17β-hydroxyl group from the corresponding ketone (Admirand, Cronholm & Sjövall, 1970). The latter observation may serve as an explanation of the observed increase in plasma concentrations of 17β-hydroxy steroid sulfates after administration of ethanol to humans, since a greater amount of NADH would be available (Admirand et al., 1970) (see Fig. 1). Other cytosolic reductases utilize NADPH and give rise to 3α-hydroxy groups (Berseus, 1967; Björkhem, 1969; Cronholm, Makino & Sjövall, 1972). Since cholesterol occurs in large quantities in brain (Rouser, Kritchevsky, Yamamoto & Baxter, 1972) these reactions are of importance to this organ. In contrast to the liver, cholesterol and related sterols are only slowly synthesized by the brain but also have a very much longer half-life (Ramsey & Nicholas, 1972) thus meeting the needs of the brain for myelin and cell membrane formation.

Recently it was reported that the glial cells are much more active than are neurons in the synthesis of sterols from 14C mevalonic acid (Jones, Nicholas & Ramsey, 1975) but, even so, incubations of several hr are required to achieve maximal incorporation of 14C into sterols in glial cells from 11-day-old rats. Since the rate-limiting step is probably formation of mevalonate from HMG CoA (see above) the rate of synthesis in vivo must be exceedingly slow compared with that in liver. An interesting observation by Edmond (1974) shows that β-hydroxybutyrate and other ketone bodies are much superior to mevalonate as precursors for sterols in developing brain.

As large concentrations of bile acids would be toxic to the brain, it is not surprising that these compounds are not synthesized to a significant extent in this tissue (Ramsey & Nicholas, 1972). This may be a reflection of the relative lack of alcohol and aldehyde dehydrogenases in the brain as compared with liver (Deitrich, 1966; Erwin & Deitrich, 1972) since these enzymes are important in the synthesis of bile acids (Okuda & Danielsson, 1965; Okuda & Takigawa, 1968, 1969, 1970; Okuda, Takigawa, Fukuba & Kuwaki, 1969; Mosbach, 1972).

(f) Steroid hormone metabolism

Conversion of cholesterol to pregnenolone involves hydroxylations at C20 and C22 followed by splitting of the cholesterol to pregnenolone and isocaproaldehyde (Burnstein & Gut, 1971). The metabolism of C21 alcohols such as corticosterone to the corresponding aldehydes is carried out by alcohol dehydrogenase in the cytoplasm. The further oxidation of the aldehyde to these acids can be catalyzed by a cytosolic aldehyde dehydrogenase from adrenals (Monder & Wang, 1973), at about 10% of the rate at which acetaldehyde is oxidized. Oxidation of corticosterone also occurs in vivo in humans as demonstrated by Bradlow, Zumoff, Monder & Hellman (1973). These investigators described the pathways by which various steroid metabolites can arise from C21 hydroxyl compounds. Generally the influence of ethanol, if any, on production of these compounds has not been studied.
However, one would expect that the oxidation of these alcohols to the aldehyde would be inhibited by ethanol.

The effects of ethanol on the biochemistry of steroid hormone synthesis may be masked or modified by the release of ACTH and consequent production of adrenocortical hormones. It is consistently observed that ethanol administration in man (Fazekas, 1966) and animals (Kalant, Hawkins & Czaja, 1963; Mendelson & Stern, 1966; Noble, Kakihana & Butte, 1971; Kakihana, 1975, personal communication) leads to increased steroid hormone levels and that this response requires a functioning pituitary (Stokes, 1971). Aberrant metabolites, produced because of the action of ethanol on the synthesis and degradation of the steroids, may alter the CNS response to ethanol.

EFFECTS OF ETHANOL ON THE BIOCHEMISTRY OF THE CNS

The delineation of the biochemistry of ethanol in the CNS is somewhat more difficult than in the periphery. Presumably all of the effects of ethanol will eventually be shown to have a biochemical, and beyond that, a physical action on the components of the brain. At this stage in our knowledge, however, we are severely restricted in pointing to well established biochemical effects of ethanol in the brain. For purposes of organization, discussion will be divided into two areas, studies of the preference for ethanol and the acute and chronic effects of ethanol ingestion. Subjects overlap and the division is not always sharp.

(a) Preference

In an attempt to duplicate man's preference for alcohol over other beverages, a number of models have been employed. We will consider only those with some biochemical basis. These involve genetic and chemical procedures. In no case, however, has it been possible to obtain animals that will voluntarily select alcohol over water to the point of addiction.

(1) Genetic. There are inbred strains of mice that show marked differences in their choice of alcohol vs water solutions. The strains most often employed are the C57 (drinkers) and the DBA (non-drinkers). Studies on such mice can utilize this difference to search for other factors which correlate with the alcohol preference. In the use of such inbred strains — since all genes are fixed — excellent correlations may be found which have nothing to do with alcohol preference. Indeed, it may even be possible to find other strains that drink for entirely different reasons and will have a different set of correlations of which only some are related to their preference for alcohol. Sheppard, Albersheim & McClearn (1970) showed that the non-drinking strain have a higher level of acetaldehyde in their blood following ethanol intake than do the drinking strains. Acetaldehyde blood levels are correlated with the amount of aldehyde dehydrogenase activity in the liver.

Another approach is to begin with a heterogeneous, outbred stock of animals and by selective breeding, develop lines that differ in their alcohol preference. This has been done by Eriksson (1972), preserving as much of the heterogeneity as possible by an outbreeding rather than an inbreeding program in his rat colony. As with the inbred mouse strains, the non-drinking strain of rats have higher blood acetaldehyde levels following ethanol intake than do the drinking animals (Eriksson, 1973).

(2) Chemical. As illustrated above, it is possible that high blood levels of acetaldehyde discourage ethanol intake. The mechanism by which acetaldehyde could induce such an
aversion to alcohol is unknown. However, relatively large amounts of acetaldehyde are produced in humans who take alcohol while on disulfiram treatment. The aversion is probably related more to the nausea, hypotension, etc., produced than to any more subtle mechanisms. (The question of elevated acetaldehyde levels in Orientals who drink alcohol was discussed earlier.) Careful measurements of the levels of acetaldehyde in both these humans on disulfiram therapy and animals who receive alcohol are required, utilizing newer methods of assay of blood acetaldehyde. Apparently the old data on acetaldehyde blood levels must be used only for comparative purposes since Sipple (1973) has shown that acetaldehyde forms spontaneously from ethanol (although this can be prevented by addition of thiourea). Previously Truitt (1970) demonstrated that acetaldehyde formed in ethanol-containing blood samples unless the samples were deproteinized quickly. Another chemical approach to elevate acetaldehyde blood levels in rats has been to administer inhibitors of aldehyde dehydrogenase and carry out alcohol preference studies in such animals (Koe & Tenen, 1970).

Myers (1963), Myers and Veale (1969) and Myers, Evans & Yaksh (1972) find that intra-ventricular infusion of small amounts of ethanol, acetaldehyde, methanol, paraldehyde or 5-hydroxytryptophol cause marked increases in the intake of alcohol in rats. Others (see Lester & Freed, 1973) have questioned some of these results.

A hotly debated chemical procedure for production of alcohol aversion is the use of p-chlorophenylalanine (pCPA) to lower brain serotonin levels (Table II). As is apparent, most investigators find a decrease in ethanol preference in heterogeneous rat strains upon treatment with pCPA. These experiments have been criticized on a number of grounds however; the results are not seen if the alcohol solutions are prepared from 95% alcohol (Cicero & Hill, 1970); the effect is an artifact which results from any noxious stimulus (Nachman, Lester & LeMagnen, 1970); the observations are the inverse of the actual situation (Geller, 1973) and the procedure for determining preference threshold is inadequate (Belenko & Woods, 1973). Conceding that the experiments are difficult and that they require much time and space, there are some obvious pharmacological principles that have not been followed by either the proponents or the detractors of the procedure: (1) Often relatively too few animals are used and these have shown widely varying alcohol preferences; (2) No one has published even the simplest dose response curve for pCPA in the effects on ethanol preference; (3) Very few investigators actually determine serotonin levels in their animals under their experimental conditions before and after pCPA. It is imperative that such data accompany all studies using pCPA or other drugs which supposedly alter brain serotonin levels (see Fig. 2).

The situation is not much better when we come to the observation that darkness increases alcohol intake in rats (Table II). The agreement that alcohol intake rises in the absence of light is reasonably good but the argument over the mechanism continues. It is well established that the pineal gland responds to lighting conditions and that the synthesis of melatonin is thus controlled (Quay, 1974). It is reasonable therefore to postulate that alcohol intake and the pineal are related. Additionally, melatonin is derived from serotonin so there is an obvious relationship between the experiments with pCPA and the experiments under different lighting conditions (Fig. 2). As a result of darkness, activation occurs of the enzymes responsible for production of melatonin in the pineal (Quay, 1974). Therefore
### Table II. Alteration of Ethanol Preference

<table>
<thead>
<tr>
<th>Compound or procedure</th>
<th>Effect on alcohol intake</th>
<th>Drug dose or condition</th>
<th>Rat strain</th>
<th>Comment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCPA</td>
<td>Decreased</td>
<td>300 mg/kg 116 and 350 mg/kg</td>
<td>Long–Evans</td>
<td>Decreased or No change</td>
<td>Myers &amp; Veale (1968)</td>
</tr>
<tr>
<td></td>
<td>Decreased</td>
<td>300 mg/kg</td>
<td>Holtzmann</td>
<td>Decreased with absolute ETOH</td>
<td>Veale &amp; Myers (1970)</td>
</tr>
<tr>
<td></td>
<td>Decreased</td>
<td>200 mg/kg</td>
<td></td>
<td>No change with 95% ETOH</td>
<td>Cizero &amp; Hill (1970)</td>
</tr>
<tr>
<td></td>
<td>No change</td>
<td></td>
<td></td>
<td></td>
<td>Myers &amp; Tytell (1972)</td>
</tr>
<tr>
<td></td>
<td>Decreased</td>
<td>300 mg/kg</td>
<td>Royal Victoria</td>
<td>Grape flavor ETOH</td>
<td>Geller (1973)</td>
</tr>
<tr>
<td></td>
<td>Increased</td>
<td>150 mg/kg</td>
<td>Sprague–Dawley</td>
<td>Effect lasted 16 days beyond treatment</td>
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</tr>
<tr>
<td></td>
<td>Decreased</td>
<td>300 mg/kg</td>
<td>Leo</td>
<td>5HT decreased</td>
<td>Frey et al. (1970)</td>
</tr>
<tr>
<td></td>
<td>Increased</td>
<td>2 mg/kg bid</td>
<td>Leo</td>
<td>Effect depends on experimental design</td>
<td>Frey et al. (1970)</td>
</tr>
<tr>
<td>Cinanserin</td>
<td>Increased</td>
<td>Not given</td>
<td>Sprague–Dawley</td>
<td>5HT receptor agonist</td>
<td>Geller et al. (1975)</td>
</tr>
<tr>
<td>Melatonin</td>
<td>Increased</td>
<td>0.5–2.5 mg/kg s.c. daily</td>
<td>Sprague–Dawley</td>
<td>Diurnal light cycle</td>
<td>Burke &amp; Kramer (1974)</td>
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<tr>
<td></td>
<td>Increased</td>
<td>0.2–1.5 mg/kg s.c. daily</td>
<td>Sprague–Dawley</td>
<td></td>
<td>Geller (1971)</td>
</tr>
<tr>
<td></td>
<td>No effect</td>
<td>1 mg/rat in beeswax</td>
<td>Sprague–Dawley</td>
<td>23.5 hr dark</td>
<td>Blum et al. (1973)</td>
</tr>
<tr>
<td></td>
<td>No effect</td>
<td>1 mg/rat ?</td>
<td>Wistar</td>
<td>Blind and pinealectomy</td>
<td>Reiter et al. (1973)</td>
</tr>
<tr>
<td>Darkness</td>
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<td>23.5 hr dark</td>
<td>Sprague–Dawley</td>
<td>Pinealectomy has little effect</td>
<td>Blum et al. (1973)</td>
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<td>Darkness</td>
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<td>Sprague–Dawley</td>
<td></td>
<td>Geller (1971)</td>
</tr>
<tr>
<td></td>
<td>Increased</td>
<td>24 hr dark</td>
<td>Sprague–Dawley</td>
<td></td>
<td>Burke &amp; Kramer (1974)</td>
</tr>
<tr>
<td></td>
<td>Increased in young</td>
<td></td>
<td>Wistar</td>
<td>Decreased in old animals</td>
<td>Sinclair (1972)</td>
</tr>
</tbody>
</table>

pCPA = p-chlorophenylalanine  
PCA = p-chloroamphetamine  
P.O. = orally  
bid = twice daily  
s.c. = subcutaneous
one would assume that pinealectomy should abolish the increased alcohol preference in darkness and, moreover, injections of melatonin should restore this preference. Such experiments have been carried out but results with pinealectomized animals (Blum, Merritt, Reiter & Wallace, 1973) are not convincing, and could well be used to argue that no blockade of alcohol preference was observed in spite of the authors’ claims to the contrary. Others, in fact, find no effect of pinealectomy (Burke & Kramer, 1974). The results of injection of melatonin on ethanol preference are impossible to interpret in any published material. The reasons for this are that no attempt has been made to carry out a dose–response relationship, perhaps because often no response is seen. An independent measure to prove that the injected melatonin is active or that it is chemically pure must be carried out. Utilizing this information one could then proceed to study its effect on alcohol preference.

(b) Effects of acute and chronic administration of ethanol

(1) Energy metabolism: glucose utilization. Because of the central role that glucose plays in brain metabolism, considerable interest has been sustained in the effects of ethanol on
glucose metabolism by the brain. Earlier studies utilizing in vivo administration of ethanol and subsequent analysis of brain metabolites could be criticized because of relatively long times between death and cessation of enzyme activity. Techniques are now available to rapidly freeze the brain after death. These methods involve freezing of the head or the whole animal in liquid nitrogen. The most rapid technique now available involves ejection of the brain contents into a liquid nitrogen-cooled container by means of compressed air (Veloso, Passonneau & Veech, 1972; Veech, 1974). The conclusions from several studies (Flock, Tyce & Owen, 1970; Roach & Reese, 1971; Veloso et al., 1972; Veech, 1974) are that: (1) Intoxicating doses of ethanol cause a considerable decrease in glucose utilization in the brain in vivo; (2) There is no marked change in the redox state of the brain, although this is not reported in all studies (Rawat & Kuriyama, 1972). A more recent paper by Rawat, Kuriyama & Mose (1973) again asserts that acute or chronic ethanol administration alters the redox state of brain. The evidence for this is not convincing for a number of reasons. These investigators carried out chronic feeding experiments using a liquid diet with sucrose isocalorically replacing ethanol. However, the animals were not pair fed. Pyrazole, an inhibitor of alcohol dehydrogenase, blocked the observed alteration of brain redox states but was administered only to the animals given alcohol and not to control animals as well. Although the statistical procedures are not detailed, recalculation using Students' t test shows that many results labeled as significant at p < 0.02 actually have p values > 0.5. Thus it is difficult, if not impossible, to evaluate such results.

The labeling and content of amino acids, especially glutamate, glutamine, aspartate and γ-aminobutyric acid from 14C glucose is also altered by ethanol (Roach, 1970; Mushakwar & Koepppe, 1972; Veloso et al., 1972; Mukherji, Kashiki, Ohyanagi & Sloviter, 1975). These changes are most likely related to the overall effect of ethanol on glucose metabolism, and as such they present a major difficulty in interpretation of the observed changes of γ-aminobutyric acid in relationship to its presumed role as an inhibitory neurotransmitter. (Gordon, 1967; Roach & Reese, 1971; Patel & Lal, 1973). The changes in glucose metabolism brought about by ethanol are most likely a result of the effect of ethanol on cell membranes in the brain and not a direct cause of the depressant effect of ethanol or other general anesthetics (see Veloso et al., 1972).

(2) Energy utilization: Na⁺ + K⁺ ATPase. There is a large body of work on the effect of ethanol on Na⁺ + K⁺ ATPase, the enzyme which is presumed to be essential to the sodium pump. These investigations are of importance since it is generally agreed that ethanol must have some influence on the permeability of cell membranes to ions and to the ability of the membranes to depolarize. Na⁺ + K⁺ ATPase is thus an obvious enzyme to receive much attention.

The original work (see references in Israel, Kalant & LeBlanc, 1966) generally established that ethanol would inhibit brain microsomal Na⁺ + K⁺ stimulated ATPase in vitro, but only at levels of ethanol that would be lethal to most animals. The inhibition is competitive with K⁺ and this observation raises the question of the availability of K⁺ to the enzyme in vivo. The inhibition may, in fact, be important to the in vivo actions of ethanol if K⁺ is low at the point of attack by ethanol (Israel et al., 1966). More recently, studies have been undertaken on the enzyme after chronic alcohol administration (Knox, Perrin & Sen, 1972). In rat and cat brain homogenates, the ATPase activity was increased by chronic
ethanol treatment. This does not occur in mouse brain (Israel & Kuriyama, 1971; Goldstein & Israel, 1972), and raises questions concerning this 'induction' of ATPase as a general explanation of tolerance and dependence in ethanol addiction. In one of these studies (Israel & Kuriyama, 1971) the mouse brain mitochondrial ATPase activity was increased after acute and chronic ethanol treatment. This activity is Mg\(^{2+}\) dependent and ouabain insensitive, in contrast to the ATPase thought to be involved in Na\(^+\) and K\(^+\) active transport.

Studies on the *in vitro* inhibition of ATPase by acetaldehyde and biogenic aldehydes (Tabakoff, 1974; Deitrich & Erwin, 1975; Kim & Erwin, 1975) show that the aldehydes derived from biogenic amines are highly potent inhibitors of brain Na\(^+\) + K\(^+\) ATPase while acetaldehyde is less so. Thus the possibility of the amplification of the effects of biogenic aldehydes on ATPase by acetaldehyde *in vivo* should be investigated.

(3) Effect of ethanol on brain neurotransmitters (Table III). Studies done several years ago on the effect of ethanol on the levels of various amine neurotransmitters yielded conflicting results, which still have not been resolved. More recently, however, techniques to

### Table III. Effect of ethanol on biogenic amines

<table>
<thead>
<tr>
<th>Amine</th>
<th>Acute</th>
<th>Chronic</th>
<th>Withdrawal</th>
<th>Comment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>Rats. No shift in metabolism, ²H tyrosine</td>
<td>Pohorecky (1974)</td>
</tr>
<tr>
<td></td>
<td>↓</td>
<td>↓</td>
<td></td>
<td>Rats. Results equivocal; baseline changes; ²H tyrosine</td>
<td>Carlsson <em>et al.</em> (1973)</td>
</tr>
<tr>
<td></td>
<td>→</td>
<td>→</td>
<td></td>
<td>Mice. Long sleep and short sleep; α-MPT</td>
<td>Collinson (1975, personal communication)</td>
</tr>
<tr>
<td></td>
<td>↓</td>
<td>↑</td>
<td>→</td>
<td>Mice. Inhalation of ethanol or acetaldehyde</td>
<td>Ortiz <em>et al.</em> (1974)</td>
</tr>
<tr>
<td></td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
<td>Rats. α-MPT</td>
<td>Hunt &amp; Majchrowicz (1974)</td>
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<tr>
<td>DA</td>
<td>↓</td>
<td>→</td>
<td>↑</td>
<td>²H tyrosine</td>
<td>Carlsson <em>et al.</em> (1973)</td>
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<tr>
<td></td>
<td>→</td>
<td>↓</td>
<td></td>
<td>SS mice. α-MPT</td>
<td>Collins (1975, personal communication)</td>
</tr>
<tr>
<td></td>
<td>→</td>
<td>↓</td>
<td>↓</td>
<td>LS mice. α-MPT</td>
<td>Collins (1975, personal communication)</td>
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<tr>
<td></td>
<td>→</td>
<td>→</td>
<td>↓</td>
<td>Rats. α-MPT</td>
<td>Hunt &amp; Majchrowicz (1974)</td>
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<td>↑</td>
<td>Mice. Inhalation of alcohol or acetaldehyde</td>
<td>Ortiz <em>et al.</em> (1974)</td>
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<tr>
<td>5HT</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>Rats. Chronic</td>
<td>Pohorecky <em>et al.</em> (1974)</td>
</tr>
<tr>
<td></td>
<td>→</td>
<td>→</td>
<td>↑</td>
<td>C57 Mice</td>
<td>Tabakoff &amp; Boggan (1974)</td>
</tr>
<tr>
<td></td>
<td>→</td>
<td>→</td>
<td>↑</td>
<td>Rats. Pargyline</td>
<td>Tyce <em>et al.</em> (1970)</td>
</tr>
<tr>
<td></td>
<td>→</td>
<td>→</td>
<td>↑</td>
<td>Mice. Pargyline</td>
<td>Kuriyama <em>et al.</em> (1971)</td>
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<tr>
<td></td>
<td>→</td>
<td>↑</td>
<td>→</td>
<td>Pargyline</td>
<td>Frankel <em>et al.</em> (1974)</td>
</tr>
<tr>
<td></td>
<td>→</td>
<td>→</td>
<td>↑</td>
<td>Mice, also did acetaldehyde; inhalation of ethanol or acetaldehyde</td>
<td>Ortiz <em>et al.</em> (1974)</td>
</tr>
</tbody>
</table>

Abbreviations: Arrows refer to: → no change, ↑ increase, ↓ decrease. α-MPT = α-methylparatyrosine.
determine the turnover of biogenic amines have become available. Such studies should present a clearer picture of the functional activity of various neurons rather than just the capacity for action that is represented by steady state levels. Since only a small fraction of the total neuronal content of these amines normally participate in synaptic transmission such turnover studies are not without difficulty.

Two recent studies on the effect of acute and chronic ethanol on norepinephrine turnover are in reasonable agreement. Pohorecky (1974) who used $^3$H tyrosine, and Hunt & Majchrowicz (1974), who used $\alpha$-MPT, found that acute doses of ethanol decrease the turnover of norepinephrine after 1–2 hr. Chronic administration of ethanol led to an increased turnover, and withdrawal of alcohol caused a further increase in turnover (Pohorecky, 1974) or a continued high turnover rate (Hunt & Majchrowicz, 1974). Hunt & Majchrowicz also investigated the turnover of dopamine under these conditions. They found a decrease in dopamine turnover upon acute or chronic ethanol administration but an increase upon withdrawal. These results are in agreement with those of Collins (1975, personal communication) as far as the acute effects of alcohol are concerned. He has utilized mice which have been selectively bred for acute tolerance to alcohol (short sleep) or acute sensitivity (long sleep), and finds no alteration in the turnover of norepinephrine but a decrease in dopamine turnover which is twice as great for long sleep mice as for short sleep mice.

Studies on the turnover of serotonin have been carried out but with conflicting results. Tyce, Flock, Taylor & Owen (1970) found a decrease in turnover upon acute administration of ethanol but both Kuriyama, Rauscher & Sze (1971) and Frankel, Khanna, Kalant & LeBlanc (1974) found no change in turnover upon acute administration of ethanol. These latter two groups differ, however, in that Kuriyama et al. (1971) found an increased turnover upon chronic ethanol treatment but Frankel et al. (1974) found no change.

Recent studies by Carmichael & Israel (1975) using superfused brain slices indicate that ethanol inhibits release of several neurotransmitters but that the release of acetylcholine was most sensitive to such inhibition. Significant inhibition of acetylcholine release was detected at 0.05 M (230 mg/100 ml), a concentration which is highly intoxicating if not lethal to most mammals. Studies such as these should be carried out utilizing dose response curves for acute administration of ethanol so that correlation between gross behavioral effects and brain biochemistry can be made. This is not usually done. Ideally, measurements of the enzymes involved in the synthesis and metabolism of the amines should be carried out. Occasionally such studies have been done (Kuriyama et al., 1971; Pohorecky, Jaffe & Berkeley, 1974; Tabakoff & Bogdan, 1974; Collins, 1975, personal communication). Studies on withdrawal should, first of all, demonstrate that the animals have measurable withdrawal symptoms (Hunt & Majchrowicz, 1974; Pohorecky, 1974; Ortiz, Griffiths & Littleton, 1974; Tabakoff & Bogdan, 1974). The question which must be answered is whether the changes in amine content and turnover are the cause or the result of the actions of ethanol on the CNS. Dose–response relationships would seem to be a useful tool in arriving at such a decision.

(4) Relationship of acetaldehyde and biogenic aldehydes to acute and chronic actions of ethanol. This subject has recently been reviewed; older references and those for the following synopsis can be found there (Deitrich & Erwin, 1975). The 'biogenic aldehyde' theory holds
that because of the escape of acetaldehyde from peripheral tissues during ethanol metabol-
ism and its potency in the CNS (Holtzman & Schneider, 1974), the effects of this metabolite
must be considered when attempting to understand the actions of ethanol on the brain.
The enzyme in the brain responsible for oxidation of acetaldehyde will also oxidize alde-
hydes derived from biogenic amines (Erwin & Deitrich, 1966), and the $K_m$ values are
relatively low so that the small amount of acetaldehyde present in blood after ethanol
ingestion is enough to interfere with the oxidation of the biogenic aldehydes. The resultant
increase in biogenic aldehyde concentration provides the opportunity for ‘amplification’
of the effects of these aldehydes referred to earlier. Acetaldehyde and these biogenic aldehydes
can react with biogenic amines to produce various alkaloids. Acetaldehyde plus dopamine
or norepinephrine results in tetrahydroisoquinolines; acetaldehyde with serotonin gives
harmaline alkaloids; 3,4-dihydroxyphenylacetaldehyde (aldehyde of dopamine) reacts
with dopamine to give tetrahydropapaveroline. While there is no doubt that such reactions
exist in vitro, with or without tissue present, until recently there was little evidence that
these compounds formed in vivo, especially in the brain (Sandler, Carter, Hunter & Stern,
1973). Collins & Bigdeli (1975) have now demonstrated the presence of a tetrahydroiso-
quinoline (salsolinol) in rat brain after treatment with ethanol and pyrogallol. The amount
of salsolinol found is about 17 ng/g in the combined midbrain, caudate nucleus and brain
stem (about 1% of the dopamine concentration in these tissues). In a similar study Turner,
Baker, Algeri, Frigerio & Garattini (1974) reported the presence of tetrahydropapaveroline
(THP) in rat brain after administration of l-DOPA with or without ethanol, but not after
ethanol alone. The quantitation was difficult but amounts in the range of 10-25 ng/g of
brain were reported.

Of critical importance to such studies is the localization of the enzymes monoamine
oxidase, aldehyde reductase and aldehyde dehydrogenase in the nervous system. Ideally
these enzymes should be found in neurones. A recent paper by Duncan, Sourkes, Dubrovsky
& Quik (1975) raises some questions about this. These investigators placed electrolytic
lesions in the medial forebrain bundle of rats and assayed for tyrosine hydroxylase, aldehyde
dehydrogenase, aldehyde reductase and acetylcholine esterase in the striatum. They found
that, while tyrosine hydroxylase was decreased to immeasurable levels, none of the other
enzymes were affected. This is in contrast to earlier studies by the same group where
aldehyde dehydrogenase activity was reduced but not abolished in the striatum of cats
after lesions in the nigrostriatal tracts (Duncan, Sourkes, Boucher, Poirier & Roberge,
1972). Similar decreases in aldehyde dehydrogenase after 6-hydroxydopamine injections
into rat brain were reported by Agid, Javoy & Youdim (1973).

Another objection to the idea that acetaldehyde is involved in the chronic effects of
ethanol has come from Sipple (1974). He finds no acetaldehyde in brain until the blood
concentration exceeds 200 µmole giving a calculated capacity of the brain for metabolism of
acetaldehyde of 0.18 µmoles/min per g of brain. Techniques are now available to utilize
perfused brain for such studies (Mukherji et al., 1975) and should yield interesting results.
Acetaldehyde, the biogenic aldehydes and their reaction products with biogenic amines
have various other actions: some bind to protein, some have effects on evoked potentials,
some function as false neurotransmitters and some have been found to inhibit Na$^+ + K^+$
ATPase strongly (see Deitrich & Erwin, 1975).
The likelihood of these aldehydes being involved in the acute intoxication effects of ethanol seems remote, but the possibility of their participation in the side effects of chronic CNS toxicity of ethanol cannot yet be dismissed.

(5) Alcoholism and ethanol receptor interaction. In studies of other addicting drugs such as morphine, the presence of highly specific receptors has long been assumed and has now been shown by several groups (Goldstein, 1973; Pert, Snowman & Snyder, 1974). Theories of addiction (e.g. Goldstein & Goldstein, 1961) often assume alteration in such receptors or associated enzymes. With ethanol, however, a different problem is faced. Because of the large amounts of ethanol required to bring about an effect we are forced to some rather unsatisfactory conclusions. Either we may assume that ethanol has no receptors in the classical sense, or that the affinity of such receptors for ethanol is very low indeed. A possible alternative approach has been to assume that it is not ethanol itself that is the depressant or addictive agent, but that some other compound, acetaldehyde, a biogenic aldehyde, a tetrahydroisoquinoline, tetrahydropapaveroline, etc., is the true addicting agent. Several recent studies promise new leads into this area. One of these is the study of interaction of ethanol with receptor–enzyme couples as exemplified by the studies of cAMP response to various agonists in brain slices from ethanol treated animals. These investigators find that brain slices taken from animals 2 hr after the last dose of ethanol show a subsensitivity of the adenylate cyclase enzyme to norepinephrine (French, Reid, Palmer, Narod & Ramey, 1974). In animals chronically treated with ethanol and then withdrawn, the opposite effect is seen, i.e. there is a supersensitivity of the adenylate cyclase to norepinephrine and histamine although not at all concentrations of histamine (French, Palmer & Narod, 1975). Related studies by Hammond & Schneider (1974) indicate that dibutyryl cyclic AMP injected intraventricularly suppressed head twitches in mice withdrawing from ethanol. This observation is not easily reconciled with those of French and his colleagues if one assumes that supersensitivity of the adenylate cyclase enzyme system to agonists is related to withdrawal symptoms. It is rather obvious that much work remains to be done here.

It is well to recall that there may not be a necessary correlation between acute ethanol depressant effects and the ‘addiction’ seen upon chronic administration. While traditional experience would suggest that addiction is a function of time vs dose relationships, the relationship may be only fortuitous and addiction may not be mechanistically related to intoxication. In other words we may need one explanation for intoxication and quite another for addiction. Some support for such an idea can be found in a study by French & Morris (1972). They administered ethanol by inhalation to rats for 1–2 weeks at a concentration that did not produce detectable blood ethanol levels. Withdrawal in such animals was assessed by the force of the jump response to electric foot shock which reached a maximum at 72 hr. This is longer than the time for development of withdrawal symptoms in mice given a higher dose of alcohol for a shorter time (Goldstein, 1972) but corresponds more closely to that seen in humans (Victor, 1970).

(6) Ethanol and calcium metabolism. An interesting, and potentially very crucial, discovery is that ethanol lowers brain calcium (Ross, Medina & Cardenas, 1974). The results are difficult to relate to either the acute or chronic effects of ethanol since morphine has a similar action, and the effects of both morphine and ethanol are blocked by naloxone, a
specific morphine antagonist. Salsolinol, the condensation product of dopamine and acetaldehyde, also lowers brain calcium and is antagonized by naloxone. Reserpine and pentobarbital also lower brain calcium but their actions are not blocked by naloxone.

There is no obvious pharmacological connection between all these drugs which can be rationalized on the basis of their effects on brain calcium. However, the importance of calcium in nerve function, muscle contraction, etc., dictates that the lowering of brain calcium by ethanol be carefully and thoroughly investigated.

A number of related observations may be relevant. One of these is the study of the hypocalcemic effect of alcohol (Peng, Cooper & Munson, 1972). Since this hypocalcemic response to acute doses of alcohol is not accompanied by an increased excretion of calcium, the conclusion is reached that the calcium was moving into some tissue, probably bone. The authors state that they did not observe a change in $^{45}$Ca in soft tissues, including brain, following injections of the radioisotope with or without alcohol treatment. They also attempted to study the effect of acetaldehyde. The results must be considered inconclusive however, since they measured calcium blood levels 1 hr after administration of acetaldehyde to rats, and the half-life of acetaldehyde is of the order of 2–3 min at a maximum (Deitrich & Siew, 1974).

It is well established that Ca$^{++}$ is essential to neurotransmitter release. This has been studied particularly in the adrenal medulla (Schneider, 1971; Rahwan, Borowitz & Miya, 1973) where it is also observed that neurotransmitter release by acetaldehyde does not require Ca$^{++}$. Freund (1973) has observed that lidocaine prevents withdrawal seizures in mice, in spite of the fact that local anesthetics are convulsants in their own right (Ritchie, Cohen & Dripps, 1970). Since local anesthetics probably act by competing with Ca$^{++}$ at the cell membrane, it would appear imperative that Ca$^{++}$ levels be studied after chronic as well as acute administration of ethanol.

Ethanol and protein synthesis. Certainly one of the more important findings in recent years has been that ethanol alters protein synthesis in the brain (see Noble & Tewari, 1975). In mice and rats forced to drink 10% ethanol as the only fluid source, but not physically dependent on it, it was found that the in vitro incorporation of $^{14}$C leucine into ribosomal protein of brain was less than that of brain from control animals. In animals made dependent on ethanol and then withdrawn, an even greater deficit was noticed. This inhibition was attributed to a deficiency in the pH 5 enzymes fraction although there were apparent changes in the polysomes of brain as well. Jarlstedt (1972) also found that animals ingesting 15% ethanol for 8 months did not incorporate $^{14}$C leucine into brain in vivo as well as did control animals. More recently Fleming, Tewari & Noble (1975) have found that the in vivo aminocacylation of t-RNA is inhibited in mice forced to drink 10% ethanol for 4–8 weeks.

The importance of these observations to the structural brain damage in chronic alcoholism is obvious. Alteration of protein synthesis may also be related to effects of alcohol on memory, tolerance and dependence, although it would be expected that defects in the synthesis of specific proteins would be more likely to be involved in these effects of ethanol.

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