1976

Effect of Continuous Inhalation of Ethanol Vapors in the Rat

Steven W. Mann
University of Rhode Island

EFFECT OF CONTINUOUS INHALATION OF ETHANOL VAPORS IN THE RAT

BY

STEVEN W. MANN

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE

REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

PHARMACOLOGY AND TOXICOLOGY

UNIVERSITY OF RHODE ISLAND

1976

MASTER OF SCIENCE THESIS

OF

STEVEN WILLIAM MANN

Approved:

Thesis Committee:

Major Professor Harbans Dal

David Lamb

Nelson J. Smith

Alan Swanson

A. A. Michel

Dean of the Graduate School

UNIVERSITY OF RHODE ISLAND

1976

ABSTRACT

Rats were exposed to continuous inhalation of ethanol vapors in an inhalation chamber plus daily injections of an alcohol dehydrogenase inhibitor pyrazole (68 mg/kg, i.p.). Ethanol vapors entered the inhalation chamber at flow rates of from 0.45 liters/min. to 0.95 liters/min. which when mixed with a constant stream of air (5.0 liters/min) produced chamber concentrations of from approximately 8.8 mg/liter to 26.5 mg/liter. Rats exposed to these conditions for 5 to 7 days developed blood ethanol levels of 0.83 $\pm$ 0.09 mg/ml/blood to 2.19 $\pm$ 0.14 mg/ml/blood. Administration of pyrazole with or without ethanol caused weight loss in these rats. Rats developed tolerance to ethanol after continuous inhalation of ethanol vapors for 5 days demonstrated by increased onset and decreased duration of ethanol-induced narcosis. After 5 days of continuous ethanol inhalation rats became physically dependent on ethanol and developed withdrawal signs of piloerection, abnormal posture, tremors, convulsions, headshakes, tail-lifts and mortality upon cessation of ethanol administration. The four withdrawal signs of piloerection, abnormal posture, tremors and convulsions were combined into an ethanol withdrawal syndrome measurement. Pretreatment of ethanol dependent rats 30 minutes prior to 36 hours of ethanol withdrawal, the period of maximum intensity of the withdrawal syndrome,

with ethanol (2.0 g/kg, orally), chlordiazepoxide (40 mg/kg, i.p.) or morphine (10 mg/kg, i.p.) significantly reduced the intensity of the ethanol withdrawal syndrome as measured during the thirty-sixth hour of ethanol withdrawal. Rats withdrawn after continuous inhalation of ethanol vapors showed intense aggression (attacks and bites, rearing and vocalizations) after small doses of apomorphine (2.5 mg/kg, i.p.) or d-amphetamine (2.0 mg/kg, i.p.), although little spontaneous aggression was seen. Other ethanol withdrawal signs returned to near control levels by 72 hours after ethanol withdrawal, however drug-induced aggression was present for at least 7 days after ethanol withdrawal. Administration of ethanol (4.0 g/kg, orally), chlordiazepoxide (80 mg/kg, i.p.) or morphine (10 mg/kg, i.p.) 30 minutes prior to apomorphine administration (2.5 mg/kg, i.p.) significantly reduced apomorphine-induced aggression in ethanol withdrawn rats. During withdrawal from ethanol, mortality was 20% among rats exposed for 5 days to ethanol inhalation and not treated with any drugs during withdrawal. None of the drugs used in this study significantly reduced the mortality rate in ethanol withdrawn rats. However, administration of chlordiazepoxide (160 mg/kg, i.p.) prior to the thirty-sixth hour of ethanol withdrawal resulted in a significant increase in the mortality to 75%. This same dose of chlordiazepoxide administered intraperitoneally to untreated rats resulted in a mortality of 66.7% of the rats treated within 36 hours after injection.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my wife, Sharon and my daughter, Dana, for their understanding and patience without which this would not have been possible.

I would also like to express my appreciation to Dr. Harbans Lal for his guidance throughout this study. I would also like to acknowledge Dr. Alvin K. Swonger and the other members of my committee for their advice and assistance.

Also, I would like to make special acknowledgement to Dr. William D. Lawing for his expert statistical advice.

I am also grateful to the faculty of the Department of Pharmacology and Toxicology for their helpful suggestions during this study.

The financial aid provided through a teaching assistantship by the Department of Pharmacology and Toxicology is gratefully acknowledged.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

FIGURE Page

INTRODUCTION

In man, alcohol dependence usually evolves over many years and consequently, its developmental antecedents are obscured by time. It has generally been agreed that development of an alcohol dependent animal would permit the study of the addictive process. Indeed several animal models of ethanol physical dependence have been reported. The methods used were schedule-induced polydipsia (Falk, 1972), liquid diets containing ethanol (Freund, 1969; Branchey et al., 1971; Pieper et al., 1972; Pieper and Skeen, 1972), gastric or nasogastric intubations (Majchrowicz, 1973; Ellis and Pick, 1970b.; Essig and Lam, 1968), intravenous administration (Deneau et al., 1969; Winger et al., 1970) and inhalation with or without pyrazole (Goldstein and Pal, 1971; Littleton et al., 1974; Roach et al., 1973). The inhalation technique has proved to be most applicable for experimental studies and has been used for testing the effects of drugs on the ethanol withdrawal reaction (Goldstein, 1972b.; 1973). The evidence indicates that maintainance of consistently high blood ethanol levels for several consecutive days is necessary for production of ethanol physical dependence (Mello and Mendelson, 1971a; 1971b.). Majchrowicz (1973) and Roach et al. (1973), outlined the withdrawal signs in rats. Although, comprehen-

sive behavioral rating scales have been developed for mice (Irwin, 1968; Goldstein, 1972a.; Freund, 1969) few have been developed for other species and none for the rat (Mello, 1973).

Behavioral tolerance to alcohol in alcoholics has been demonstrated (Isbell et al., 1955; Newman, 1941). However, the biochemical mechanisms underlying tolerance to ethanol are unknown. Metabolic tolerance to alcohol in animals (Hawkins et al., 1966) and man (Mendelson et al., 1966) has been shown, however metabolic changes cannot adequately explain the tolerance seen in animals or man (Mendelson, 1971; Mello 1972; LaBlanc et al., 1969) and a role for cellular adaption in the central nervous system is necessary.

The mechanisms underlying the expression of withdrawal signs upon cessation of ethanol administration are unknown. Roles for hypomagnesemia and respiratory alkalosis (Victor, 1973), sleep disturbances (Gross et al., 1974), and denervation supersensitivity (Jaffe and Sharpless, 1968) have been proposed as relating to the expression of withdrawal signs. Theories about the underlying mechanism involved have included enzyme repression and derepression (Goldstein and Goldstein, 1967), increased receptor sites (Collier, 1965), formation of morphine-like alkaloids within the central nervous system (Walsh et al., 1970), formation of false adrenergic transmitters (Cohen, 1973a.; 1973b.) and inhibition of nerve impulses (Wallgren, 1973; Tabakoff et al., 1973).

Treatment of the acute alcohol withdrawal syndrome has been reviewed by Gross et al. (1974). Goldstein (1972b.), compared several drugs in the treatment of ethanol withdrawal signs including ethanol, chlordiazepoxide, promazine and barbiturates. It has been shown that ethanol does act as expected in reducing the withdrawal severity of alcohol dependent and withdrawn animals (Goldstein, 1972b.; Ellis and Pick, 1972b.), although such treatment in man is not advised (Isbell et al., 1955; Golbert et al., 1967). Chlordiazepoxide is considered one of the most effective treatments for delirium tremens (Gross et al., 1974; Favazza and Martin, 1974) and is effective in animals as well (Goldstein, 1972b.).

The use of haloperidol in the treatment of acute alcohol withdrawal has been studied, although not extensively (Gross et al., 1974), however morphine has not been reported in the treatment of acute alcohol withdrawal. Gross et al. (1974), presented evidence that haloperidol is at least as effective as paraldehyde or chlordiazepoxide in reducing withdrawal severity during the first 48 hours of alcohol withdrawal in man. It has been proposed that ethanol, via its metabolite acetaldehyde, may cause formation of morphine-like alkaloids in the central nervous system (Walsh et al., 1970) and that acute morphine administration blocks dopamine receptors similar to haloperidol, but, is less potent (Puri et al., 1973). Therefore, the use of ethanol,

chlordiazepoxide and morphine in the treatment of acute alcohol withdrawl in rats is of scientific interest.

Rats made physically dependent on morphine show spontaneous aggression during withdrawal without any other stimulation (Puri and Lal, 1973). This spontaneous aggression is enhanced by administration of apomorphine or amphetamine and is blocked by the narcotic methadone and the dopamine receptor blocking neuroleptic haloperidol (Puri and Lal, 1973). It has also been shown that chronic haloperidol administration increases the sensitivity of rats to induction of aggression by small doses of apomorphine (Gianutsos et al., 1974a.). Apomorphine is a known stimulator of dopamine receptors (Anden et al., 1967; Ernst, 1967). It has also been shown that morphine dependent rats withdrawn for protracted periods of up to 30 days show evidence of latent dopaminergic supersensitivity as represented by aggressive behavior upon aggregation (Gianutsos et al., 1974b.).

This investigation was undertaken to develop an animal model of ethanol physical dependence in the rat using a modification of the inhalation procedure described by Goldstein and Pal (1971). Also, undertaken were investigations of the effect of drugs in the treatment of the acute effects associated with ethanol withdrawal and the possible relationship between physical dependence on ethanol and morphine.

Based upon this proposal, the following predictions

which were undertaken for testing are:

1. That continuous inhalation of ethanol vapors by rats along with daily injections of an alcohol dehydrogenase inhibitor pyrazole will result in maintainance of high blood ethanol levels and result in ethanol tolerance and physical dependence.

2. That tolerance, either metabolic or cellular will result in alterations in ethanol-induced narcosis.

3. That physical dependence will be demonstrated by the presence of withdrawal signs upon cessation of chronic ethanol administration and that:

   a) withdrawal sign intensity could be measured by the use of an intensity rating scale.

   b) withdrawal signs will follow a similar time course of development in intensity.

   c) withdrawal signs related to ethanol withdrawal will be reduced by readministration of ethanol.

   d) withdrawal signs related to ethanol can be combined into an ethanol withdrawal syndrome measurement.

4. That drugs known to have cross-tolerance with ethanol will be effective in reducing the severity of the ethanol withdrawal reaction (ex. chlordiazepoxide).

5. That administration of morphine will reduce the intensity of the ethanol withdrawal syndrome.

6. That some sign of morphine withdrawal will be seen in ethanol dependent and withdrawn rats.

a) aggressive behavior

b) aggression will be enhanced by dopaminergic stimulating agents (ex. apomorphine, d-amphetamine).

c) aggression reduced by ethanol, chlordiazepoxide and morphine

It was therefore undertaken to subject animals to all the aforementioned conditions or treatments and to measure the ethanol withdrawal reaction in order to establish the relative value of this model of ethanol physical dependence in the study of alcohol addiction and to test the hypotheses proposed.

LITERATURE SURVEY

ALCOHOLISM AS AN ADDICTION

Alcoholism has been shown to be a form of addiction, as defined in terms of the traditional pharmacological criteria of tolerance and physical dependence (Isbell et al., 1955; Mendelson, 1964; Victor and Adams, 1953). Recognition that alcoholism is an addictive disorder has proceeded slowly and at one time it was thought that the alcohol withdrawal syndrome reflected intercurrent illness, vitamin or nutritional deficiencies (Victor and Adams, 1953). Now it has been shown that alcohol withdrawal signs and symptoms occur in healthy, well nourished alcoholics (Mendelson, 1964) and in experimental animals (Goldstein and Pal, 1971), solely as a function of withdrawal of alcohol.

The crucial determinants in the development of alcohol addiction are unknown and the nature of the addictive process remains a matter of conjecture. It has generally been agreed that only the development of an alcohol dependent animal would permit the study of the addictive process at a behavioral, biochemical and neurophysiological level. In man, alcohol dependence usually evolves over many years and consequently, its developmental antecedents are obscured by time.

ETHANOL TOLERANCE

Data obtained over 35 years ago demonstrated that the severity of intoxication at similar blood alcohol levels was less severe in alcoholics than in abstainers (Jetter, 1938). The development of tolerance in alcoholics has been demonstrated by a number of investigators (Isbell et al., 1955; Newman, 1941). However, the biochemical mechanisms subserving increased tolerance are unknown, but two general mechanism have been postulated: an enhanced rate of metabolism and/or an increased degree of cellular adaption to ethanol in the central nervous system.

Metabolic Tolerance

Both experimental animals (Hawkins et al., 1966) and man (Mendelson et al., 1966) may develop increased rates of ethanol metabolism following chronic ethanol administration. The increased ethanol metabolism in experimental animals has been correlated with an increase in hepatic alcohol dehydrogenase activity (Hawkins et al., 1966; Mendelson et al., 1965). However, differences in metabolic rate alone do not adequately explain the high quality of tolerance seen in animals and man (Mendelson, 1971; Mello, 1972).

Cellular Tolerance

Since there is no good evidence that metabolic processes can adequately account for the degree of tolerance observed in alcoholics, cellular adaption to ethanol appears to be a more likely explanation for the phenomena of

tolerance. Axelrod (1968), has reviewed several mechanisms which may underlie cellular adaption. Although, the models used were based upon possible actions of narcotics, they have relevance for other centrally acting drugs such as ethanol. Three possible factors are: 1. reduced activity of the drug receptor. 2. depletion of endogenous substances which indirectly cause or mediate drug action and 3. enhanced metabolism and/or inactivation of the drug at its site of action in the central nervous system. Mendelson (1971), has reviewed the evidence relating to the possible role of ethanol as related to each possible mechanism, concluding that little evidence exists supporting any of the proposed mechanisms of cellular tolerance discussed by Axelrod (1968). Furthermore, there is little conclusive evidence supporting the many hypotheses (see Mechanisms of Tolerance and Physical Dependence) advanced to explain tolerance (Mendelson, 1971). A general consensus of many neurophysiologists and neurochemists is that ethanol acts on membrane structure and function, which reflects the lack of decisive evidence available (Mendelson, 1971).

ETHANOL DEPENDENCE

The occurrence of withdrawal signs upon the cessation of ethanol administration is evidence of physical dependence. Dependence is the second major pharmacological criteria of alcohol addiction. The critical determinants of

the onset of withdrawal are unclear since either a relative decrease in blood ethanol levels or abrupt withdrawal may precipitate abstinence signs. Abstinence signs have been observed in man (Isbell et al., 1955; Mello and Mendelson, 1970) and animals (Goldstein, 1972a.) while blood ethanol levels are still high. Goldstein (1972a.), found that withdrawal signs after a single large dose of alcohol began when blood ethanol levels were as high as 3 mg/ml and convulsions were seen in some mice with blood ethanol levels greater than 1 mg/ml.

Hypomagnesemia and Respiratory Alkalosis

There has been a growing body of data on the relationship between signs associated with alcohol withdrawal on the one hand and hypomagnesemia and respiratory alkalosis on the other. This area has recently been reviewed by Victor (1973), who has been intricately involved in research in this area. Victor (1973), noted that three separate studies, of which two were clinical and one experimental, involving a total of 58 patients, the only two consistent chemical abnormalities during withdrawal were hypomagnesemia and respiratory alkalosis. Furthermore, he observed that the severity of these chemical abnormalities were related and followed a similar time course as the seizures associated with the withdrawal. As to the mechanism involved Victor (1973), suggested that it may involve rebound suppression of the respiratory centers during alcohol intoxication with respiratory alkalosis and magnesium shift in response

to the alkalosis. He also suggested that a concomitant mechanism might be cerebral hypoxia secondary to the respiratory alkalosis.

Sleep Disturbances

Considerable research in the past decade has been directed to the study of the relationship between sleep disturbances and the phenomenology of the acute alcohol withdrawal syndrome. Clinical investigations have demonstrated consistently that patients admitted in acute alcohol withdrawal states who were hallucinating were likely, if they could sleep, to have elevation in Stage I REM (rapid eye movement), that part of sleep associated with vivid dreaming (Gross et al., 1966; Gross and Goodenough, 1968; Greenberg and Pearlman, 1967; Johnson et al., 1970). Levels as high as 100% Stage I REM (normally 20%) were observed in hallucinating patients in full-blown delirium tremens (Gross et al., 1966; Johnson et al., 1970). Thus, in alcoholics there appears to be a relationship between REM and the hallucinations of withdrawal. Alcohol also reduces REM in normals (Yules et al., 1966) and in chronic alcoholics (Gross and Goodenough, 1968). This suggests that an important mechanism involved in the hallucinogenesis during acute alcohol withdrawal may be rebound of REM and its associated dream activity. If REM rebound were great enough it might break through into the waking state as hallucinations (Fisher and Dement, 1963) as was once observed (Gross et al., 1966). There is also a marked reduction in delta

sleep which may be an important condition permitting very high levels of REM (Johnson et al., 1970). Johnson et al. (1970), suggested that the sleep changes may result from the effect of ethanol on brain biogenic amines. This position is shared by Williams and Salamy (1972), Lester et al., (1973) and Kissin et al. (1973).

Denervation Supersensitivity

Jaffe and Sharpless (1968), pointed out that many withdrawal phenomena involve an exaggeration of behavior that is ordinarily suppressed by the agent which induces dependence. For example, morphine ingestion in man is associated with myosis while mydriasis is observed during morphine withdrawal. In addition to the concept of rebound hyperexcitability exists during the time the agent is ingested (Jaffe and Sharpless, 1968).

The phenomena of denervation supersensitivity has been used as a model to explain central nervous system (CNS) seizure activity in severe abstinence syndromes. It has been suggested that chronic CNS depression by barbiturates, morphine, alcohol, etc., is tantamount to "disuse" (analogous to peripheral nervous system (PNS) denervation. Upon removal of the drug, there is a consequent rebound hyperexcitability of the functional systems depressed (Jaffe and Sharpless, 1965). Therefore, drugs that elevate thresholds for chemically induced seizures would be expected to exhibit lowered seizure thresholds during withdrawal. The formula-

tion of "disuse supersensitivity" is supported by the finding that while barbiturates are known to increase the threshhold for pentylenetetrazol (PTZ)-induced seizures, barbiturate withdrawal in the addicted cat is associated with a decreased threshold for PTZ-induced seizures (Jaffe and Sharpless, 1965). Jaffe and Sharpless (1965), concluded that the lowered threshold for seizures was consistent with a "disuse supersensitivity" model of physical dependence and that the time course for the development of physical dependence is similar to that of denervation supersensitivity in the PNS as observed by Fleming and Trendelenburg (1961).

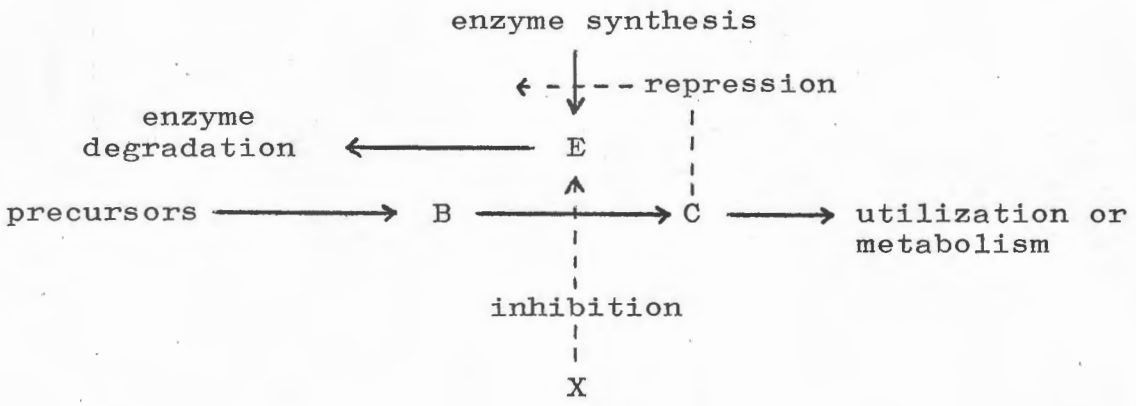
MECHANISMS OF TOLERANCE AND PHYSICAL DEPENDENCE

Tolerance and physical dependence have been discussed as separate entities although both are essential criteria for addiction. Tolerance and physical dependence may represent phenomena subserved by a common mechanism or may represent different biochemical and physiological processes. It is well known that certain centrally acting drugs produce tolerance but no physical dependence. For example, amphetamine produces no clear-cut dependence, while tolerance to increasing doses is common (Jaffe, 1970). Way et al. (1969), has presented strong evidence that a common mechanism underlies the development of tolerance and physical dependence to morphine in mice. However, prior to similar experiments being done in alcohol addiction a satisfactory animal model of ethanol physical dependence is needed (Mendelson, 1971).

Enzyme Expansion Theory

Goldstein and Goldstein (1967), have developed an enzyme expansion theory of drug tolerance and physical dependence. This theory consists of tolerance and physical dependence as a unitary mechanism based upon represssion and derepression of enzyme synthesis. Substance C in Figure 1 is considered excitatory at some regional or cellular level in the central nervous system. Substance C is synthesized via precursor B catalyzed by enzyme E. Levels of C are

Figure 1



maintained via feedback inhibition or repression of synthesis of enzyme E. Drugs such as ethanol are proposed to inhibit enzyme E thereby reducing synthesis of substance C. The reduced levels of C result in increased levels of synthesis of E restoring normal levels of excitatory substance C. At this time both high levels of drug (ethanol) and enzyme exists (tolerance) but with normal CNS function. With-

drawal of ethanol results in release of inhibition of enzyme E which results in increased levels of C and overexcitation and the withdrawal phenomena (physical dependence).

Increased Receptor Sites

Collier (1965), proposed a theory based upon the hypothesis that drugs with addictive potency induce an increase in receptor sites. This theory proposes that active receptor sites exist in cells which bind with the drug (possibly receptors for endogenous substances ex. neuroamines). When the drug is administered in increasing dosage, new receptors are made to replace those bound by the drug. Thus as more receptors develop more drug is needed to bind them, hence tolerance. Removal of the drug results in availability of an excess of receptors which are active and results in excitation and withdrawal signs.

Acetaldehyde, Alcohol and Biogenic Amines

There have been a number of suggestions that the phenomena of tolerance and physical dependence on alcohol may not be directly related to the action of ethanol on neural tissue, but rather related to a crucial metabolite. Truitt and Walsh (1971) have stressed the importance of the ethanol metabolite acetaldehyde in the actions of ethanol. Furthermore, acetaldehyde may have a direct role in the development of tolerance and physical dependence upon ethanol (Ortiz et al., 1974).

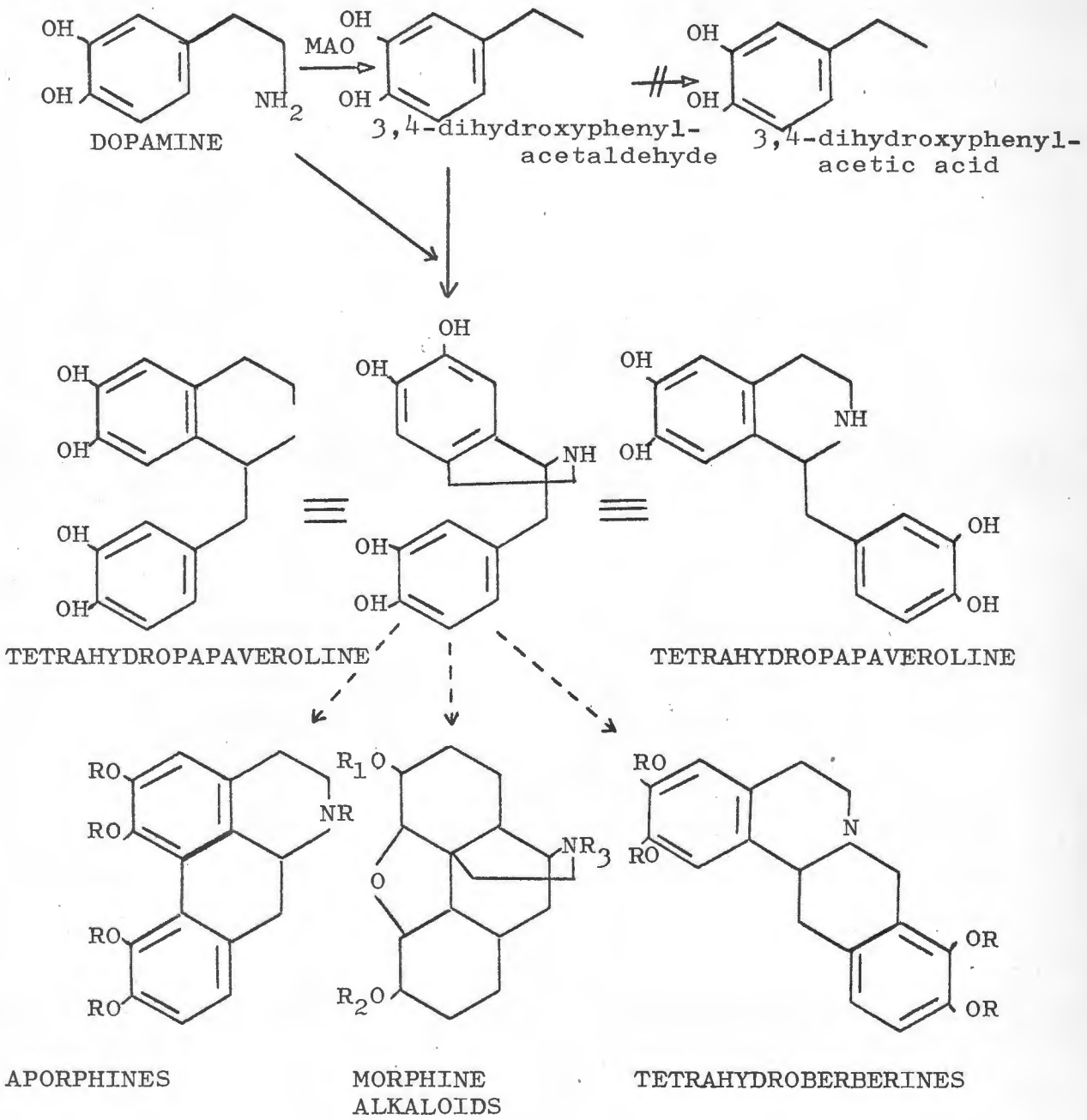
Although some authors believe that ethanol's effect on

nerve membranes is primarily involved in the production of physical dependence upon ethanol either directly (Wallgren, 1973) or indirectly via binding of biogenic amine aldehydes (Tabakoff et al., 1973), synaptic events involving catecholamines are not dismissed.

Chronic ethanol administration is known to cause sustained release of norepinephrine in the brain (Hunt and Majchrowicz, 1974), which has lead to investigation and speculation that this release of norepinephrine is directly responsible for at least some of the characteristics of ethanol tolerance and physical dependence (Reis, 1973; French et al., 1974). Davis et al. ($_1$967b.), found that ethanol and acetaldehyde cause release of norepinephrine and alter its metabolism from the normal oxidative pathway towards a reductive one of a glycol excretion product (methoxyhydroxyphenylglycol, MHPG) a similar shift involving serotonin was also seen (Davis et al., 1967a.). However, although the metabolism of dopamine via the oxidative route was reduced no increase in the reductive pathway was seen (Davis, 1971). The shift in catecholamine metabolism is believed to be due to the inhibition of aldehyde reductase in the brain by acetaldehyde formed from the metabolism of ethanol via alcohol dehydrogenase, catalase and the microsomal ethanol oxidizing system proposed by Lieber and DeCarli (1968). The feasibility of a role for acetaldehyde in alcoholism has recently been given support

from Korsten et al. (1975), who found that the blood levels of acetaldehyde were elevated in alcoholics compared to normals after equal doses of ethanol and at similar blood ethanol levels. Hasumura et al. (1975), presented evidence that the elevation in acetaldehyde levels in alcoholics may result from the chronic intake of ethanol, since it was found that in rats chronic administration of ethanol inhibited acetaldehyde oxidation by liver mitochondria.

Although the shift in catecholamine metabolism, previously cited, may not itself be important, the fact that dopamine metabolism does not increase through the reductive pathway focused attention on this neuroamine. Walsh et al. (1970), found that under conditions which limited the metabolism of the dopamine derived aldehyde (3,4-dihydroxyphenylacetaldehyde) an alkaloid metabolite, tetrahydropapaveroline (THP) was formed from the condensation of dopamine with its aldehyde metabolite. Davis et al. (1970), found that alcohol or acetaldehyde considerably augmented the formation of THP in vitro. It is known that in plants THP is the requisite intermediate in the biosynthesis of complex alkaloids including morphine, aporphine and papaverine alkaloids (Battersby, 1961; Spenser, 1966). Davis et al. (1970), proposed that the formation of such alkaloids may represent the mechanism for the addiction liability of ethanol (see Figure 2 for proposed mechanism). A recent review of the background for this hypothesis has recently been published



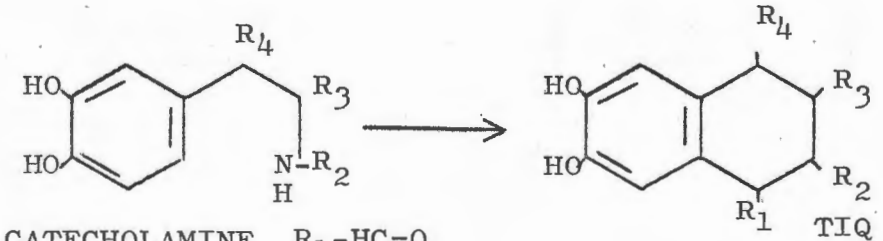
	R_1	R_2	R_3
normorphine	H	H	H
morphine	H	H	CH_3
norcodeine	H	CH_3	H
codeine	H	CH_3	CH_3

Figure 2. Reproduced from Davis et al., (1970).

(Walsh, 1973). This hypothesis has been criticized by Seevers (1970) and by Goldstein and Judson (1971), based upon the failure of naloxone to induce a typical narcotic withdrawal sign (jumping) in ethanol dependent mice.

There has also been presented in the literature, almost simultaneously to the above studies, studies which have shown that acetaldehyde or formaldehyde will condense, via the simple Pictet-Spengler reaction, with dopamine or norepinephrine to form tetrahydroisoquinoline alkaloids (THIs) (Cohen, 1971; Yamanaka et al., 1970). Figure 3 shows the possible alkaloids.

Figure 3



Formation of 1,2,3,4-tetrahydroisoquinoline (TIQ) alkaloids from catecholamines by condensation with aldehydes. The aldehydes are formaldehyde (R_1 = H) and acetaldehyde (R_1=CH_3). The catecholamines are dopamine (R_2=R_3=R_4=H), norepinephrine (R_2=R_3=H; R_4= OH) and epinephrine (R_2=CH; R_3=H; R_4=OH).

Cohen and his co-workers after much experimentation proposed that these condensation products act as false adrenergic transmitters and meet the essential requirements of synthesis, uptake, storage and release by the adrenal and peripheral adrenergic nerves (Cohen, 1973a.; 1973b.). Recent work

has shown that these THIs are differentially secreted from the adrenals, such that in the presence of Ca^{++} both catecholamines and THIs are released, however when Ca^{++} is absent only the catecholamines are released (Rahwan et al., 1974).

These hypotheses concerning acetaldehyde are very provocative and have created much furor in the past few years. Similarities between the actions of morphine and ethanol are not unknown in the literature. Ross et al. (1974), has shown that both morphine and ethanol as well as salsolinol (a THI) and reserpine deplete regional brain Ca^{++}, however, only the effects of morphine, ethanol and salsolinol were antagonized by naloxone a narcotic antagonist. Swartz et al. (1974), showed that acute doses of ethanol inhibit the uptake and binding of Ca^{++} by cardiac microsomes. Therefore, if one examines the available data: 1. ethanol and salsolinol deplete regional brain calcium, possibly via inhibition of uptake and binding 2. the depletion of calcium would favor release of catecholamines (NE) over THIs 3. the release of NE after ethanol administration is already well known as is the diversion of its metabolism in the presence of ethanol or acetaldehyde. Furthermore, it has been shown that barbiturates also induce a similar shift in norepinephrine metabolism as does ethanol or acetaldehyde (Davis et al., 1974) presumed to be due to inhibition of aldehyde reductase as shown by Tabakoff and Erwin (1970).

Thus, signs which are used to categorize the withdrawal syndrome consist of a mixture of central, peripheral and derivative metabolic effects which are not easily explained by enzyme derepression, receptor induction or neuroamine alteration theories. At the present time it appears as if tolerance and physical dependence on alcohol have multiple determinants whose mechanisms relate to complex interactions of neural, endocrine and metabolic functions.

ASSESSMENT OF ETHANOL TOLERANCE

Kalant et al. (1971), has reviewed the many techniques for assessing tolerance to ethanol. Various investigators have used electroshock seizure thresholds (Allan and Swinyard, 1949) and motor performance (Gibbins et al., 1968; Moskowitz and Wapner, 1964; LaBlanc et al., 1969). In most instances changes in metabolic distribution cannot explain the degree of tolerance observed (LaBlanc et al., 1969; Mendelson, 1971).

Increases in the rate of ethanol elimination have been shown in man (Mendelson et al., 1966) and animals (Gibbins et al., 1966). Animals made physically dependent on ethanol have also been shown to have increased rates of ethanol elimination (Mello, 1973). However, the behavioral methods cited above have been carried out in non-dependent animals, possibly due to the interference of the withdrawal reaction with the performance of such tasks as rotor rod per-

formance. Tolerance to alcohol in non-dependent mice did not return to normal for at least 14 days and not for 3-4 weeks when tolerance was at its maximum following ethanol treatments (LaBlanc et al. 1969).

ASSESSMENT OF ETHANOL WITHDRAWAL

For many years, investigators have concentrated upon devising techniques for inducing preference for alcohol in animals. There has also been an unfortunate tendency to equate a transitory alcohol preference with addiction even though no withdrawal signs and symptoms occurred upon cessation of chronic drinking. Removal of the factors which accelerated alcohol preference (ex. noxious stimuli), is usually accompanied by a decreased alcohol intake. Reviews of this area have been published (Lester, 1966; Mello, 1968; Myers and Veale, 1972; Woods and Winger, 1971).

Recently, several groups have produced physical dependence upon alcohol in animals using oral, intragastric, intravenous and inhalation routes of administration. Although, there is some interspecies variability in the types of withdrawal signs, there has been reasonable agreement from different laboratories (Mello, 1973). Majchrowicz (1973) and Roach et al. (1973), have outlined most of the ethanol withdrawal signs in rats. Withdrawal after several days of 3-5 fractional daily doses of ethanol 12-15 g/kg/day in rats resulted in withdrawal signs of "squealing, hyper-

activity, ventromedialdistal forepaw flexion, spascity, tremors, teeth chattering, wet shakes, induced and spontaneous convulsions" (Majchrowicz, 1973). Withdrawal after 7 days of inhalation of ethanol without pyrazole resulted in tremors, forward tail-arching, hypersensitivity to sound and touch, abnormal postures and convulsions (Roach et al., 1973). However, neither investigators attempted to do more than group the withdrawn rats based upon an overall subjective interpretation of withdrawal severity, rather than by systematically assessing each sign.

Although, comprehensive behavioral rating scales have been developed for withdrawal signs in mice (Irwin, 1968; Goldstein, 1972a.; Freund, 1969) few have been developed for other species and none for the rat (Mello, 1973). Since, the grossly observable withdrawal signs presumably represent central nervous system hyperexcitability directly by examining the seizure threshold to electro-convulsive shock (McQuarrie and Fingl, 1958), audiogenic stimuli and convulsive drugs (Ratcliffe, 1972) and the startle response (Gibbins et al., 1971).

Oral and Intragastric Administration

Falk (1972), reported successful application of a behavioral technique, schedule-induced polydipsia, in producing ethanol physical dependence in the rat. The polydipsia phenomena was first reported by Falk (1961). Substitution of a 5 or 6% ethanol solution for water resulted in daily intakes of between 11 and 15 g/kg with blood levels

maintained above 1 mg/ml. Physical dependence resulted after 3 months of exposure to the polydipsia technique. Freund (1969), feed liquid diets containing 35% of the caloric intake as ethanol to mice on a food-restricted diet. Although, this procedure induced gross intoxication and physical dependence in four days, the severe weight reduction, to 65% of their free-feeding weight, prior to ethanol administration introduces some serious inadequacies into this model (Ogata et al., 1972). It has been shown both in animals and man that the rate of ethanol metabolism is reduced by as much as 50% in fasted organisms (Forsander et al., 1965; Mendelson, 1970; Owens and Marshall, 1950; Smith and Newman, 1959). The Freund method has also been applied to rats (Branchey et al., 1971).

Gastric intubations of 3-5 fractional daily intubations of 12-15 g/kg/day of ethanol in rats has produced physical dependence in approximately 7 days (Majchrowicz, 1973).

Several unsuccessful studies using the polydipsia technique with rhesus monkeys to induce physical dependence did yield the important result that maintenance of consistently high blood ethanol levels on successive days is important for induction of physical dependence on ethanol (Mello and Mendelson, 1971a.; 1971b.).

Use of one to seven-month-old chimpanzees (Pan troglodytes) given liquid diets with 45% of the calories as ethanol at standard feeding times has resulted in physical

dependence (Pieper et al., 1972). The chimpanzees maintained normal weight gain, consumed ethanol at 2-8 g/kg/day with blood levels ranging between 0.5-3.0 mg/ml for 6-10 weeks and displayed hyperreflexia, irritability, spastic rigidity and tonic-clonic convulsion upon abrupt withdrawal of ethanol (Pieper et al., 1972). This liquid diet procedure was extended to adult rhesus monkeys with comparable results including significant increases in the rate of ethanol metabolism (Pieper and Skeen, 1972).

Forced alcohol administration procedures have proved to be consistently effective in producing alcohol dependence in monkeys (Ellis and Pick, 1969; 1970b.; 1971) and in dogs (Ellis and Pick, 1970a.; Essig and Lam, 1968; 1971). Ellis and Pick (1969), were the first to report that nasogastric intubation of alcohol (25%) in 2-3 daily doses of 4-8 g/kg for 10-18 days produced physical dependence in rhesus monkeys with increases in ethanol metabolism. Essig and Lam (1968), were the first to report ethanol physical dependence in dogs following prolonged administration via surgically implanted gastric cannula. Upon withdrawal the authors described states of apparent hallucinations and disrupted sleep as well as tremulousness and convulsions (Essig and Lam, 1968; 1971).

Intravenous Administration

Yanagita et al. (1965), were the first to report ethanol physical dependence in monkeys using a paradigm in

which a monkey could lever press to self-administer ethanol. Their original work was confirmed and extended (Deneau et al., 1969; Winger et al., 1970; Woods and Winger, 1971; Woods et al., 1971). Consistent delivery of 6-8 g/kg/day for 10 weeks was sufficient to produce withdrawal signs upon withdrawal of ethanol injections (Deneau et al., 1969). There are striking similarities between the patterns of intravenous self-administration of ethanol by the rhesus monkey and spontaneous drinking patterns in human alcoholics (Deneau et al., 1969; Mello and Mendelson, 1972; Nathan et al., 1970; Nathan et al., 1971; Woods et al., 1971). Human alcoholics, given an operant task, frequently alternate drinking episode of 3-6 days with relatively abstinent work periods associated with partial withdrawal signs lasting 2-3 days (Mello and Mendelson, 1972; Nathan et al., 1970; Nathan et al., 1971). Alcohol self-administration in monkeys is also punctuated by periods or spontaneous abstinence and partial withdrawal signs (Deneau et al., 1969; Woods et al., 1971). This raises some basic questions concerning the ambiguous relationship between physical dependence and subsequent drug self-administration. It is usually assumed that once an addictive drug-taking pattern is established, the avoidance of withdrawal signs and symptoms is a critical factor in maintenance of drug use. However, avoidance of withdrawal per se does not appear to be the only factor involved in the maintenance of alcohol self-administration.

Inhalation Administration

Goldstein and Pal, (1971), reported alcohol dependence in mice exposed to inhalation of ethanol vapors and daily injections of pyrazole (68 mg/kg) for 3-4 days. Chamber ethanol concentrations were between 10-16 mg/liter which resulted in blood levels of approximately 1.8 mg/ml (Goldstein and Pal, 1971). Upon removal from inhalation of ethanol withdrawal signs of tremors, convulsions to handling, tail-lifts, startle reactions and spontaneous seizures were seen with peak intensity occurring 10-12 hours after withdrawal (Goldstein and Pal, 1971; Goldstein, 1972b.). This model has the advantage of producing large numbers of physically dependent animals in a relatively short time. Goldstein has used this model to study the relationship between alcohol dose and the intensity of withdrawal signs, effects of drugs which modify neurotransmission and the effect of other drug on the withdrawal reaction (ex. chlordiazepoxide) (Goldstein, 1972a.; 1972b.; 1973). Roach et al. (1973), has reported production of physical dependence in rats by inhalation of ethanol without the use of pyrazole after 7 days of inhalation at concentrations of 15-30 mg/liter. Upon withdrawal the rats were subdivided into 3 groups, slightly dependent, moderately dependent and severely dependent based upon an overall subjective evaluation of the withdrawal signs. Blood levels varied widely with means of from 0.8 mg/ml to 3 mg/ml of ethanol.

Alcohol and Pyrazole

Goldstein (1972a.), found that the use of pyrazole was necessary, since without it blood ethanol levels fluctuated drastically resulting in a high mortality rate during ethanol inhalation.

Pyrazole inhibits the metabolism of ethanol both *in vitro* and *in vivo*. Theorell *et al*. (1969), reported that pyrazole inhibited alcohol dehydrogenase by formation of a ternary complex with ADH and nicotinamide adenine dinucleotide (NAD). Lester *et al*. (1968) and Goldberg and Rydberg (1969), showed that pyrazole inhibited ethanol metabolism in rats and that the minimal lethal dose was approximately 18 mmole/kg (1200 mg/kg). Lester and Benson (1970), found that the metabolism of other alcohols including ethanol are inhibited by pyrazole as well as some oximes and amides. Rydberg *et al*. (1972), has studied the kinetics of pyrazole and reported that not only does pyrazole inhibit ethanol metabolism, but that administration of ethanol increased the half-life of pyrazole from 13 to 21 hours. Rydberg *et al*. (1972), suggested that ethanol might inhibit a microsomal mechanism for pyrazoles elimination (Rubin *et al*., 1971). Bustos *et al*. (1970) and Morgan and DiLuzio (1970) have dealt with the induction of fatty-livers in animals treated with pyrazole and ethanol.

Pyrazole is known to have hepatotoxic effects (Lelbach, 1969; Lieber *et al*., 1970) and some synergistic action with

ethanol upon the central nervous system (Goldberg et al., 1972). However, overall pyrazole is well tolerated in rats at doses which are highly effective at reducing ethanol metabolism (Lester and Benson, 1970) and in most cases toxic effects are not seen until the dose of pyrazole is quite high, usually greater than several hundred milligrams per kilogram of body weight.

TREATMENT OF ETHANOL WITHDRAWAL

There is a vast literature on the use of a wide variety of drugs in the specific rather than the supportive treatment of the alcohol withdrawal syndrome. The rationale for the usefulness of these agents has followed two basic directions. One direction has been to find effective central nervous system depressants which, by their cross-tolerance to alcohol, would theoretically be effective in the treatment of acute withdrawal. This has been emphasized by Isbell et al. (1955). The other direction has been the use of major and minor tranquilizers to calm and sedate patients. The former presumably attacks the underlying mechanism, the latter symptoms of anxiety, agitation and hallucinations.

Specific Treatment of Acute Alcohol Withdrawal

The data suggest that specific treatment applies to the impending delirium tremens than to delirium tremens (Gross et al., 1974). Cross-tolerant drugs were signifi-

cantly more effective in preventing delirium tremens and in reducing seizures than non-cross-tolerant drugs (Golbert et al., 1967; Kaim et al., 1969). In the case of delirium tremens, the characteristic of cross-tolerance may not be as important and it is the opinion of many clinicians that the difference between the clinical efficacy of cross-tolerant and non-cross-tolerant drugs may be less critical than the experience of the clinician in detecting complications and correcting such problems as electrolyte imbalance (Gross et al., 1974; Victor, 1966). However, use of paraldehyde is still extensive and is vigorously supported for treatment of delirium tremens (Victor, 1966). Although a recent survey of physicians by Favazza and Martin (1974), found that chlordiazepoxide was favored by almost 2 to 1 as the drug of choice in the treatment of delirium tremens, other experimental workers have found no difference between chlordiazepoxide and paraldehyde. This is of interest, since the major metabolite of paraldehyde is acetaldehyde.

Ethanol in the Treatment of Alcohol Withdrawal

Withdrawal reactions follow when chronic administration of a dependence producing drug stops, and the signs should therefore be relieved by reinstating the same drug or its pharmacological equivalent. Isbell et al. (1955), pointed out that ethanol should relieve alcohol abstinence, but stressed the limitations of such therapy in practice (rapid metabolism, low margin of safety). According to Golbert

et al. (1967), alcohol treatment does not always prevent the appearance of delirium tremens in man. Goldstein (1972b.) in mice and Ellis and Pick (1970b.) in monkeys, showed that ethanol does act as expected in reducing withdrawal severity. Goldstein (1972b.), has also shown that paraldehyde, meprobamate and barbiturates, which have similar pharmacological properties to ethanol, are also effective in treating withdrawal in mice.

Comparison of Major and Minor Tranquilizers in Acute Alcohol Withdrawal

A comparative study of chlordiazepoxide, paraldehyde and haloperidol found that in the treatment or uncomplicated acute alcohol withdrawal in man the rate of improvement was significantly greater with chlordiazepoxide over the long run (fourth day of observation), however, during the first 48 hours all drugs worked effectively with no significant difference between them (Gross et al., 1974).

Other major tranquilizers have been tested in the treatment of alcohol withdrawal. The use of chlorpromazine or promazine were compared with paraldehyde and/or chlordiazepoxide in the treatment of delirium tremens (Kaim et al., 1969; Thomas and Freedman, 1964). In all cases chlorpromazine and promazine were markedly inferior. While promazine was no different than chlordiazepoxide in several symptoms other than delirium tremens, promazine failed to suppress convulsions (Chambers and Schultz, 1965). Thomas and

Freedman (1964) and Golbert et al. (1967) reported an alarming increase in mortality after promazine compared to paraldehyde or chlordiazepoxide. These results are in agreement with Goldstein (1972b.), who found that administration of promazine or chlorpromazine increased the severity of the withdrawal in ethanol dependent and withdrawn mice. Although Goldstein (1972b.), found chlordiazepoxide, diazepam and long-acting barbiturates highly effective in reducing withdrawal signs in mice, it was also reported that a significiant increase in mortality in mice treated with these long-acting drugs. The cause for the increased mortality was unknown and it was suggested that the disorder may have been one which is routinely corrected in a clinical situation, such as electrolyte or fluid imbalance. However, more seriously they may add to the brain damage caused by several days of intoxication and due to their cross-tolerant nature be equivalent to further days of intoxication and subsequently increase the withdrawal severity (Goldstein, 1972b.).

It appears that the clinical use of drugs is a very complex problem. Although, paraldehyde and chlordiazepoxide appear to emerge as consistently effective treatments, clinical management appears to have at least as much importance (Gross et al., 1974).

WITHDRAWAL AGGRESSION

Rats made physically dependent upon morphine display intense aggression during withdrawal without any other stimulation (Puri and Lal, 1973). This spontaneous aggression is enhanced by direct and indirect acting dopamine receptor stimulants, such as apomorphine and amphetamine and is blocked by the narcotic methadone or the dopamine receptor blocking neuroleptic haloperidol (Puri and Lal, 1973). It has been proposed that morphine withdrawal aggression indicates the presence of dopamine receptor supersensitivity (Puri and Lal, 1973). Apomorphine is known to be a direct dopamine receptor stimulator (Anden et al., 1967; Ernst, 1967) and capable of inducing aggression itself, but at doses several fold higher than doses which intensify morphine withdrawal aggression (Gianutsos, 1974). Dopamine receptor supersensitivity appears to be latent for protracted periods of up to at least 30 days after withdrawal of morphine, demonstrated by intense aggression in morphine dependent and withdrawn rats after 30 days (Gianutsos etal., 1974b). The involvement of dopamine receptor supersensitivity in the production of aggression has been further supported by evidence that chronic blockade of dopamine receptors by the neuroleptic haloperidol results in increased sensitivity to apomorphine-induced aggression (Gianutsos et al., 1974a.). It has been proposed that acute adminstration of morphine can result in blockade of dopamine receptors similar to haloperidol, but less potent (Puri et al., 1973).

EXPERIMENTAL

Animals:

Long-Evans strain, male hooded rats weighing 150-400 grams (Charles River Breeding Laboratory, Wilmington, Mass.) were used in this study. They were housed in animal quarters maintained at 21-23°C with room lights alternated on a 12-hour light-dark cycle. The rats were housed in groups of 15 or less with food and water available ad libitum at all times unless specified otherwise.

Materials:

Analytical grade chemicals or equivalent were used throughout the study. Ethanol (95%) used for production of ethanol vapors and animal dosing was obtained from U.S. Industrial Chemical Co., New York, N.Y. Sodium pyrophosphate, sodium carbonate and glycine used for the blood alcohol assay were obtained from Mallinckrodt Chemical Works, St. Louis, Mo. Apomorphine-hydrochloride was obtained from Mallinckrodt Chemical Works. Alcohol dehydrogenase-(Alcohol: NAD oxidoreductase; E.C. No. 1.1.1.1) from yeast; crystallized and lyophilized powder, β -diphosphopyridine nucleotide (β -DPN; β -NAD)- yeast and aminooxy acetic acid (hemi-hydrochloride) were obtained from Sigma Chemical Co., St. Louis, Mo. Librium (Chlordiazepoxide-HCl Lot #670110) was obtained from Hoffman-La Roche, Inc., Nutlet, N.J., Morphine sulfate (U.S.P. Crystalline) was obtained from Merck Chemical Division, Rahway, N.J. Pyrazole (99%) was obtained from Aldrich Chemical Co., Inc., Milwaukee, Wis.

Ethanol Vapor Chamber:

The ethanol vapor chamber consisted of a flat bottom, cylindrical glass bell jar placed horizontally on a wooden platform. It contained a perforated metal grid floor, drinking water bottle and food pellets. The open end of the jar was closed by a wooden frame sealed by a cork liner and fitted with a rubber stopper (2" diameter) having four holes. The rubber stopper was fitted with two inlets (one for air and one for ethanol vapors) an outlet and a thermometer. Compressed air (20 psi) was directed via a Y-tube to two flow-meters. The flow-meter regulating air flow had a range of 0-11.5 liters/min; the flow-meter regulating ethanol vapors a range of 0-0.95 liters/min (Fisher and Porter Co., Warminster, Pa.). The air flow-meter was connected directly to the ethanol vapor chamber, while the ethanol flow-meter was connected to a 500 cc gas-washing bottle (Thomas Co., Philadelphia, Pa.) containing 95% ethanol. The ethanol vapors produced flowed into the ethanol vapor chamber where it was mixed with the air flow.

Determination of Ethanol-Vapor Concentration:

The concentration of ethanol in the inhalation chamber was determined using a Packard 810 Gas Chromatograph (Packard Instrument Co., Inc., Downers Grove, Ill.). Column packing was Carbosieve-B GSC, mesh 45/65 (Superlco, Inc., Bellefonte, Pa.). The inlet temperature was 200°C, column 190°C and detector 200°C. The nitrogen carrier flow was 90 cc/minute.

Standard analysis was done by injecting 1 ul of absolute ethanol. Air flow to the chamber was constant at 5.0 liters/min and ethanol vapor flow-rates were set from 0.2-0.9 liters/min. The chamber was allowed to equilibrate for 30 minutes prior to sampling. Unpublished results from our lab have shown that chamber ethanol concentrations are stable 15 minutes after ethanol vapor flow-rates are changed (Drawbaugh et al., 1973). Samples of chamber air (1 ml) were withdrawn from the outlet tube using a Hamilton air-tight syringe (Hamilton Co., Inc., Calif.) and injected on to the column.

Ethanol comes off Carbosieve-B as a distinct, but somewhat broad peak. Therefore, analysis was based upon the weight of each curve. The curve was cut out and weighted to the nearest milligram using a Mettler H 20 balance (Arthur Scientific Co., Philadelphia, Pa.). Chamber ethanol concentrations were determined as follows:

$$\frac{(1\text{ml})\text{ sample weight x attenuation}}{(1\text{ul})\text{ standard weight x attenuation}} \times 1000 \times 0.8\ \text{mg/ul} = \text{mg/liter/ethanol}$$

Figure 4 demonstrates the relationship between ethanol vapor flow-rate and chamber ethanol concentrations.

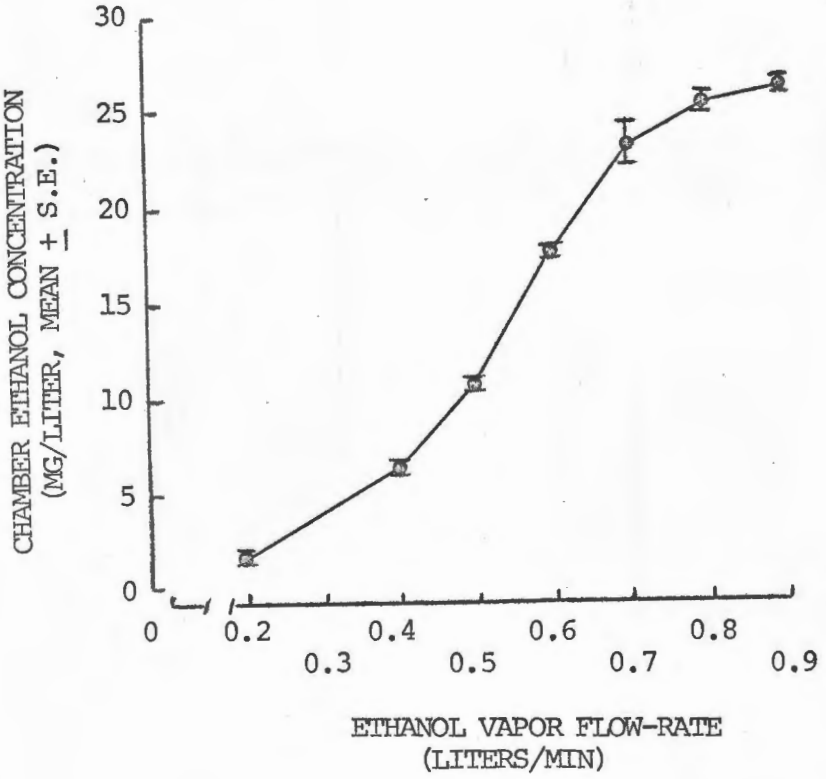


Figure 4. RELATIONSHIP BETWEEN ETHANOL VAPOR FLOW-RATE AND CHAMBER ETHANOL VAPOR CONCENTRATION. Each point represents the mean ± S.E. of 3 samples of vapor taken from the outlet of the ethanol vapor chamber at ethanol flow-rates of 0.2, 0.4, 0.5, 0.6, 0.7 and 0.8 liters/min. Air flow-rate was constant at 5.0 liters/min.

Determination of Blood Ethanol Levels:

The assay procedure was that of Jones et al. (1970). Rats were removed from the inhalation chamber and anesthestized with ether and a small blood sample collected from the tail vein in a heparinized vial. A sample of 100 ul was deproteinized with perchloric acid (4.9 ml, 3.4%). The supernatant obtained after centrifugation at 3000 rpm for 10 minutes was used directly for analysis. The supernatant (100 ul) was added to 2.6 ml of ice-cooled reagent in capped vials containing per liter 0.03 mole of sodium pyrophosphate, 0.04 mole glycine, 0.11 mole sodium carbonate, 0.03 mole aminooxyacetic acid, 0.0017 mole nicotinamide adenine dinucleotide (NAD^+; alcohol free grade) and 25 units of crystalline yeast alcohol dehydrogenase per assay (pH 8.8-9.2)[1] The reaction mixture was then incubated at 30°C in a constant temperature bath with shaking for 10 minutes. The reaction mixture was then allowed to sit at room temperature for 15 minutes at which time the absorption was measured at 340 mu. The optical density, after subtraction of a non-ethanol blank value, was proportional to the concentration of ethyl alcohol in the blood. The standard analysis was made by addition of 100 ul of ethanol standard solution containing 0.25, 0.5, 1.0 and 2.0 mg/ml ethanol in

[1]Preparation of 50 ml of reagent was done by adding 0.699 g sodium pyrophosphate, 0.1500 g glycine, 0.682 g sodium carbonate, 0.164 g aminooxyacetic acid, 0.064 g NAD^+ and 1.09 mg (25 units) of alcohol dehydrogenase to 50 ml of distilled water.

distilled water. The standard solutions of ethanol were prepared as follows: 1 ml absolute ethanol = 0.8 g ethanol, 2.5 ml/absolute ethanol = 2.0 g ethanol. Therefore, 2.5 ml/absolute ethanol q.s. to 1000 ml contains 2.0 mg/ml ethanol; 500 ml of this stock solution q.s. 1000 ml contains 1.0 mg/ml etc. for 0.5 and 0.25 mg/ml standard solutions. Figure 5 demonstrates the standard curve for quantitative determination of ethanol by the enzymatic method of Jones et al., (1970). The least squares regression line is linear the Y intercept is 0.00128 the slope 0.085, therefore ethanol levels may be determined by:

$X = \frac{Y - b}{M}$ where, X is ethanol/mg/ml: Y is optical density; b is the Y intercept and M is the slope.

Ethanol Intoxication:

The animals were exposed continuously for 5 or 7 days in the ethanol vapor chamber to an air-ethanol mixture obtained by mixing two streams of air; one directly to the chamber at a rate of 5.0 liters/min. and another bubbled through 95% ethanol in a gas-washing bottle at a rate of from 0.45 liters/min to 0.95 liters/min.

Rats were exposed to ethanol at a specific rate for the first 24 hours followed by gradual increases in the ethanol vapor flow-rate every 24 hours until the final day of the intoxication period. Three protocols of ethanol vapor exposure were emplo ed as follows: 1. First 24 hours ethanol vapor flow-rate was 0.45 liters/min, 24-48 hours

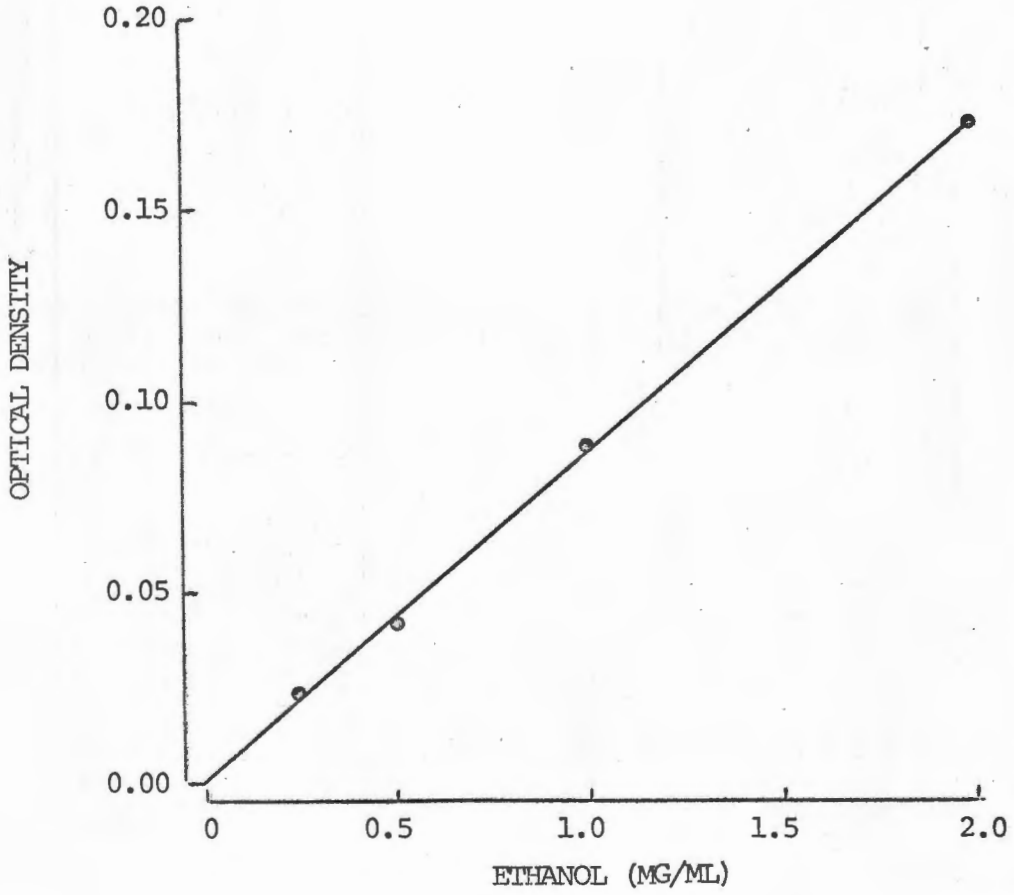


Figure 5. STANDARD CURVE FOR QUANTITATIVE DETERMINATION OF ETHANOL IN BLOOD. The enzymatic method of Jones et al., (1970) was used.

0.5 liters/min, 48-72 hours 0.55 liters/min, 72-96 hours 0.6 liters/min and 96-120 hours 0.6 liters/min. 2. First 24 hours ethanol vapor flow-rate was 0.55 liters/min, 24-48 hours 0.6 liters/min, 48-72 hours 0.7 liters/min, 72-96 hours 0.8 liters/min and 96-120 hours 0.1 liters/min. 3. First 24 hours ethanol vapor flow-rate was 0.55 liters/min, 24-48 hours 0.6 liters/min, 48-72 hours 0.7 liters/min, 72-96 hours 0.8 liters/min, 96-120 hours 0.85 liters/min, 120-144 hours 0.9 liters/min and 144-168 hours 0.95 liters/min. The three ethanol vapor exposure-protocols were subsequently denoted as 5 DAY LOW, 5 DAY HIGH and 7 DAY HIGH, respectively.

All animals were exposed to ethanol vapors in groups of six rats. Prior to exposure and every 24 hours thereafter all animals were weighed and injected with pyrazole (68 mg/kg, i.p.), except on the final day when weight was recorded but no pyrazole was administered. Goldstein and Pal (1971), found that pyrazole stabilizes the blood ethanol levels in mice inhaling ethanol vapors as well as inhibiting ethanol metabolism as shown by Theorell et al., (1969) and Lester et al., (1968).

The air-ethanol flow-rates and daily doses of pyrazole maintained blood ethanol levels in 5 DAY LOW exposed rats at 0.83 $\pm$ 0.09 mg/ml/blood after 24 hours to 1.80 $\pm$ 0.05 mg/ml/blood after 120 hours of ethanol inhalation. In 5 DAY HIGH exposed rats levels were 0.78 $\pm$ 0.14 mg/ml/blood after 24 hours to 1.98 $\pm$ 0.16 mg/ml/blood after 120 hours

of ethanol inhalation. In 7 DAY HIGH exposed rats levels were 0.93 ± 0.20 mg/ml/blood after 24 hours to 2.19 ± 0.14 mg/ml/blood after 168 hours of ethanol inhalation. Table 1 shows the mean blood ethanol levels on each day of ethanol inhalation in groups inhaling ethanol under each of the three protocols. Figure 6 demonstrates a linear relationship between the blood ethanol level and the ethanol vapor concentration within the inhalation chamber. The data represented in Figure 6 was prepared by plotting the mean blood ethanol levels of rats after exposure for 24 hours to ethanol vapors at flow-rates of 0.45-0.9 liters/min regardless of the duration of ethanol inhalation at other flow-rates. For example, rats exposed to the 5 DAY LOW protocol inhaled ethanol vapors at flow-rates of 0.6 liters/min from 72-120 hours of exposure, while rats in the 5 DAY HIGH group inhaled ethanol vapors at a flow-rate of 0.6 liters/min from 24-48 hours of exposure. The blood levels of all rats at these flow-rates were used for mean determinations. Chamber ethanol concentrations were taken from Figure 4. Figure 7 demonstrates a linear relation between blood ethanol levels and the cumulative doses of pyrazole. Since, both ethanol vapor concentration and pyrazole are increasing during ethanol inhalation it is not possible, from these retrospective comparisons, to conclude that pyrazole itself is not responsible for the increasing blood ethanol levels. However, Goldstein (1972a.), has shown that pyrazole does not cause increases in the blood

TABLE 1

BLOOD ETHANOL LEVELS IN RATS INHALING ETHANOL VAPORS

	MEAN BLOOD ETHANOL ± S.E. (MG/ML)							
	DAYS							
TREATMENTS	1	2	3	4	5	6	7	WITHDRAWN 24 HOURS
5 DAY LOW	0.83 ± 0.09 (3)	0.95 ± 0.06 (3)	1.61 ± 0.02 (3)	1.63 ± 0.05 (3)	1.80 ± 0.05 (3)	-	-	0.09 ± 0.02 (3)
5 DAY HIGH*	0.78 ± 0.14 (3)	0.99 ± 0.02 (3)	1.85 ± 0.07 (3)	1.86 ± 0.10 (3)	1.98 ± 0.16 (3)	-	-	0.18 ± 0.04 (6)
7 DAY HIGH**	0.93 ± 0.02 (3)	1.27 ± 0.16 (3)	1.77 ± 0.08 (3)	1.81 ± 0.11 (3)	1.98 ± 0.13 (3)	1.90 ± 0.12 (3)	2.19 ± 0.14 (6)	0.36 ± 0.01 (3)

ANALYSIS OF VARIANCE

SOURCE	d.f.	SS	MS	F	P
DAYS	4	8.2745	2.0686	73.16	<0.005
CONCENTRATIONS	2	0.2861	0.1431	5.06	<0.025
INTERACTION	8	0.1793	0.0224	0.79	N.S.
ERROR	30	0.8482	0.0283		
TOTAL	44	9.5881			

* $P < 0.05$; ** $P < 0.005$ vs. 5 DAY LOW

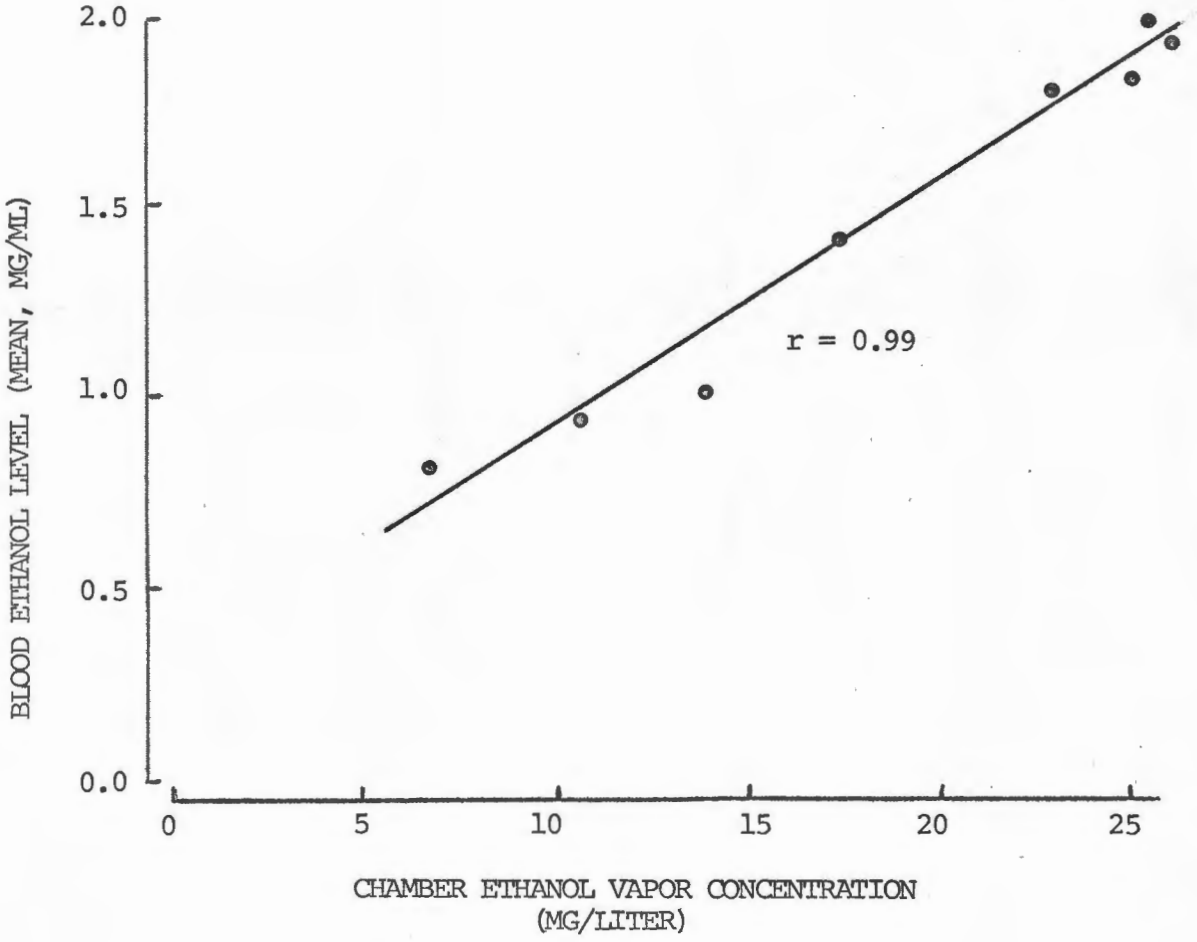


Figure 6. RELATIONSHIP BETWEEN CHAMBER ETHANOL VAPOR CONCENTRATION AND THE MEAN BLOOD ETHANOL LEVELS OF RATS INHALING ETHANOL. Each point represents the mean blood ethanol level for 3, 3, 9, 12, 6, 6, 3 and 6 rats corresponding to chamber ethanol vapor concentrations of 8.8, 10.8, 14.0, 17.5, 23.0, 25.1, 25.5 and 26.1 mg/liter, respectively.

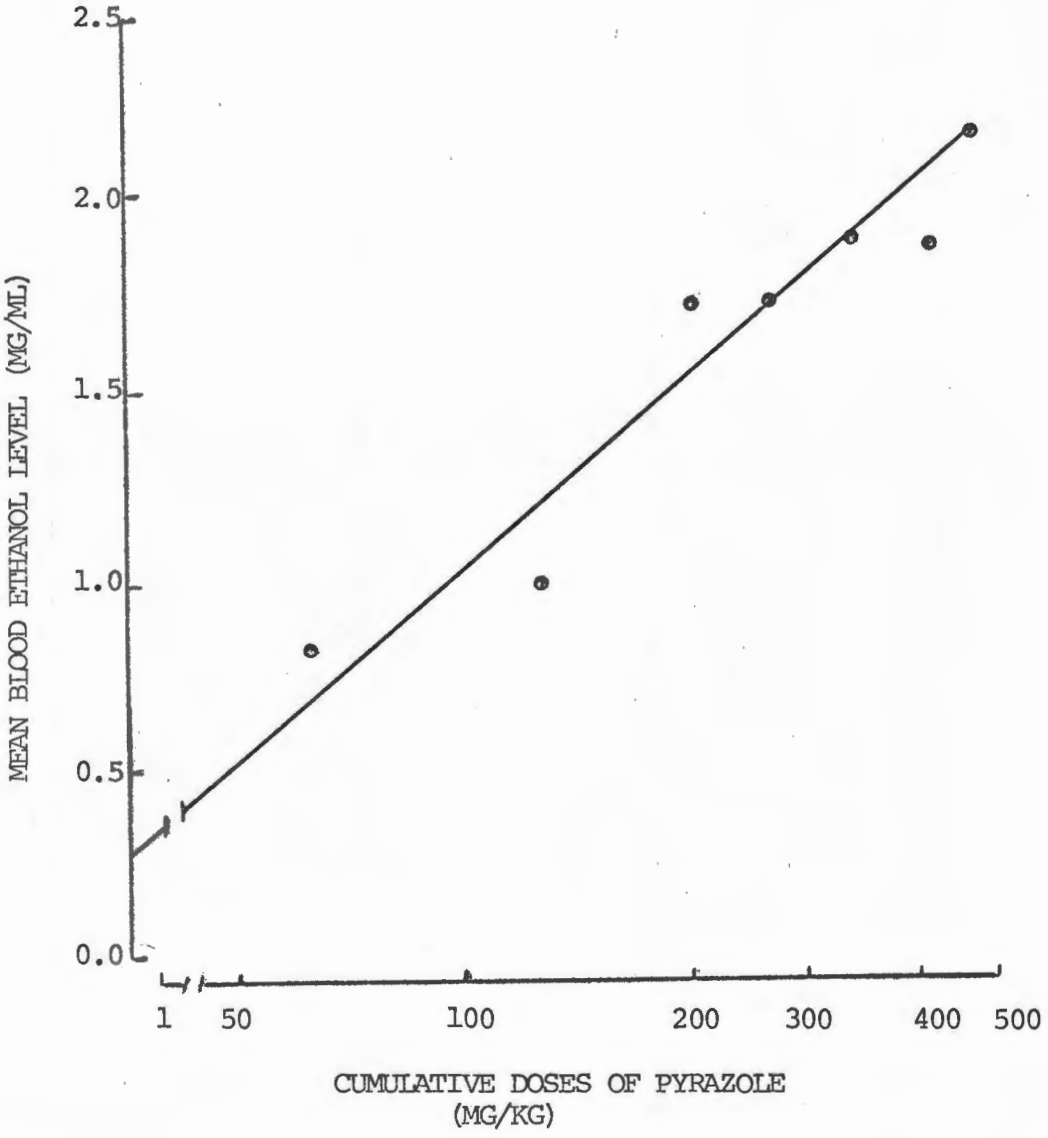


Figure 7. RELATIONSHIP BETWEEN THE CUMULATIVE DOSES OF PYRAZOLE AND THE MEAN BLOOD ETHANOL LEVELS OF RATS INHALING ETHANOL. Rats were administered ethanol by either the 5 DAY LOW, 5 DAY HIGH or 7 DAY HIGH protocol and administered pyrazole 68 mg/kg/day, i.p. for 5 or 7 days. Each point represents the mean blood ethanol level of 9 rats, except after a cumulative dose of pyrazole of 408 and 476 mg/kg where the N = 3 and 6, respectively.

ethanol concentrations when injected daily while ethanol concentrations in the inhalation chamber are held constant.

Ethanol Tolerance:

Measurement of tolerance to ethanol was done by measuring the onset and duration of ethanol-induced narcosis. Ethanol-induced narcosis was measured in rats after either 72 hours of ethanol withdrawal after exposure to the 5 DAY HIGH protocol, withdrawal for 96 hours after five days of pyrazole treatment (68 mg/kg/day, i.p.), corresponding to 72 hours of ethanol withdrawal or after no pretreatment. Onset of narcosis was defined as the interval between injection and the loss of the righting reflex, duration as the interval between onset and recovery of the end point, which was defined as two rightings within one minute. For example, when a rat lying on its back spontaneously righted itself it was immediately placed upon its back again if it again righted itself in one minute time was recorded if it did not time was continued. Rats were starved 24 hours prior to ethanol administration (5 g/kg, 50% in saline, i.p.).

Ethanol Withdrawal:

Rats were withdrawn after continuous inhalation of ethanol vapors 24 hours after their lastinjection of pyrazole and placed in individual cages. At first rats were observed every 2 hours for 16 hours then at longer intervals thereafter. Final intervals for observation were at 0, 12, 24, 36, 48 and 72 hours of withdrawal; observation

duration was one hour. Measurement of withdrawal intensity was done via a ethanol withdrawal intensity scale (see Results, Table 3).

Withdrawal Aggression:

Rats were administered apomorphine or d-amphetamine at various doses and at vari us time periods during ethanol withdrawal. Apomorphine or d-amphetamine were dissolved in distilled water and injected intraperitoneally. The rats were aggregated immediately after injection in groups or three rats and observed for aggressive behaviors. Three parameters of aggression were measured, attacks and bites which were counted and displayed digitally, rearing which was measured in seconds and displayed digitally and vocalizations which were measured automatically via audio relay. Attacks and bites and rearing were measured with the help of Lehigh Valley Electronics programmable digital counters and ATCOTROL clock, while vocalizations were measured using Audio Threshold Detection Relay-761G Scientific Prototype Mfg. Corp., New York, N.Y. Pretreatment with either ethanol, chlordiazepoxide or morphine was administered 30 minutes prior to injection of apomorphine in experiments designed to measure the effectiveness of ethanol, chlordiazepoxide or morphine in reducing apomorphine-induced aggression in ethanol withdrawn rats.

Statistical Analysis:

Statistical analysis performed on data obtained using the ordinal rating scale were done using the non-parametric Mann-Whitney U Test and the Kruskal-Wallis Non-Parametric One-Way Analysis of Variance (Siegel, 1956). Parametric analysis were performed using the Student's "t" Test, Regression Analysis, One and Two-Way Analysis of Variance with preplanned one degree of freedom comparisons (Snedecor and Cochran, 1967).

RESULTS

The results obtained from the study of continuous inhalation of ethanol vapors in rats are described in the following sections:

A) Ethanol-induced narcosis

B) Ethanol withdrawal signs

C) Drug treatment of acute ethanol withdrawal

D) Drug-induced aggression during ethanol withdrawal

E) Drug treatment of apomorphine-induced aggression during ethanol withdrawal

F) Mortality during ethanol withdrawal

A) EFFECT OF ETHANOL INHALATION ON ETHANOL-INDUCED NARCOSIS IN RATS

The onset and duration of ethanol-induced narcosis was measured in rats 72 hours after withdrawal from the 5 DAY HIGH protocol or 96 hours after five days of pyrazole pretreatment (68 mg/kg/day, i.p.) corresponding to 72 hours of ethanol withdrawal or no pretreatment. Onset of narcosis was defined as the interval between injection of ethanol and the loss of the righting reflex. Duration of ethanol narcosis was defined as the interval between onset and recovery of the end point which was defined as 2 rightings within one minute. For example, when a rat lying on its back spontaneously righted itself it was immediately placed on its back again, if it again righted itself time was recorded, if it did not time was continued. Rats were starved 24 hours prior to ethanol administration (5 g/kg, 50% in saline, i.p.). Table 2 summarizes the effects of the above pretreatments on the onset and duration of ethanol-induced narcosis. Pretreatment of ethanol plus pyrazole (5 DAY HIGH) significantly increased the onset and decreased the duration of ethanol-induced narcosis. Pyrazole pretreatment alone did not alter ethanol-induced narcosis.

B) ETHANOL WITHDRAWAL SIGNS

Seven signs of withdrawal were observed in rats withdrawn from inhalation of ethanol vapors along with daily injections of pyrazole. They are piloerection, abnormal posture, tremors, convulsions, headshakes, tail-lifts and

TABLE 2

EFFECT OF PYRAZOLE OR ETHANOL PLUS PYRAZOLE ON ETHANOL-INDUCED NARCOSIS

PRETREATMENT[1]	N	NUMBER DEAD	ETHANOL NARCOSIS[2] (MINUTES, MEAN±S.E. ONSET	P[3]	DURATION	P[3]
AIR + SALINE	16	5	66 ± 8	-	825 ± 56	-
AIR + PYRAZOLE	17	5	70 ± 8	n.s.	846 ± 64	n.s.
ETHANOL + PYRAZOLE	19	6	86 ± 7	<0.05	438 ± 35*	<0.001

[1]Pretreatment was that of the 5 DAY HIGH protocol.
[2]5 g/kg ethanol 50% in saline administered (i.p.) after 72 hours of ethanol withdrawal, 96 hours after the last pyrazole injection.
[3]Student's "t" Test vs Air + Saline
*Student's "t" Test vs Air + Pyrazole $P < 0.001$

deaths. Piloerection, abnormal posture, tremors and convulsions were observed at regular intervals during withdrawal and their intensity measured by the rating scale shown in Table 3. The withdrawal signs or headshakes (rapid shaking of the head and occasionally the entire body) and tail-lifts (sudden arching of the tail over the back) were counted and recorded at regular intervals. All withdrawal signs were measured during an observation period, which was one hour in duration at each observation time, except deaths which were cumulated up to and including 72 hours after withdrawal. The ethanol withdrawal syndrome, which consisted of the withdrawal signs of piloerection, abnormal posture, tremors and convulsions, used here as an expression of the intensity of the ethanol withdrawal reaction, was calculated by summing the scores each rats received for the four rated withdrawal signs and then obtaining a median value at each of the hours of ethanol withdrawal observation.

1. Effect of Different Ethanol Inhalation Protocols on the Intensity of the Ethanol Withdrawal Syndrome.

The intensity of the ethanol withdrawal syndrome in rats withdrawn after three different protocols of ethanol inhalation is shown in Figure 8. The intensity of the ethanol withdrawal syndrome in rats, as measured by the ethanol withdrawal scale (Table 3), after withdrawal from the 5 DAY LOW protocol was relatively mild. The peak in-

TABLE 3

ETHANOL WITHDRAWAL INTENSITY RATING SCALE

WITHDRAWAL SIGN	DESCRIPTION	RATING
PILOERECTION	NO PILOERECTION	0
	SLIGHT PILOERECTION	1
	MARKED PILOERECTION	2
	SEVERE "PUFFED APPEARANCE" PILOERECTION	3
ABNORMAL POSTURE	NO ARCHED BACK, TIP-TOE STANCE OR ROTATION OF HINDLIMBS	0
	SLIGHTLY ARCHED BACK	1
	MARKED ARCHING OF BACK	2
	MAXIMUM ARCHED BACK WITH EITHER OR BOTH A TIP-TOE STANCE OR OUTWARD ROTATION OF THE HINDLIMBS	3
TREMOR	NO TWITCHES, JERKS OR TREMBLING	0
	SPORATIC TWITCHING, JERKING OR TREMBLING	1
	MARKED JERKING AND TREMBLING INVOLVING THE ENTIRE BODY	2
	SEVERE, VIOLENT JERKING AND TREMBLING OF LONG DURATION, MAY VOCALIZE, APPROACHES SPONTANEOUS CONVULSION	3
CONVULSION INDUCED BY AIR-BLAST	NO CONVULSION	0
	CLONIC RUNNING	1
	TONIC-CLONIC CONVULSION	2
	TONIC-CLONIC CONVULSION RESULTING IN DEATH	3
	MAXIMUM	12

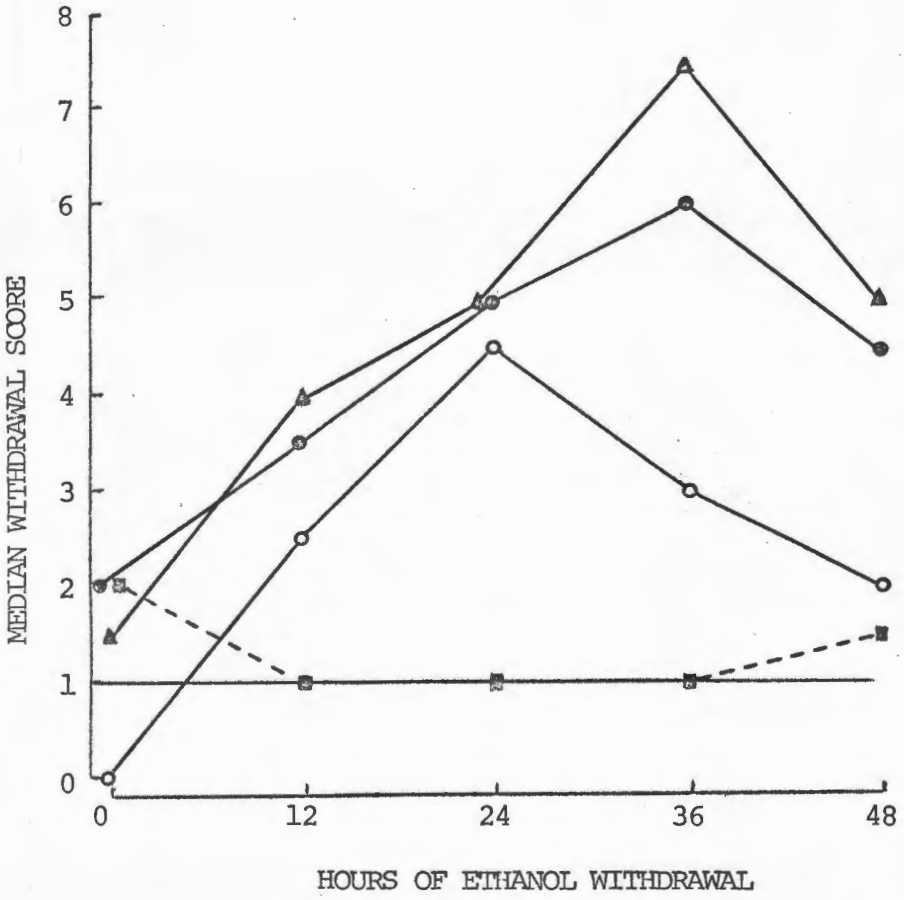


Figure 8. EFFECT OF THREE ETHANOL EXPOSURE PROTOCOLS ON THE INTENSITY OF THE ETHANOL WITHDRAWAL SYNDROME. Rats were withdrawn from inhalation of ethanol vapors after exposure to either the 5 DAY LOW protocol (0.45-0.60 liters/min o—o), the 5 DAY HIGH protocol (0.55-0.90 liters/min ●—●), or the 7 DAY HIGH protocol (0.55-0.95 liters/min ▲—▲). Control rats were administered pyrazole (■---■) or no treatment (——). Rats withdrawn from ethanol inhalation and controls were observed and scored from 0-3 for piloerection, abnormal posture, tremors and convulsions at regular hourly intervals. Scores were combined and the median score determined. Untreated control N = 30 at all time periods, pyrazole treated N = 18 at all time periods, 5 DAY LOW N = 6 at 0, 12, and 24 hours; N = 5 at 36 and 48 hours, 5 DAY HIGH N = 48, 36, 42, 35, and 40 at 0, 12, 24, 36, and 48 hours, respectively, 7 DAY HIGH N = 6, 6, 18, 10 and

tensity occurred after 24 hours of withdrawal with a median score of 4.5. The peak intensity of the ethanol withdrawal syndrome in rats after withdrawal from either the 5 DAY HIGH or 7 DAY HIGH protocols occurred at 36 hours of withdrawal with median scores of 6.0 and 7.5, respectively. The peak intensity of the ethanol withdrawal syndrome in rats exposed to the 5 DAY LOW protocol was significantly less than rats exposed to the 5 DAY HIGH or 7 DAY HIGH protocols ($P < 0.001$ Mann-Whitney U Test). The peak intensity of the 5 and 7 DAY HIGH protocols were not significantly different. Therefore, the 5 DAY HIGH protocol was selected for most studies, since the longer inhalation protocol did not significantly increase the withdrawal intensity. Goldstein and Pal (1971), have shown that some ethanol withdrawal signs can occur at low levels of intensity in untreated and pyrazole treated mice. Therefore, control rats were administered pyrazole for 5 days and control rats which were not pretreated were observed and rated as were ethanol withdrawn rats. These results are also seen in Figure 8. See Appendix I for individual values contained in Figure 8.

2. Effect of Pyrazole on the Body Weight of Rats Inhaling Ethanol

Table 4 summarizes the effects of chronic administration of pyrazole or ethanol plus pyrazole on the body weight of rats. Food and water were available ad libitum at all times to these rats. Untreated rats gained 6% of their starting weight over the five-day period. Rats ad-

TABLE 4

EFFECT OF PYRAZOLE ON THE BODY WEIGHT OF RATS INHALING ETHANOL

GROUP	TREATMENT[1]	N	MEAN ± S.E. PRE-DRUG WEIGHT	MEAN ± S.E. POST-DRUG WEIGHT	WEIGHT CHANGE GRAM	WEIGHT CHANGE %	P[2]
1	NONE	20	311 ± 5	329 ± 5	+ 19 ± 1	+ 6 ± 0.4	
2	AIR + PYRAZOLE	48	295 ± 10	248 ± 10	- 47 ± 2	- 17 ± 0.7	1 vs 2, < 0.001 2 vs 3, < 0.05
3	ETHANOL + PYRAZOLE	30	290 ± 7	254 ± 7	33 ± 4	12 ± 1.4	1 vs 3, < 0.001

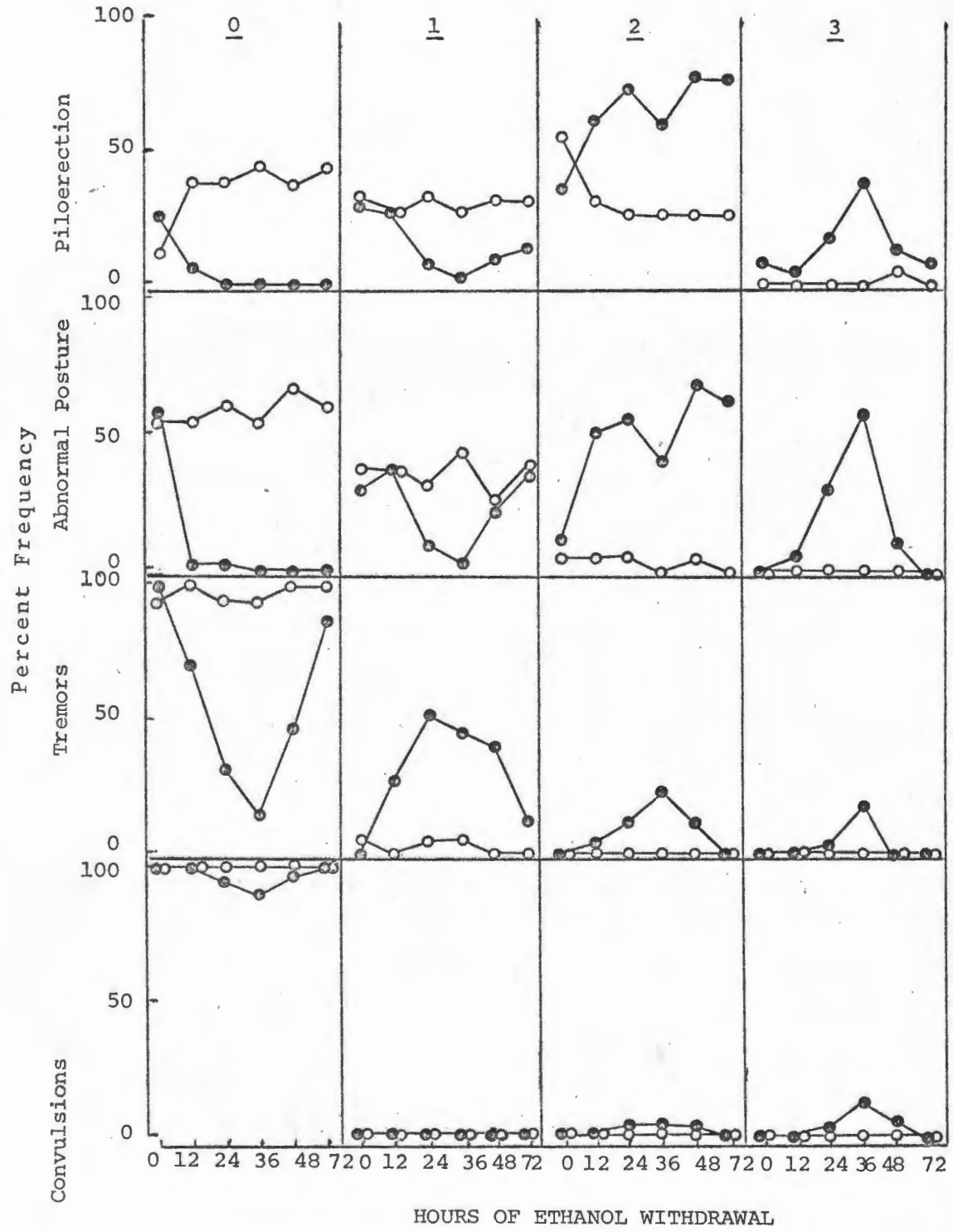
[1]Treatment was that of the 5 DAY LOW protocol.

[2]Student's "t" Test.

ministered pyrazole (68 mg/kg/day) lost 17% of their starting weight after five days of treatment and weighted significantly less than untreated controls. Although rats inhaling ethanol along with daily injections of pyrazole lost 12% of their starting weight and weighted significantly less than untreated controls their weight loss was significantly less than rats treated with only pyrazole. Data expressed as weight change (Table 4) was calculated as follows: grams lost or gained was calculated by subtracting the pre-drug weight from the post-drug weight of each rat and then obtaining a mean and standard error for all rats; % weight change was calculated by dividing the grams lost by the pre-drug weight of each rat and then obtaining a mean and standard error for all rats.

3. Effect of Ethanol Withdrawal Duration on the Intensity of Ethanol Withdrawal Signs

The data in Figure 9a. demonstrates the effect of the duration of ethanol withdrawal on the frequency with which each rating of withdrawal intensity was assigned to the four withdrawal signs piloerection, abnormal posture, tremors and convulsions. In order to clearly identify the hour at which each withdrawal sign reaches peak intensity the data in Figure 9a is represented in Figure 9b. as the percent frequency with which ratings of either 0 or 1 and 2 or 3 were assigned. The highest frequency of maximum (2 or 3) and lowest frequency of minimum (0 or 1) scores


0
1
2
3
Piloerection
Abnormal Posture
Tremors
Convulsions
Percent Frequency
100
50
0
0 12 24 36 48 72
HOURS OF ETHANOL WITHDRAWAL

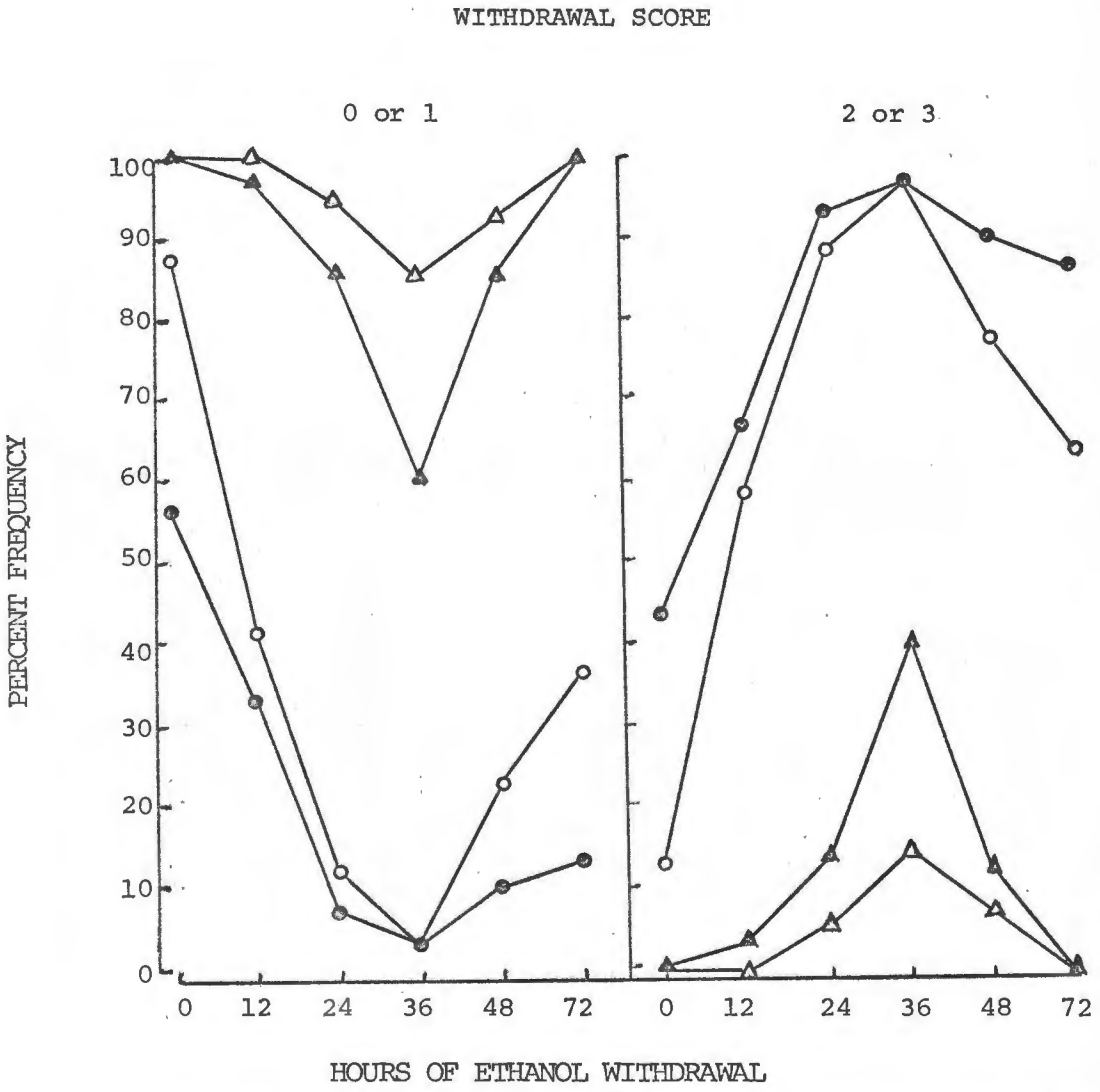


Figure 9b. EFFECT OF WITHDRAWAL DURATION UPON THE INTENSITY OF ETHANOL WITHDRAWAL SIGNS. Shown are the percent frequency with which withdrawal scores were assigned to rats withdrawn from ethanol inhalation under the 5 DAY HIGH protocol for the withdrawal signs of piloerection (o——o), abnormal posture (o——o), tremors (Δ——Δ) and convulsions (Δ——Δ). See Figure 9a. for N at each time period.

occurred at 36 hours of ethanol withdrawal. See Appendix II for data expressed in Figures 9a and 9b.

Figure 10 demonstrates the effect of the duration of ethanol withdrawal on the occurrence of headshakes. Although the mean number of headshakes are maximum at 36 hours of withdrawal the variability is high. Although, untreated rats show a low frequency of headshakes, rats treated with pyrazole display a relatively high frequency of headshakes which are significantly greater than the frequency observed in untreated rats ($P < 0.0005$, $P < 0.0005$, $P < 0.0005$, $P < 0.0005$ $P < 0.0005$ and $P < 0.005$ at 0, 12, 24, 36, 48 and 72 hours. Withdrawal headshakes are significantly less than pyrazole treated rats at 0 hours of withdrawal ($P < 0.0005$) and greater than pyrazole treated rats at 36 and 72 hours of withdrawal ($P < 0.001$ and $P < 0.05$, respectively).

Figure 11 demonstrates the effect of the duration of ethanol withdrawal on the frequency of tail-lifts. Tail-lifts were not seen in untreated controls and only at very low levels at 0 hours of withdrawal in pyrazole treated rats. However, in ethanol withdrawn rats the mean number of tail-lifts are maximum at 24 hours of withdrawal, but do not decline until 48 hours of ethanol withdrawal. Tail-lifts are significantly greater than pyrazole controls at 12, 24 and 36 hours of ethanol withdrawal ($P < 0.05$, $P < 0.05$ and $P < 0.01$, respectively).

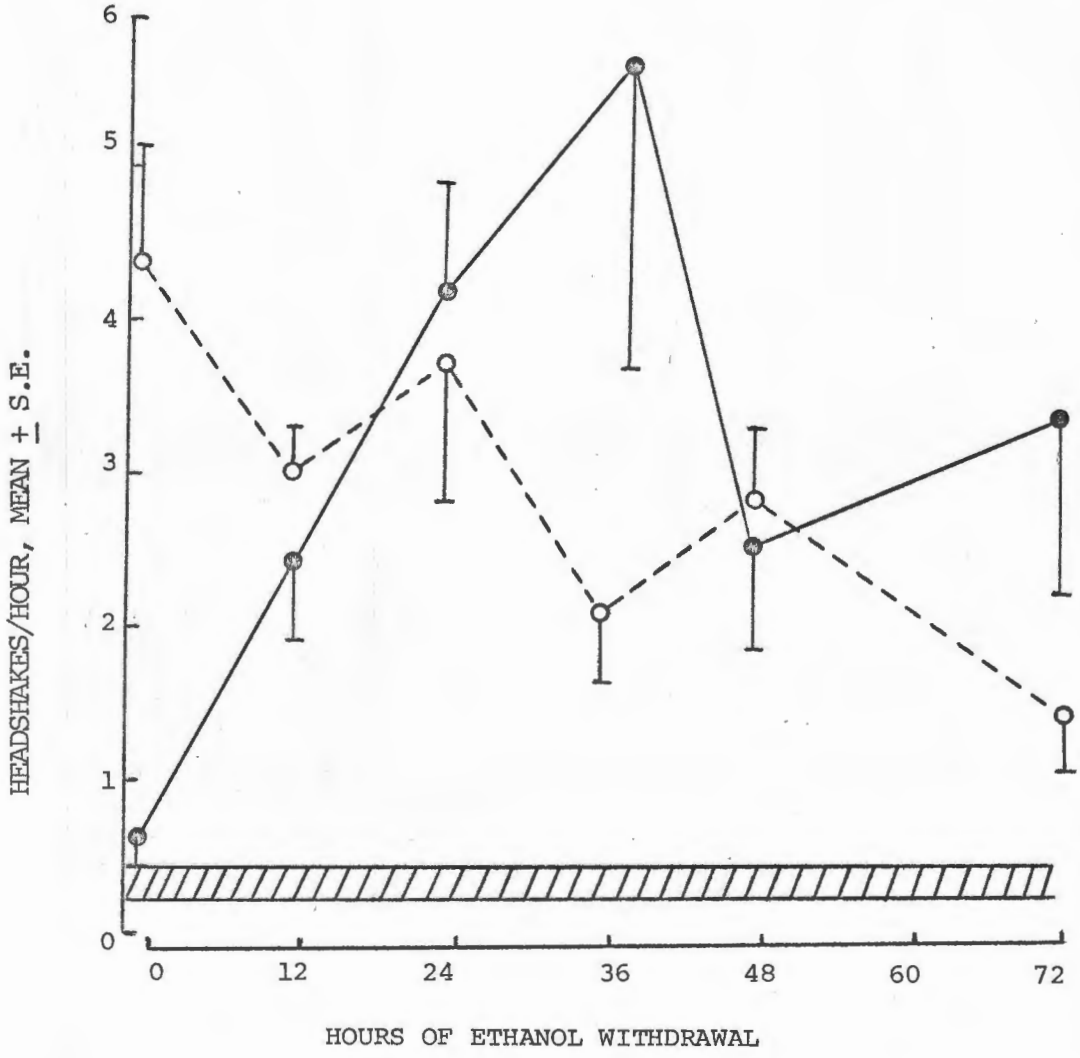


Figure 10. EFFECT OF THE DURATION OF ETHANOL WITHDRAWAL ON THE OCCURENCE OF HEADSHAKES. Rats withdrawn from inhalation of ethanol vapors under the 5 DAY HIGH protocol (●——●), pyrazole treated rats (o——o) and untreated rats (——) were observed for one hour for headshakes. Untreated rats were observed at one time period. 5 DAY HIGH and pyrazole treated rats were observed at six time periods over a 72 hour period. Pyrazole treated rats N = 18 at all time periods, untreated rats N = 30 and ethanol exposed rats N = 48, 36, 42, 35, 40 and 22 at 0, 12, 24, 36, 48 and 72 hours of ethanol withdrawal.

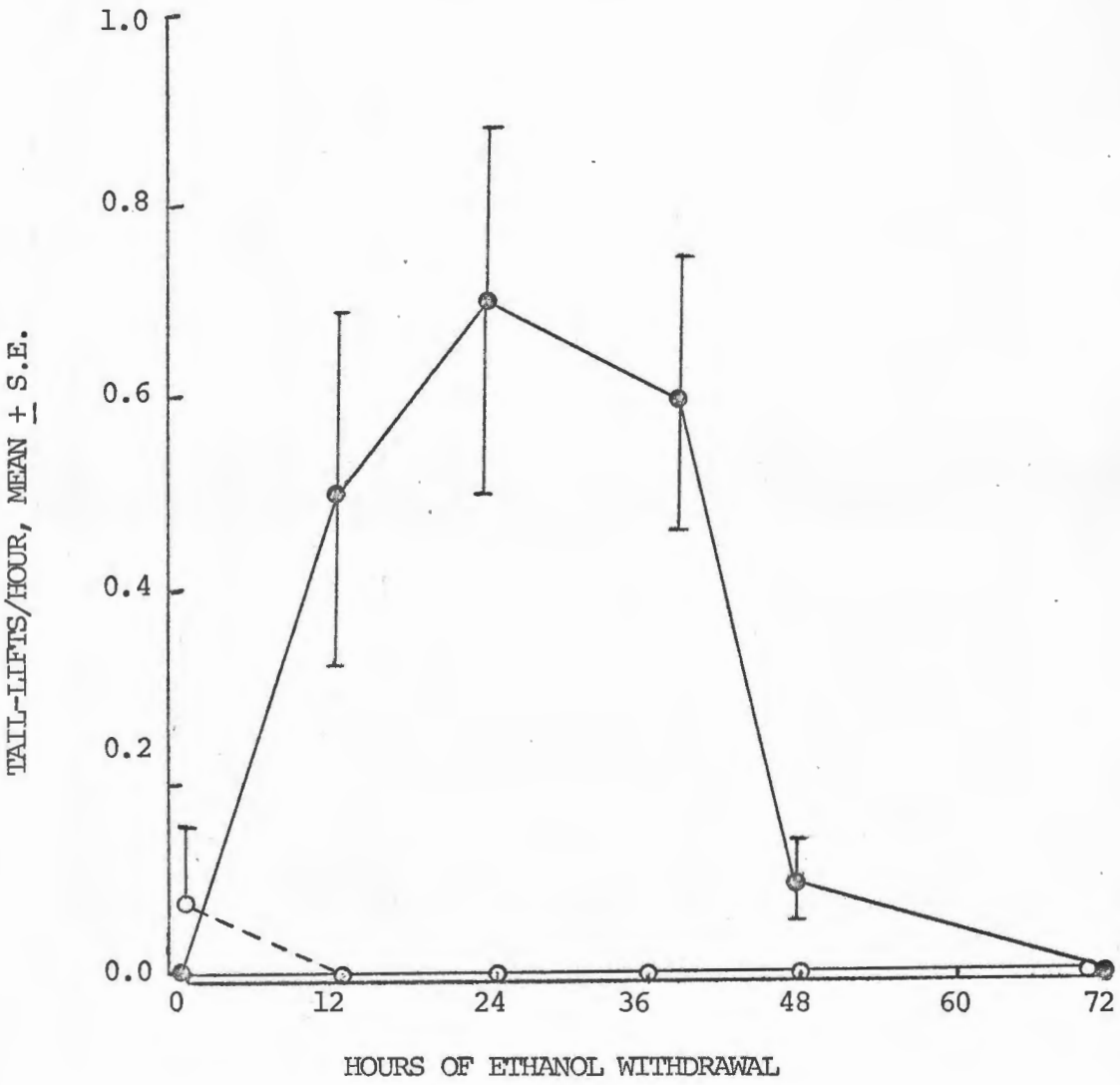
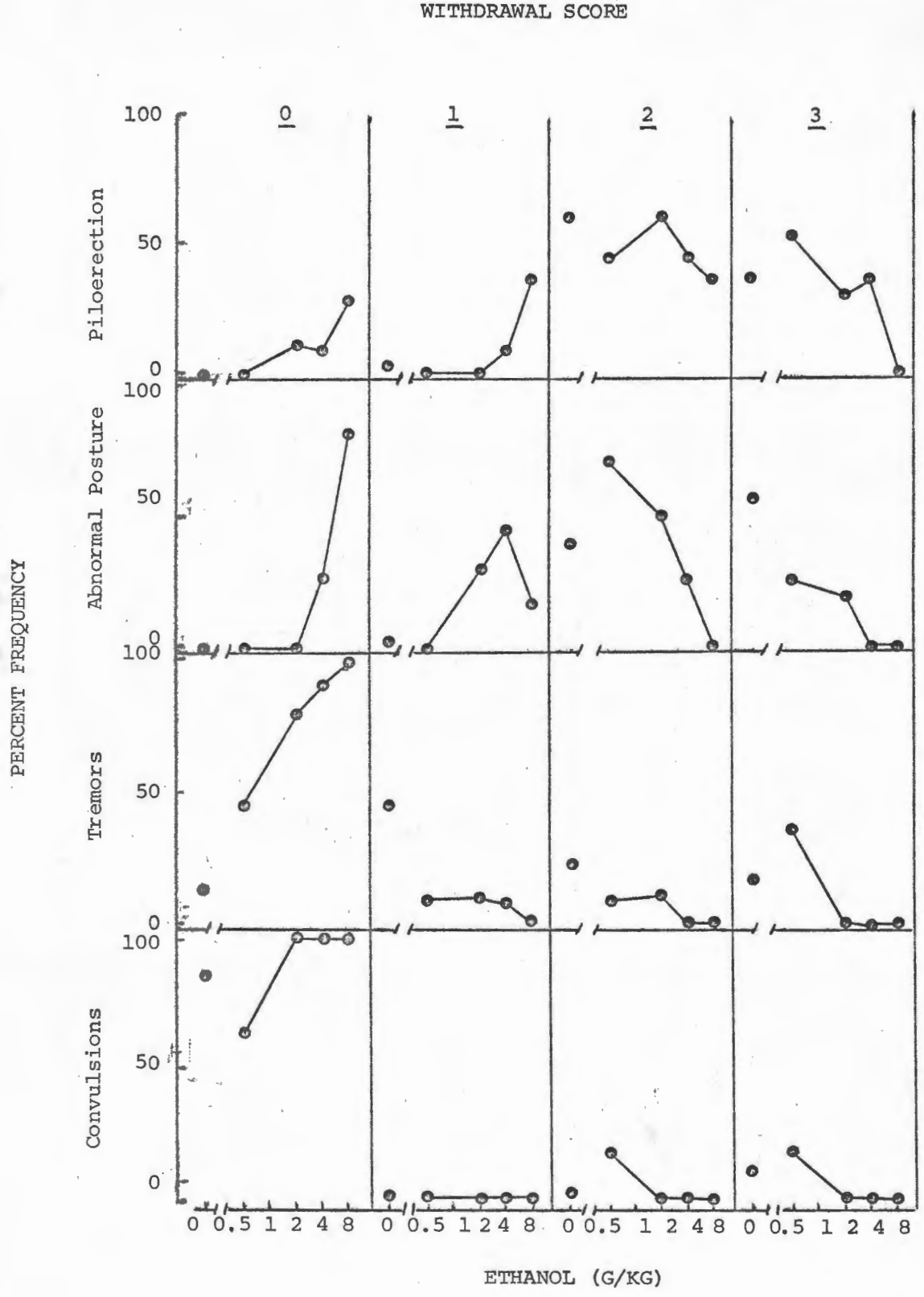


Figure 11. EFFECT OF THE DURATION OF ETHANOL WITHDRAWAL ON THE OCCURRENCE OF TAIL-LIFTS. Rats withdrawn from inhalation of ethanol vapors under the 5 DAY HIGH protocol (o——o), pyrazole treated rats (o——o) and untreated rats (——) were observed for one hour for tail-lifts. Untreated rats were observed at one time period. 5 DAY HIGH and pyrazole treated rats were observed at six time periods over a 72 hour period. Pyrazole treated rats N = 18 at all time periods, untreated rats N = 30 and ethanol exposed rats N = 48, 36, 42, 35, 40 and 22 at 0, 12, 24, 36, 48 and 72 hours of ethanol withdrawal.

c) DRUG TREATMENT OF ACUTE ETHANOL WITHDRAWAL IN RATS

1. Effect of Ethanol on the Ethanol Withdrawal Signs

Figure 12a. demonstrates the effect of orally administered ethanol on the ethanol withdrawal signs. Ethanol (50% in saline, p.o.) was administered 30 minutes prior to observation of withdrawal signs after 36 hours of ethanol withdrawal. In order to clearly identify the effect of ethanol on the ethanol withdrawal signs the data in Figure 12a. is expressed in Figure 12b. as the percent frequency of rats rated either 0 or 1 and 2 or 3. It can be seen in Figure 12b. that as the dose of ethanol administered increases the frequency with which minimum scores (0 or 1) were assigned increases, while the frequency of maximum scores (2 or 3) decreases representing a decrease in the intensity of the ethanol withdrawal signs. See Appendix III for data contained in Figure 12a. and 12b. All signs of ethanol withdrawal were significantly reduced by administration of ethanol (piloerection $P < 0.02$, abnormal posture $P < 0.001$, tremors $P < 0.001$ and convulsions $P < 0.001$, Kruskal-Wallis Non-parametric One-Way Analysis of Variance). It can be seen in Figures 9a and 9b that all of the rated ethanol withdrawal signs follow a similar time course in intensity development and in Figure 12a and 12b that all of these ethanol withdrawal signs are reduced by reinstating ethanol administration. Therefore, all four rated ethanol withdrawal signs were combined into an ethanol withdrawal


WITHDRAWAL SCORE
0
1
2
3
PERCENT FREQUENCY
Piloerection
Abnormal Posture
Tremors
Convulsions
100
50
0
0 0.5 1 2 4 8
ETHANOL (G/KG)

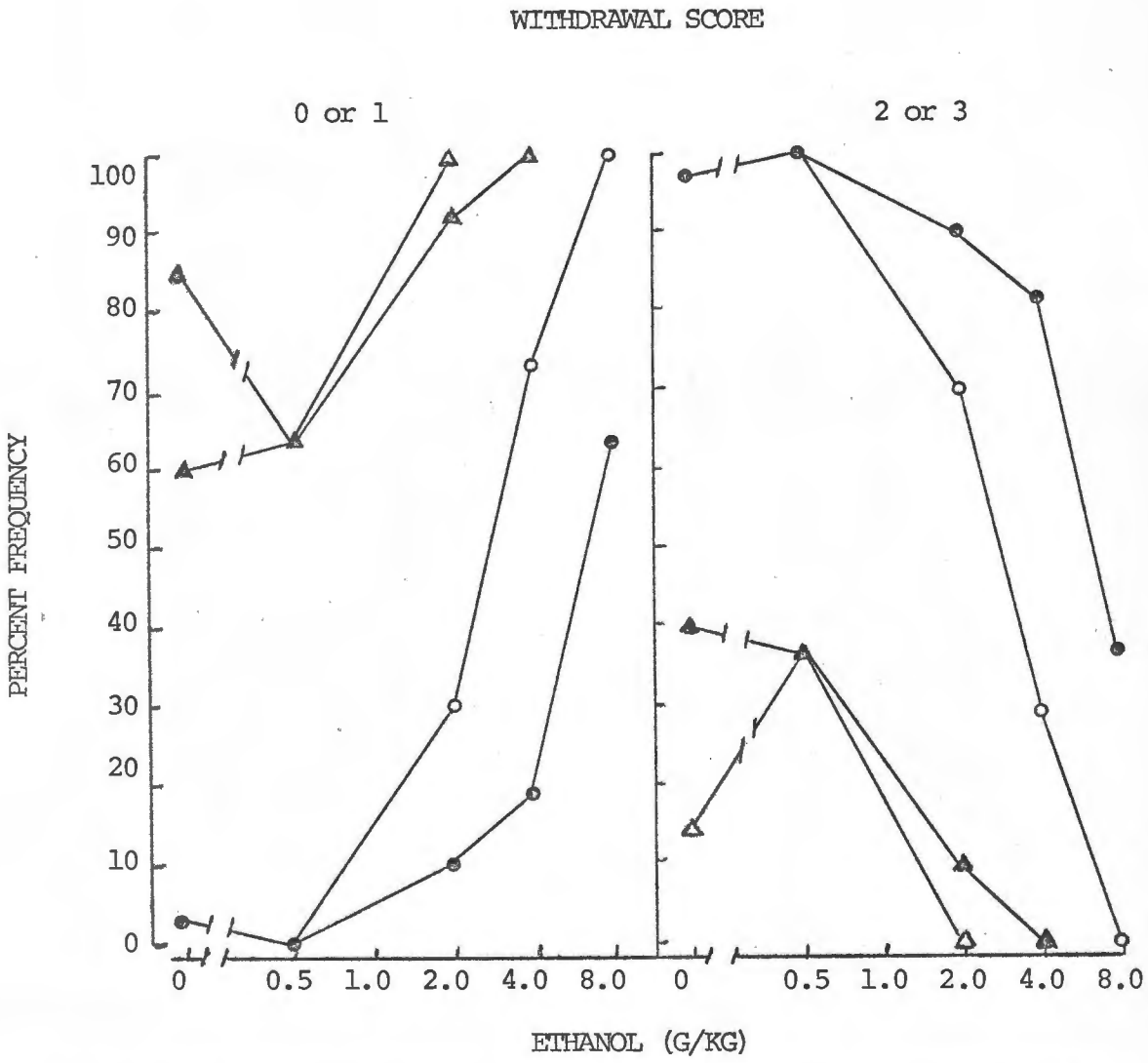


Figure 12b. EFFECT OF ORALLY ADMINISTERED ETHANOL ON ETHANOL-WITHDRAWAL SIGNS. Shown are the withdrawal scores for rats withdrawn 36 hours after exposure under the 5 DAY HIGH protocol for the withdrawal signs of Piloerection (o——o), Abnormal Posture (o——o), Tremors (▲——▲) and Convulsions (△——△). Ethanol was administered orally 30 minutes prior to observation for one hour. See Figure 12a. for N at each dose shown.

syndrome measurement, used here as a simplified expression of the intensity of the ethanol withdrawal reaction. This was done by summing the scores each rat obtained for each of the four rated withdrawal signs (maximum 12) and then obtaining a median withdrawal score. Figure 13 demonstrates the effect of ethanol on the ethanol withdrawal syndrome. Doses of 2.0 g/kg or greater of orally administered ethanol significantly reduced the ethanol withdrawal syndrome ($P < 0.001$ Mann-Whitney U Test). The effect of ethanol on the ethanol withdrawal syndrome was dose-dependent ($r=0.96$, $P < 0.025$, regression analysis). See Appendix IV for data contained in the ethanol withdrawal syndrome Figure 13.

Table 5 summarizes the effect of orally administered ethanol on the frequency of headshakes. Ethanol was administered 30 minutes prior to observation at 36 hours of ethanol withdrawal. A dose of ethanol 2.0 g/kg, 4.0 g/kg and 8.0 g/kg significantly reduced the frequency of headshakes compared to untreated ethanol withdrawn rats ($P < 0.005$, $P < 0.001$ and $P < 0.0005$, respectively Student's "t" Test).

Table 6 summarizes the effect of orally administered ethanol on the frequency of tail-lifts. Ethanol was administered 30 minutes prior to observation at 36 hours of ethanol withdrawal. A dose of 4.0 g/kg and 8.0 g/kg significantly reduced the frequency of tail-lifts compared to untreated ethanol withdrawn rats ($P < 0.01$ and $P < 0.0005$, respectively Student's "t" Test).

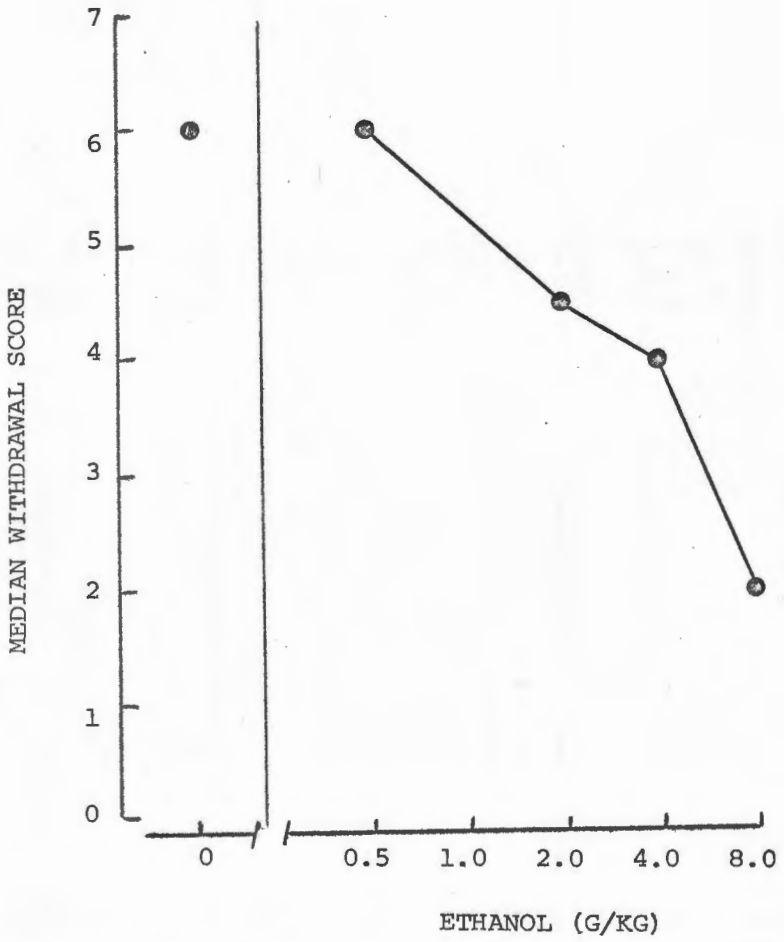


Figure 13. DOSE-DEPENDENT EFFECT OF ORALLY ADMINISTERED ETHANOL ON THE ETHANOL WITHDRAWAL SYNDROME. Rats were withdrawn from inhalation of ethanol vapors for 36 hours then administered ethanol (50% p.o.) 30 minutes prior to 1 hour of observation for withdrawal signs of piloerection, abnormal posture, tremors and convulsions. Scores were combined and the median determined at each dose. Ethanol administration significantly reduced the withdrawal scores (Kruskal-Wallis Non-parametric One-Way Analysis of Variance $P < 0.001$). Reduction of withdrawal scores were significantly less in ethanol treated rats at a dose of 2.0 g/kg, 4.0 g/kg and 8.0 g/kg ($P < 0.001$ Mann-Whitney U Test). N = 35, 11, 10, 11 and 11 at ethanol doses of 0, 0.5, 2.0, 4.0 and 8.0 g/kg, respectively.

TABLE 5

EFFECT OF ETHANOL UPON THE FREQUENCY OF HEADSHAKES

ETHANOL[1] (G/KG)	N	HEADSHAKES/HR, MEAN ± S.E.	P[2]
0	35	5.7 ± 1.14	
0.5	11	6.1 ± 2.17	N.S.
2.0	10	2.3 ± 0.98	< 0.005
4.0	11	1.4 ± 0.54	< 0.001
8.0	11	1.1 ± 0.49	< 0.0005

[1]Ethanol administered orally (50% in saline) 30 minutes prior to 1 hour of observation at 36 hours of ethanol withdrawal.

[2]Student's "t' Test vs 0 ethanol.

TABLE 6

EFFECT OF ETHANOL UPON THE FREQUENCY OF TAIL-LIFTS

ETHANOL[1] (G/KG)	N	TAIL-LIFTS/HR, MEAN + S.E.	P[2]
0	35	0.60 ± 0.175	
0.5	11	0.82 ± 0.325	N.S.
2.0	10	0.30 ± 0.153	N.S.
4.0	11	0.09 ± 0.091	< 0.01
8.0	11	0.00 ± 0.00	< 0.0005

[1]Ethanol administered orally (50% in saline) 30 minutes prior to 1 hour of observation after 36 hours of ethanol withdrawal.

[2]Student's "t" Test vs 0 Ethanol.

2. Effect of Chlordiazepoxide on the Ethanol Withdrawal Syndrome

Figure 14 demonstrates the effect of intraperitoneally injected chlordiazepoxide on the ethanol withdrawal syndrome in rats withdrawn from inhalation of ethanol. See Appendix III for percent frequency for which each rating of withdrawal intensity was assigned for the four rated ethanol withdrawal signs piloerection, abnormal posture, tremors and convulsions. A dose of chlordiazepoxide (i.p.) 40 mg/kg, 80 mg/kg and 160 mg/kg significantly reduced the ethanol withdrawal syndrome ($P < 0.005$, $P < 0.001$ and $P < 0.001$ Mann-Whitney U Test). See Appendix IV for data expressed in Figure 14. Chlordiazepoxide was administered (i.p.) 30 minutes prior to observation for 1 hour at 36 hours of ethanol withdrawal. The reduction of the ethanol withdrawal syndrome by chlordiazepoxide was dose-dependent ($r=0.98$, $P<0.001$, regression analysis).

Table 7 summarizes the effect of administration of chlordiazepoxide on the frequency of headshakes. Chlordiazepoxide was administered (i.p.) 30 minutes prior to observation for 1 hour at 36 hours of ethanol withdrawal. A dose of chlordiazepoxide 160 mg/kg significantly reduced the frequency of headshakes compared to untreated ethanol withdrawn rats ($P < 0.0005$, Student's "t" Test).

Table 8 summarizes the effect of administration of chlordiazepoxide on the frequency of tail-lifts. Chlor-

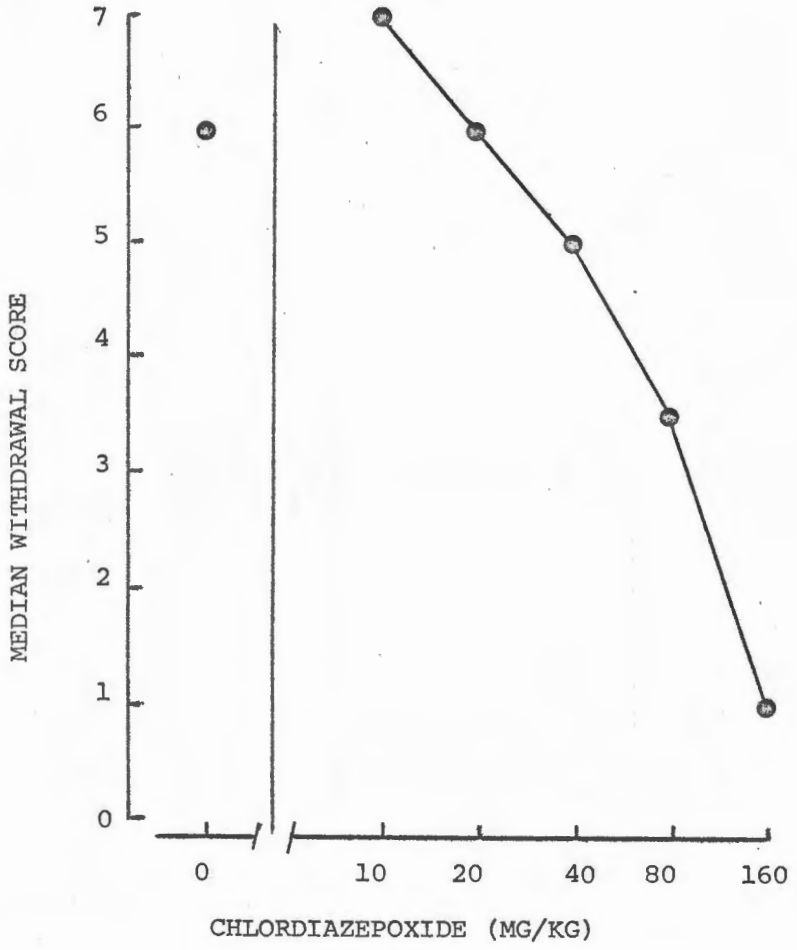


Figure 14. DOSE-DEPENDENT EFFECT OF INTRAPERITONEALLY ADMINISTERED CHIORDIAZEPOXIDE ON THE ETHANOL WITHDRAWAL SYNDROME. Rats were withdrawn from inhalation of ethanol vapors for 36 hours then administered chlordiazepoxide (i.p.) 30 minutes prior to 1 hour of observation for withdrawal signs of piloerection, abnormal posture, tremors and convulsions. Scores were combined and the median determined at each dose. Chlordiazepoxide administration significantly reduced the withdrawal scores (Kruskal-Wallis Non-parametric One-Way Analysis of Variance $P < 0.001$). Reduction of withdrawal scores were significantly less in chlordiazepoxide treated rats at a dose of 40 mg/kg, 80 mg/kg and 160 mg/kg ($P < 0.005$, $P < 0.001$ and $P < 0.001$, respectively, Mann-Whitney U Test). N = 35, 12, 10, 12, 12, and 12 at chlordiazepoxide doses of 0, 10, 20, 40, 80 and 160 mg/kg, respectively.

TABLE 7

EFFECT OF CHLORDIAZEPOXIDE UPON THE FREQUENCY OF HEADSHAKES

CHLORDIAZEPOXIDE[1] (MG/KG)	N	HEADSHAKES/HR, MEAN ± S.E.	P[2]
0	35	5.7 ± 1.14	
10	12	6.0 ± 1.70	N.S.
20	10	5.4 ± 1.48	N.S.
40	12	4.4 ± 0.80	N.S.
80	12	3.6 ± 0.92	N.S.
160	12	0.0 ± 0.00	< 0.0005

[1]Chlordiazepoxide administered (i.p.) 30 minutes prior to 1 hour of observation at 36 hours of ethanol withdrawal.

[2]Student's "t" test vs 0 mg/kg Chlordiazepoxide.

TABLE 8

EFFECT OF CHLORDIAZEPOXIDE UPON THE FREQUENCY OF TAIL-LIFTS

CHLORDIAZEPOXIDE[1] (MG/KG)	N	TAIL-LIFTS/HR, MEAN ± S.E.	P[2]
0	35	0.60 ± 0.175	
10	12	0.75 ± 0.304	N.S.
20	10	0.50 ± 0.220	N.S.
40	12	0.25 ± 0.130	N.S.
80	12	0.00 ± 0.00	< 0.001
160	12	0.00 ± 0.00	< 0.001

[1]Chlordiazepoxide administered (i.p.) 30 minutes prior to observation for 1 hour after 36 hours of ethanol withdrawal.

[2]Student's "t" Test vs 0 mg/kg Chlordiazepoxide.

diazepoxide on the frequenty of tail-lifts. Chlordiaze-poxide was administered (i.p.) 30 minutes prior to observation for 1 hour at 36 hours of ethanol withdrawal. A dose of 80 mg/kg and 160 mg/kg of chlordiazepoxide significantly reduced the frequency of tail-lifts when compared to untreated ethanol withdrawn rats ($P < 0.001$ and $P < 0.001$, respectively Student's "t" Test).

3. Effect of Morphine Sulfate on the Ethanol Withdrawal Syndrome

Figure 15 demonstrates the effect of intraperitoneally administered morphine sulfate on the ethanol withdrawn syndrome in rats withdrawn from inhalation of ethanol. See Appendix III for percent frequency for which each rating of withdrawal intensity was assigned for the four rated ethanol withdrawal signs piloerection, abnormal posture, tremors and convulsions. A dose of morphine sulfate (i.p.) 10 mg/kg, 20 mg/kg and 40 mg/kg significantly reduced the ethanol withdrawal syndrome ($P < 0.01$, $P < 0.001$ and $P < 0.001$, respectively Mann-Whitney U Test). See Appendix IV for data expressed in Figure 15. Morphine sulfate was administered (i.p.) 30 minutes prior to observation for 1 hour at 36 hours of ethanol withdrawal. The reduction of the ethanol withdrawal syndrome by morphine sulfate was dose-dependent ($r = 0.99$, $P < 0.05$ regression analysis).

Table 9 summarizes the effect of administration of morphine sulfate on the frequency of headshakes. Morphine

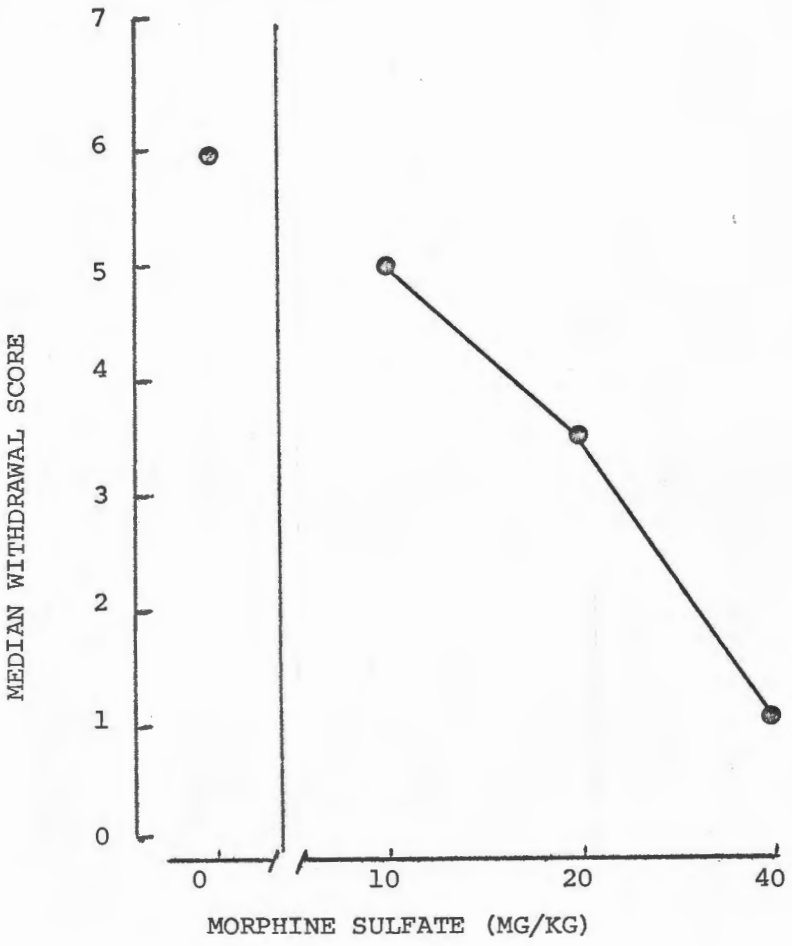


Figure 15. DOSE-DEPENDENT EFFECT OF INTRAPERITONEALLY ADMINISTERED MORPHINE SULFATE ON THE ETHANOL WITHDRAWAL SYNDROME. Rats were withdrawn from inhalation of ethanol vapors for 36 hours then administered morphine sulfate (i.p.) 30 minutes prior to 1 hour of observation for withdrawal signs of piloerection, abnormal posture, tremors and convulsions. Scores were combined and the median determined for each dose. Morphine sulfate significantly reduced the withdrawal scores (Kruskal-Wallis Non-parametric One-Way Analysis of Variance $P < 0.001$). Reduction of withdrawal scores were significantly less in morphine sulfate treated rats at a dose of 10 mg/kg, 20 mg/kg and 40 mg/kg ($P < 0.01$, $P < 0.001$ and $P < 0.001$, respectively, Mann-Whitney U Test). N = 35, 12, 12, 11 at doses of morphine sulfate,of 0, 10, 20, and 40 mg/kg, respectively.

TABLE 9

EFFECT OF MORPHINE SULFATE UPON THE FREQUENCY OF HEADSHAKES

MORPHINE[1] (MG/KG)	N	HEADSHAKES/HR, MEAN ± S.E.	P[2]
0	35	5.7 ± 1.14	
10	12	3.2 ± 0.90	< 0.05
20	12	0.7 ± 0.26	< 0.0005
40	11	0.0 ± 0.00	< 0.0005

[1]Morphine sulfate administered (i.p.) 30 minutes prior to 1 hour of observation at 36 hours of ethanol withdrawal.

[2]Student's "t" Test vs 0 mg/kg Morphine Sulfate.

sulfate was administered (i.p.) 30 minutes prior to observation for 1 hour at 36 hours of ethanol withdrawal. A dose of morphine sulfate 10 mg/kg, 20 mg/kg and 40 mg/kg significantly reduced the frequency of headshakes compared to untreated ethanol withdrawn rats ($P < 0.05$, $P < 0.0005$ and $P < 0.0005$, respectively Student's "t" Test).

Table 10 summarizes the effect of administration of morphine sulfate on the frequency of tail-lifts. Morphine sulfate was administered (i.p.) 30 minutes prior to observation for 1 hour at 36 hours of ethanol withdrawal. A dose of 20 mg/kg and 40 mg/kg significantly reduced the frequency of tail-lifts when compared to untreated ethanol withdrawn rats ($P < 0.025$ and $P < 0.0005$, respectively Student's "t" Test).

D) DRUG-INDUCED AGGRESSION DURING ETHANOL WITHDRAWAL IN RATS

1. Effect of the Duration of Ethanol Withdrawal on Apomorphine-Induced Aggression in Ethanol Withdrawn Rats

Rats withdrawn from inhalation of ethanol vapors were placed in individual cages until the time of aggression testing. Table 11 summarizes the effect of administration of apomorphine (2.50 mg/kg) to rats withdrawn from ethanol for various durations of time. Rats were addicted to ethanol using the 5 DAY LOW protocol. Analysis of variance of each of the parameters of aggression (attacks and bites, rearing and vocalizations) shows a significant effect of

TABLE 10

EFFECT OF MORPHINE SULFATE UPON THE FREQUENCY OF TAIL-LIFTS

MORPHINE[1] (MG/KG)	N	TAIL-LIFTS/HR, MEAN ± S.E.	P[2]
0	35	0.60 ± 0.175	
10	12	0.33 ± 0.140	N.S.
20	12	0.17 ± 0.112	< 0.025
40	11	0.00 ± 0.000	< 0.0005

[1]Morphine sulfate administered (i.p.) 30 minutes prior to 1 hour of observation at 36 hours of ethanol withdrawal.

[2]Student's "t" Test vs 0 mg/kg Morphine Sulfate.

TABLE 11

EFFECT OF THE DURATION OF ETHANOL WITHDRAWAL ON APOMORPHINE-INDUCED AGGRESSION

TREATMENT	HOURS[1] WITHDRAWN	N[2]	RESPONSES/HOUR (MEAN ± S.E.) ATTACKS + BITES	REARING	VOCALIZATIONS
AIR	24	4	0 + 0	0 ± 0	13 ± 2
AIR + PYRAZOLE	72	8	0 ± 0	0 ± 0	0 ± 0
ETHANOL	24	4	150 ± 46	927 ± 426	643 ± 242
+	48	2	174 ± 26	2211 ± 719	1659 ± 446 *
PYRAZOLE	72	4	308 ± 33**	2500 ± 228**	1767 ± 184 **

Analysis of Variance of Attacks and Bites

SOURCE	d.f.	SS	MS	F	P
HOURS	2	55129	27564	4.88	< 0.05
ERROR	7	39576	5654		
TOTAL		94705			

Analysis of Variance of Rearing

SOURCE	d.f.	SS	MS	F	P
HOURS	2	5342717	2671359	4.86	< 0.05
ERROR	7	3843884	549126		
TOTAL		9186601			

Analysis of Variance of Vocalizations

SOURCE	d.f.	SS	MS	F	P
HOURS	2	2858786	1429393		
ERROR	7	1504630	214947	6.65	< 0.025
TOTAL	9	4363416			

[1]Apomorphine (2.50 mg/kg) administered (i.p.) immediately prior to aggregation.

[2]N = number of groups, each group contains 3 rats.

* $P < 0.05$; ** $P < 0.025$; *** $P < 0.01$, comparisons among 24, 48 and 72 hour withdrawn rats after ethanol + pyrazole treatment under 5 DAY LOW protocol.

the duration of ethanol withdrawal on the intensity of apomorphine-induced aggression. Rats withdrawn for 72 hours display the most intense aggression which is significantly greater than all three parameters of aggression at 24 hours of ethanol withdrawal.

2. Effect of the Dose of Apomorphine on Apomorphine-Induced Aggression in Ethanol Withdrawn Rats

Table 12 summarizes the effect of various doses of apomorphine on apomorphine-induced aggression in rats withdrawn 72 hours after inhalation of ethanol vapors (5 DAY HIGH). The analysis of variance shows a significant effect of the dose of apomorphine on all parameters of aggression. Further analysis using one degree of freedom comparisons revealed that a dose of 1.25 mg/kg apomorphine did not significantly increase the intensity of any parameter of aggression when compared to rats receiving no apomorphine treatment. However doses of 2.50 and 5.00 mg/kg apomorphine significantly increased all parameters of aggression compared to rats receiving no apomorphine treatment. Comparisons among treatments revealed that doses of 2.50 and 5.00 mg/kg apomorphine significantly increased the intensity of aggression on all parameters when compared to rats treated with 1.25 mg/kg apomorphine. However, a dose of 5.00 mg/kg apomorphine did not significantly increase the number of attacks and bites when compared to rats treated with 2.50 mg/kg apomorphine and in fact reduced

TABLE 12

APOMORPHINE-INDUCED AGGRESSION IN ETHANOL WITHDRAWN RATS

APOMORPHINE (MG/KG)	N[2]	RESPONSES/HOUR, MEAN ± S.E. ATTACKS + BITES	REARING	VOCALIZATIONS
0	15	3 ± 1	62 ± 51	21 ± 14
1.25	3	18 ± 6	304 ± 196	74 ± 36
2.50	13	164 ± 20	2052 ± 198	1565 ± 182
5.00[3]	3	185 ± 17	1328 ± 80	709 ± 20
		P	P	P
0 vs 1.25		n.s.	n.s.	n.s.
0 vs 2.50		< 0.005	< 0.005	< 0.005
0 vs 5.00		< 0.005	< 0.005	< 0.005
1.25 vs 2.50		< 0.005	< 0.005	< 0.005
1.25 vs 5.00		< 0.005	< 0.005	< 0.025
2.50 vs 5.00		n.s.	< 0.005	< 0.005
APOMORPHINE (MG/KG)	N	RESPONSES 0-30 MIN, MEAN + S.E. ATTACKS + BITES	REARING	VOCALIZATIONS
2.50	13	98 ± 15	1023 ± 156	821 ± 131*
5.00	3	141 ± 8**	1050 ± 59	552 ± 13
		RESPONSES 30-60 MIN, MEAN + S.E.		
		ATTACKS + BITES	REARING	VOCALIZATIONS
2.50	13	65 ± 10	975 ± 114***	745 ± 94***
5.00[3]	3	43 ± 10	278 ± 42	160 ± 24

ANALYSIS OF VARIANCE OF ATTACKS AND BITES/HOUR

SOURCE	d.f.	SS	MS	F	P
DOSES	3	226263	75421	30.76	< 0.005
ERROR	30	73548	2452		
TOTAL	33	299811			

ANALYSIS OF VARIANCE OF REARING/HOUR

SOURCE	d.f.	SS	MS	F	P
DOSES	3	29308542	9769514	126.35	< 0.005
ERROR	30	2319598	77320		
TOTAL	33	31628140			

ANALYSIS OF VARIANCE OF VOCALIZATIONS/HOUR

SOURCE	d.f.	SS	MS	F	P
DOSES	3	17794972	5931657	34.12	< 0.005
ERROR	30	5215120	173837		
TOTAL	33	23010092			

[1]Apomorphine administered (i.p.) at 72 hours of ethanol withdrawal, 5 DAY HIGH protocol.
[2]N = number of groups, each group contains 3 rats.
[3]In 2/3 groups at least one rat was dead from injuries during observation.
* P<0.05, *** P<0.005 significantly greater than 5.00 mg/kg apomorphine
**P<0.025 significantly greater than 2.50 mg/kg apomorphine, Student's "t" Test.

significantly rearing and vocalizations. Since, a dose of 5.00 mg/kg apomorphine did not significantly increase aggression intensity, but in fact decreased the intensity of both rearing and vocalizations compared to a dose of 2.50 mg/kg apomorphine the one-hour aggression measurement was reanalyzed by breaking out the first 30 minutes and the last 30 minutes of the one hour of aggression measurement (note: during one hour of aggression measurement each parameter was recorded at 10 minute intervals). Analysis of the first 30 minutes of aggression after 2.50 or 5.00 mg/kg apomorphine showed that rats treated with 5.00 mg/kg apomorphine displayed significantly greater numbers of attacks and bites and significantly less vocalizations, while rearing was not significantly increased compared to the first 30 minutes of rats treated with 2.50 mg/kg apomorphine. During the last 30 minutes of aggression measurement there was no significant difference between attacks and bites, but rearing and vocalizations were significantly less in rats treated with 5.00 mg/kg apomorphine than in rats treated with 2.50 mg/kg apomorphine. Furthermore, in the first of the three groups treated with 5.00 mg/kg apomorphine one rat was dead within the first 30 minutes, the other two were dead within the last 30 minutes with none surviving the last 10 minutes of observation (death was due to the severe injuries sustained from bites received during intense aggression). In the second group of the

three treated with 5.00 mg/kg apomorphine one rat died during the first 30 minutes leaving only two rats remaining during the last 30 minutes of aggression measurement. In the third group treated with 5.00 mg/kg apomorphine no rats died during aggression measurement. A dose of 2.50 mg/kg apomorphine was selected for further studies with apomorphine-induced aggression in ethanol withdrawn rats (see discussion for interpretation of results).

3. D-Amphetamine-Induced Aggression in Ethanol Withdrawn Rats

D-Amphetamine was administered (i.p.) to rats withdrawn 72 hours after inhalation of ethanol vapors (5 DAY HIGH) or to rats treated for 5 days with pyrazole (68 mg/kg/day) or to rats administered only air in the inhalation chamber for 5 days. It was found that after administration of d-amphetamine to ethanol withdrawn rats the most consistent and intense aggression was seen 60-120 minutes after injection. Therefore, this time period was selected for measurement of amphetamine-induced aggression. Table 13 summarizes the effects of various doses of d-amphetamine administered to air-only controls, pyrazole controls and ethanol plus pyrazole treated and withdrawn rats. Untreated rats exposed to only air in the inhalation chamber were administered either 32 or 64 mg/kg d-amphetamine in an attempt to induce aggression. No aggression was seen and a dose of 64 mg/kg d-amphetamine administered to air-

TABLE 13

AMPHETAMINE-INDUCED AGGRESSION

TREATMENTS	D-AMPHETAMINE[1] (MG/KG)	N[2]	RESPONSES/HOUR (MEAN ± S.E.) ATTACKS + BITES	REARING	VOCALIZATIONS
AIR	32	4	0 ± 0	0 ± 0	6 ± 3
	64[3]	2	0 ± 0	0 ± 0	43 ± 3
AIR	4	4	0 ± 0	0 ± 0	7 ± 3
	8	4	0 ± 0	0 ± 0	2 ± 1
	16	4	0 ± 0	0 ± 0	6 ± 1
	32[4]	2	0 ± 0	0 ± 0	4 ± 2
PYRAZOLE	64[4]	2	0 ± 0	0 ± 0	16 ± 1
ETHANOL	0	15	3 ± 1	62 ± 51	21 ± 14
+	2	7	15 ± 8**	1492 ± 535***	601 ± 244**
PYRAZOLE	4	6	36 ± 7***	1283 ± 154***	690 ± 101***

[1]Administered (i.p.) after 72 hours of withdrawal 1 hour prior to aggregation, 5 DAY HIGH protocol.

[2]N = number of groups, each group contains 3 rats.

[3]Lethal to 5/6 rats tested.

[4]Lethal to 6/6 rats tested.

*P < 0.05, ** P<0.001, *** P<0.0005 Student's "t" Test vs 0 mg/kg d-amphetamine.

only controls proved fatal to 5 of 6 of these rats. In rats administered only daily doses of pyrazole d-amphetamine was administered at 4, 8, 16, 32 and 64 mg/kg, which also failed to induce aggression and proved fatal to all rats administered 32 or 64 mg/kg d-amphetamine. However, rats withdrawn 72 hours after inhalation of ethanol vapors and administered either 2 or 4 mg/kg d-amphetamine displayed aggression at levels significantly higher on all parameters of aggression compared to rats withdrawn from ethanol inhalation and administered saline.

4. Protracted Drug-Induced Aggression in Ethanol Withdrawn Rats

Rats which had previously fought at 72 hours of ethanol withdrawal after either apomorphine or d-amphetamine were housed individually until the seventh day of withdrawal. These rats were then administered either apomorphine (2.50 mg/kg, i.p.), d-amphetamine (4.00 mg/kg, i.p.) or saline. Rats which had fought at 72 hours of ethanol withdrawal after apomorphine administration were administered apomorphine or saline on the seventh day of withdrawal, while rats which had fought after d-amphetamine at 72 hours of ethanol withdrawal were administered d-amphetamine on the seventh day of withdrawal. Aggression was observed immediately after injection in rats administered apomorphine or saline and from 60-120 minutes after injection with d-amphetamine as was done at 72 hours of ethanol with-

drawal. Administration of either apomorphine or d-amphetamine to seven day ethanol withdrawn rats significantly increased the intensity of all parameters of aggression when compared to rats which had fought at 72 hours of ethanol withdrawal after apomorphine, but administered only saline after seven days of ethanol withdrawal (see Table 14).

E) EFFECT OF DRUG TREATMENT ON APOMORPHINE-INDUCED AGGRESSION IN ETHANOL WITHDRAWN RATS

1. Effect of Ethanol on Apomorphine-Induced Aggression in Ethanol Withdrawn Rats

Table 15 summarizes the effect of orally administered ethanol in rats withdrawn for 96 hours after inhalation of ethanol vapors (5 DAY HIGH). A small number of groups of rats withdrawn from ethanol fail to display aggression after apomorphine (2.50 mg/kg, i.p.) at 72 hours of withdrawal. Therefore, rats were tested at 72 hours of withdrawal with a dose of 2.50 mg/kg apomorphine, if aggression was not seen within a 30-minute period, these groups were omitted from aggression studies at 96 hours of withdrawal. Ethanol was administered orally at various doses 30 minutes prior to administration of apomorphine (2.50 mg/kg, i.p.) at 96 hours of ethanol withdrawal. The analysis of variance shows a significant effect of ethanol on all parameters of apomorphine-induced aggression. Oral administration of ethanol at doses of 4.0 and 8.0 g/kg signficantly reduced apomorphine-induced aggression when

TABLE 14

DRUG-INDUCED AGGRESSION IN SEVEN DAY ETHANOL WITHDRAWN RATS

TREATMENT	DRUG[1] (MG/KG)	N[2]	RESPONSES/HOUR, MEAN ± S.E. ATTACKS+BITES	REARING	VOCALIZATIONS
AIR + PYRAZOLE	APOMORPHINE (2.50)	4	0 ± 0	0 ± 0	0 ± 0
ETHANOL	SALINE	5	0 ± 0	0 ± 0	0 ± 0
+	APOMORPHINE (2.50)	9	40 ± 11	1328 ± 324	911 ± 284
PYRAZOLE	D-AMPHETAMINE (4.00)	5	46 ± 14	1809 ± 200	1087 ± 260
			P[3]	P[3]	P[3]
ETHANOL + PYRAZOLE	APOMORPHINE (2.50)	9	< 0.001	< 0.001	< 0.005
	D-AMPHETAMINE (4.00)	5	< 0.01	< 0.0005	< 0.001

[1]Administered (i.p.) after 7 days of ethanol withdrawal in rats which fought at 72 hours of ethanol withdrawal (5 DAY HIGH).

[2]N = Number of groups, each group contains 3 rats.

[3]Significantly greater than saline treated rats Student's "t" Test.

TABLE 15

REDUCTION OF APOMORPHINE-INDUCED AGGRESSION BY ETHANOL

ETHANOL[1] (G/KG)	N[2]	RESPONSES/HOUR (MEAN ± S.E.) ATTACKS + BITES	REARING	VOCALIZATIONS
0	8	167 ± 23	2669 ± 121	1841 ± 108
0.5	2	129 ± 35	2378 ± 522	1373 ± 103*
2.0	2	216 ± 17	2810 ± 34	2012 ± 90
4.0	2	23 ± 23**	236 ± 236**	54 ± 54**
8.0	2	1 ± 1**	2 ± 2**	8 ± 2**

Analysis of Variance of Attacks and Bites

SOURCE	d.f.	SS	MS	F	P
DOSES	4	82557	20639	6.91	< 0.005
ERROR	11	32876	2989		
TOTAL	15	115433			

Analysis of Variance of Rearing

SOURCE	d.f.	SS	MS	F	P
DOSES	4	19387860	4846965	36.16	< 0.005
ERROR	11	1474378	134034		
TOTAL	15	20862238			

Analysis of Variance of Vocalizations

SOURCE	d.f.	SS	MS	F	P
DOSES	4	9765752	2441438	33.66	< 0.005
ERROR	11	694697	63154		
TOTAL	15	10460449			

[1]Administered at 96 hours of withdrawal 30 min prior to Apomorphine (2.50 mg/kg, i.p.). Ethanol administered orally.
[2]N = Number of groups, each group contains 3 rats.
* $P < 0.025$; ** $P < 0.005$ vs. 0 mg/kg Ethanol

compared to rats administered only apomorphine at 96 hours of ethanol withdrawal.

2. Effect of Chlordiazepoxide on Apomorphine-Induced Aggression in Ethanol Withdrawn Rats

Table 16 summarizes the effect of administration of chlordiazepoxide (i.p.) on apomorphine-induced aggression. Chlordiazepoxide was administered 30 minutes prior to administration of apomorphine (2.50 mg/kg, i.p.) at 96 hours of ethanol withdrawal. The analysis of variance shows a significant effect of chlordiazepoxide on all parameters of apomorphine-induced aggression in ethanol withdrawn rats. Chlordiazepoxide administration at a dose of 40 mg/kg significantly reduced the parameters of attacks and bites and vocalizations, while a dose of 80 mg/kg significantly reduced all parameters of apomorphine-induced aggression when compared to rats administered only apomorphine at 96 hours of ethanol withdrawal.

3. Effect of Morphine Sulfate on Apomorphine-Induced Aggression in Ethanol Withdrawn Rats

Table 17 summarizes the effect of administration of morphine sulfate (i.p.) on apomorphine-induced aggression in ethanol withdrawn rats. Morphine sulfate was administered 30 minutes prior to administration of apomorphine (2.50 mg/kg, i.p.) at 96 hours of ethanol withdrawal. The analysis of variance shows a significant effect of morphine sulfate on all parameters of apomorphine-induced aggression

TABLE 16

REDUCTION OF APOMORPHINE-INDUCED AGGRESSION BY CHLORDIAZEPOXIDE

CHLORDIAZEPOXIDE[1] (MG/KG)	N[2]	RESPONSES/HOUR (MEAN ± S.E.) ATTACKS + BITES	REARING	VOCALIZATION
0	8	167 ± 23	2669 ± 121	1841 ± 108
10	3	137 ± 49	2210 ± 159	1776 ± 291
20	2	186 ± 16	2267 ± 161	1685 ± 28
40	2	74 ± 14*	2712 ± 648	588 ± 212**
80	2	4 ± 1**	110 ± 18**	58 ± 1**

Analysis of Variance of Attacks and Bites

SOURCE	d.f.	SS	MS	F	P
DOSES	4	55966	13991	3.83	< 0.05
ERROR	12	43851	3654		
TOTAL	16	99817			

Analysis of Variance of Rearing

SOURCE	d.f.	SS	MS	F	P
DOSES	4	10991053	2747763	17.74	< 0.005
ERROR	12	1858873	154906		
TOTAL	16	12849926			

Analysis of Variance of Vocalizations

SOURCE	d.f.	SS	MS	F	P
DOSES	4	7006626	1751656	16.82	< 0.005
ERROR	12	1249607	104134		
TOTAL	16	8256233			

[1]Administered at 96 hours of withdrawal 30 min prior to Apomorphine (2.50 mg/kg, i.p.). Chlordiazepoxide administered intraperitoneally.
[2]N = Number of groups, each group contained 3 rats.
* $P < 0.05$; ** $P < 0.005$ vs. 0 mg/kg Chlordiazepoxide.

TABLE 17

REDUCTION IN APOMORPHINE-INDUCED AGGRESSION BY MORPHINE

MORPHINE (MG/KG)	N	RESPONSES/HOUR (MEAN ± S.E.) ATTACKS + BITES	BEARING	VOCALIZATIONS
0	8	167 ± 23	2669 ± 121	1841 ± 108
5	3	149 ± 10	2312 ± 116*	1717 ± 67
10	2	5 ± 1**	108 ± 22**	40 ± 19*
20	3	4 ± 3**	87 ± 52**	49 ± 32**

Analysis of Variance of Attacks and Bites

SOURCE	d.f.	SS	MS	F	P
DOSES	3	86584	28861	11.77	< 0.005
ERROR	12	29425	2452		
TOTAL	15	116009			

Analysis of Variance of Rearing

SOURCE	d.f.	SS	MS	F	P
DOSES	3	21352448	7117483	93.43	< 0.005
ERROR	12	914201	76183		
TOTAL	15	22266649			

Analysis of Variance of Vocalizations

SOURCE	d.f.	SS	MS	F	P
DOSES	3	10700632	3566877	62.48	< 0.005
ERROR	12	685014	57085		
TOTAL	15	11385646			

[1]Administered at 96 hours of withdrawal 30 min prior to Apomorphine (2.50 mg/kg, i.p.).

[2]N = Number of groups, each group contains 3 rats.
* $P < 0.05$; ** $P < 0.005$ vs. 0 mg/kg morphine sulfate.

in ethanol withdrawn rats. Morphine sulfate administration at a dose of 5 mg/kg significantly reduced the aggression parameter of rearing, while doses of 10 mg/kg and 20 mg/kg of morphine sulfate significantly reduced all parameters of apomorphine-induced aggression in ethanol withdrawn rats when compared to rats administered only apomorphine at 96 hours of ethanol withdrawal.

F) MORTALITY DURING ETHANOL WITHDRAWAL

1. Mortality After Various Ethanol Exposure Protocols

Table 18 summarizes the frequency of mortality occurring during withdrawal from ethanol after each of the three exposure protocols used in this study. Statistically none of the three protocols significantly changed the frequency of deaths during ethanol withdrawal. However, mortality occurring after exposure to the 5 DAY HIGH and 7 DAY HIGH protocols fell only slightly short of reaching significance (Chi square) vs 5 DAY LOW protocol.

2. Mortality During Ethanol Withdrawal After Drug Treatment During Ethanol Withdrawal

Shown in the second part of Table 18 are the frequency (percentage) of mortality occurring during ethanol withdrawal in rats treated with either ethanol, chlordiazepoxide or morphine sulfate 30 minutes prior to the thirty-sixth hour of ethanol withdrawal. As was mentioned in the Experimental section of this thesis, mortality was recorded up to and including the seventy-second hour of ethanol withdrawal. Rats

TABLE 18

MORTALITY DURING ETHANOL WITHDRAWAL IN UNTREATED RATS AND RATS ADMINISTERED ETHANOL, CHLORDIAZEPOXIDE OR MORPHINE DURING ETHANOL WITHDRAWAL

TREATMENT	N	PERCENTAGE DEATHS[1]
5 DAY LOW	6	16.7
5 DAY HIGH	48	20.8
7 DAY HIGH	18	44.4

WITHDRAWAL[2] TREATMENT	DOSE		PERCENTAGE DEATHS[1]
ETHANOL	0.5 g/kg		41.7
	2.0 g/kg		41.7
	4.0 g/kg		50.0
	8.0 g/kg		8.3
CHLORDIAZEPOXIDE	10 mg/kg		25.0
	20 mg/kg		25.0
	40 mg/kg		41.7
	80 mg/kg		33.3
	160 mg/kg		75.0*
	(160 mg/kg	untreated	66.7)
MORPHINE	10 mg/kg		16.7
	20 mg/kg		16.7
	40 mg/kg		8.3

[1]Ethanol administered orally, chlordiazepoxide and morphine administered intraperitoneally 30 minutes prior to observation at 36 hours of ethanol withdrawal (5 DAY HIGH); N = 12 at each dose.

[2]Total deaths occurring up to and including 72 hours of ethanol withdrawal.

* $P < 0.001$ Chi square vs. 5 DAY HIGH.

undergoing untreated ethanol withdrawal were not observed to display any mortality after 72 hours of ethanol withdrawal nor were any rats treated during ethanol withdrawal. Seventy-two hours of ethanol withdrawal in rats treated during ethanol withdrawal would correspond to 36 hours post-injection. In most cases the number of animals was not sufficient to achieve statistical significance and it was not feasible to increase the numbers solely for study of mortality during ethanol withdrawal. However, in one extreme case a dose of 160 mg/kg chlordiazepoxide was administered which resulted in 75% mortality, which was significantly greater than rats withdrawn after exposure to the 5 DAY HIGH protocol and left untreated during ethanol withdrawal. Since, a dose of chlordiazepoxide 160 mg/kg proved to be significantly toxic to rats undergoing ethanol withdrawal, this same dose was administered (i.p.) to untreated control rats. As can be seen in the second part of Table 18 a dose of chlordiazepoxide 160 mg/kg administered intraperitoneally was also toxic to untreated rats resulting in the death of 66.7% of these rats by the thirty-sixth hour post-injection. There is no significant difference between the mortality seen in ethanol withdrawn rats and untreated rats administered a dose of 160 mg/kg chlordiazepoxide (i.p.)

DISCUSSION

The development of tolerance to ethanol in alcoholis has been demonstrated (Isbell et al., 1955; Newman, 1941). However, the biochemical mechanisms subserving increased tolerance are unknown, but two general mechanisms have been postulated: an enhanced rate of metabolism and/or an increased degree of cellular tolerance or adption to ethanol in the central nervous system. Both, experimental animals (Hawkins et al., 1966) and man (Mendelson et al., 1966) develop increased rates of ethanol metabolism following chronic administration. The increased ethanol metabolism in experimental animals has been correlated with increased hepatic alcohol dehydrogenase activity (Hawkins et al., 1966; Mendelson et al., 1965). However, changes in the rate of ethanol metabolism can not adequately explain the degree of tolerance observed (Mendelson, 1971). Furthermore, LeBlanc et al. (1969), showed that tolerance after several doses of ethanol was not associated with an increased metabolic rate and suggested some form of cellular adaption as the basis of the tolerance seen. Sleeping time (narcosis) can be altered by increased elimination (Lal and Shah, 1968) and/or decreased sensitivity of the central nervous system (ex. hyperexcitability) (Patel and Lal, 1973).

In our experiments tolerance to ethanol was measured as a reduction in the duration or an increase in the onset

of ethanol-induced narcosis in rats withdrawn 72 hours after continuous ethanol inhalation (5 DAY HIGH) at which time pyrazole administered during inhalation would be at negligible levels (Rydberg et al., 1972). Preliminary experiments demonstrated that a dose of 5.5 g/kg, i.p. proved fatal to 6/8 rats treated, while a dose of 4.5 g/kg, i.p. induced narcosis in only 4/8 rats treated. Therefore, a dose of 5.0 g/kg, i.p. was selected for induction of ethanol narcosis. Although, it is usually common practice to assign a maximum score to animals which fail to recover, some animals died shortly after injection, while others never recovered and subsequently died. Since, the number of deaths in each experimental group (5/18 air + saline, 5/18 air + pyrazole and 6/23 ethanol + pyrazole) were similar these animals were omitted from the study. From a reduction in the duration of ethanol induced narcosis or an increase in onset time, as seen in our experiments, it is not possible to separate metabolic from cellular tolerance.

The occurrence of withdrawal signs upon cessation of ethanol administration is evidence of physical dependence. Inhalation of ethanol vapors along with daily injections of an alcohol dehydrogenase inhibitor pyrazole (68 mg/kg, i.p.) has been shown to produce ethanol physical dependence in mice (Goldstein and Pal, 1971; Littleton et al., 1974). Goldstein and Pal (1971), developed a method for rating the intensity of the ethanol withdrawal syndrome

based upon several withdrawal signs in mice. This ethanol withdrawal intensity scale was subsequently used to analyze the effects of drugs on the alcohol withdrawal reaction (Goldstein, 1972b.; 1973). Majchrowicz (1973), outlined the withdrawal signs in rats made physically dependent upon ethanol by daily intubations, but no effort was made to measure their intensity. Although, Roach et al., (1973), categorized ethanol withdrawn rats, based upon the overall intensity, no effort was made to base the ranking upon the measurement of individual withdrawal signs. Therefore, we have developed a method for the measurement of the intensity of each withdrawal signs in rats made physically dependent on ethanol by continuous inhalation of ethanol vapors and daily injections of pyrazole. The intensity of each of these withdrawal signs can subsequently be combined as a measurement of the intensity of the ethanol withdrawal syndrome, which can be used to express the effects of drugs on the withdrawal reaction in rats.

Unlike the development of alcoholism in man, the production of physical dependence on ethanol in animals is very rapid, ranging from a few days (Goldstein and Pal, 1971) to several months (Falk, 1972). Animal models of physical dependence on ethanol are generally classified as behavioral (self-administration) and pharmacological (forced-administration). Forced administration models provide a valuable tool for assessing the end-product of

addiction, physical dependence. However, they afford little information about the factors which initiate and maintain drug administration as seen in human alcoholics. Implicit in this notion is the fact that behavioral models may prove more valuable in the future study of alcoholism. Forced-administration models do provide information about the biochemical and neurophysiological concomitants of alcohol dependence which may prove valuable in the future in the development of satisfactory behavioral models.

For the purpose of this study, a modification of the technique developed by Goldstein and Pal (1971), was adopted. It shares the disadvantages common to all forced-administration models, but provides advantages which are lacking in most other models. The Goldstein-Pal model allows the production of relatively large numbers of dependent animals in short time, which are necessary for meaningful and practical experimental studies. Furthermore, the inhalation method provides the production of an environment which is identical for all experimental subjects within a group, such that although chamber ethanol concentrations may vary somewhat from group to group all animals within an experimental group are exposed to identical ethanol levels and only individual biological variation can effect the within group variation.

Justification for the use of pyrazole is necessary. Pyrazole is known to produce liver toxicity (Lelbach, 1969;

Lieber et al., 1970) and its half-life in the body is increased by ethanol administration (Rydberg et al., 1972). Pyrazole also has some synergistic action with ethanol on the central nervous system when the doses used are higher than those used in this research (Goldberg et al., 1972). Pyrazole, at the doses used in this research are several-fold lower than the doses shown by Lester and Benson (1970), to be toxic in rats, therefore it is unlikely that pyrazole would produce toxic effects in our experiments. Furthermore, careful controlling of experiments should prevent against erroneous conclusions from synergistic actions of pyrazole and ethanol. Goldstein (1972a), used pyrazole to stabilize blood ethanol levels which markedly fluctuated when pyrazole was not used. These fluctuations resulted in high mortality during inhalation of ethanol vapors (Goldstein, 1972a).

Roach et al. (1973), produced physical dependence in rats using inhalation of ethanol vapors without pyrazole, however, the blood ethanol levels were highly variable as was the intensity of the withdrawal reaction. Large variability in the intensity of the withdrawal reaction induces unnecessarily high variability in the measurement of drug effects on the withdrawal reaction and tends to obscure significant information about drug effectiveness in the treatment of the ethanol withdrawal reaction. The method of Goldstein and Pal (1971) provides for control of this variability.

Reliable detection of withdrawal signs as being present or absent can be done accurately (Mello, 1973) and carefully developed rating scales, such as the one developed by Goldstein (1972a.) for mice, can provide valuable information about the relative severity of the ethanol withdrawal reaction. Table 3 shows the ordinal rating scale used to measure the intensity of the ethanol withdrawal signs which are not readily measurable by quantitative measures. Statistically meaningful inferences can be made from rating or "ranking" scales using non-parametric analysis (Siegel, 1956).

Figure 8 represents the withdrawal intensity seen after three different exposure procedures carried out as preliminary experiments to determine the conditions sufficient for production of physical dependence. Goldstein (1972a), showed that the intensity of the alcohol withdrawal reaction was related to the total dose of alcohol administered. The results obtained in this study are in agreement with Goldstein's work, although no attempt was made to calculate the total dose of alcohol, since the overall purpose of this work was not comparison among treatments. This is supported by the following (see Table 1) : 1. the mean blood ethanol levels are significantly higher in 5 DAY HIGH and the first five days of the 7 DAY HIGH exposure protocols than in the 5 DAY LOW protocol. 2. the higher blood ethanol levels resulted in greater severity of the withdrawal reaction in

rats exposed to the 5 and 7 DAY HIGH protocols. The 5 DAY HIGH protocol was selected for subsequent studies because the degree of physical dependence was satisfactory and the mortality during ethanol withdrawal was not excessive (see Table 18).

Goldstein (1972a.), reported weight loss in mice after either ethanol or pyrazole treatment, but did not report any results of both ethanol and pyrazole treatment on weight loss. Littleton et al. (1974), reported weight loss in mice after pyrazole treatment whether or not ethanol was administered, however he did report that there was no weight loss after ethanol alone. Table 4 demonstrates the effect of pyrazole or pyrazole plus ethanol on the body weight of rats after five days of treatment. Preliminary experiments carried out earlier agreed with Littleton's work. Rats exposed to only inhalation of ethanol vapors for five days did not lose body weight, but did prevent any gain in body weight over this period of time. As seen in Table 4 treatment of rats with pyrazole or ethanol plus pyrazole reduced body weight, however, ethanol administration had a sparing effect on the pyrazole-induced body weight loss, possibly due to the caloric value of the inhaled ethanol.

The withdrawal syndrome seen upon removal of a chronically administered physical dependence-producing drug is a dynamic process. Abstinence signs have been observed in man (Isbell et al., 1955; Mello and Mendelson, 1970) and

in experimental animals (Goldstein, 1972a.), while blood ethanol levels are still high. Majchrowicz (1973), has shown that the peak intensity of the withdrawal reaction occurs some time after blood ethanol levels fall to negligible levels. Withdrawal signs specific to ethanol withdrawal should follow similar changes in intensity as the duration of withdrawal increases. As seen in Figures 9a. and 9b. withdrawal signs seen after removal of rats from inhalation of ethanol vapors follows a similar time course of development in intensity. The peak intensity occurring at 36 hours after withdrawal. Headshakes occurring after withdrawal of rats from ethanol inhalation also follows a similar time course of development in intensity with the peak intensity occurring at 36 hours after withdrawal (see Figure 10). However, it can also be seen in Figure 10 that headshakes are the only ethanol withdrawal sign observed to be associated with the administration of pyrazole alone at levels significantly greater than untreated control rats. Majchrowicz (1973), using an ethanol intubation procedure without pyrazole for production of physical dependence in rats also reported shakes during ethanol withdrawal. Therefore, it is highly unlikely that pyrazole administration during ethanol inhalation is solely responsible for the increased occurrence of headshakes during ethanol withdrawal, although some synergistic action may be involved. As seen in Figure 11 tail-lifts reach their peak intensity at 24 hours after ethanol with-

drawal, however they do not decline significantly until 48 hours after withdrawal, similar to the other ethanol withdrawal signs.

Withdrawal reactions follow when chronic administration of a dependence producing drug stops, and the signs of withdrawal should therefore be relieved by reinstating the same drug or its pharmacological equivalent. Goldstein (1972b.) and Ellis and Pick (1970b.), showed that ethanol does reduce the severity of the ethanol withdrawal reaction. Since, the mechanism of ethanol physical dependence and the subsequent withdrawal reaction are not known it is not clear which signs are specifically due to ethanol withdrawal. However, one may rule out signs which are not reduced by ethanol administration as being non-specific. As seen in Figures 12a. and 12b. all signs seen in rats after withdrawal from inhalation of ethanol vapors are significantly reduced by readministration of ethanol. Therefore, since all withdrawal signs follow a similar time course in intensity development and are all reduced by readministration of ethanol the scores of the four ranked signs of piloerection, abnormal posture, tremors and convulsions were combined and summed for each rat tested to obtain a value representative of the intensity of the overall withdrawal syndrome. Figure 13 demonstrates the effect of readministration of ethanol on the ethanol withdrawal syndrome, represented as the median value of the sum of the four

ranked signs versus the dose of ethanol administered orally. As seen in Table 5 headshakes occurring during ethanol withdrawal are also significantly reduced by readministration of ethanol. Tail-lifts as seen in Table 6 were also significantly reduced by readministration of ethanol. Therefore, all withdrawal signs observed and measured appear to be specifically related to the withdrawal of ethanol, although there is some question as to what significance, if any, pyrazole has on the intensity of the headshakes seen during ethanol withdrawal, since pyrazole alone induces significantly higher frequencies of headshakes than observed in untreated control rats.

The use of chlordiazepoxide in the treatment of acute alcohol withdrawal in man is well established (Gross et al., 1974). Chlordiazepoxide is also effective in reducing the severity of the ethanol withdrawal signs in animals (Goldstein, 1972b.). In our experiments chlordiazepoxide was also effective in reducing the severity of the ethanol withdrawal reaction in rats. However, a dramatic increase in the mortality rate was seen after the highest dose of chlordiazepoxide administered (160 mg/kg, i.p.). Goldstein (1972b.), also reported an increase in mortality in ethanol withdrawn mice after chlordiazepoxide (50 mg/kg, i.p.). The LD_{50} values for chlordiazepoxide in mice are 95 mg/kg (i.v.), 268 mg/kg (i.p.), 530 mg/kg (s.c.), 720 mg/kg (p.o.) and in rats 165 mg/kg (i.v.), 800 mg/kg (s.c.) and 2000 mg/kg (p.o.) (Barnes and Ellherington, 1965). It has also been

reported that 240 mg/kg/day (p.o.) chlordiazepoxide for 2 years is well tolerated in rats (Zbinden, 1961) as well as 100 mg/kg/day (i.p.) for five days (Hoogland, 1966). To my knowledge there has not been reported an LD_{50} value for intraperitoneally administered chlordiazepoxide in the rat. However, as can be seen the doses in the rat are 2-3 times as great as for mice, therefore one would expect the LD_{50} for chlordiazepoxide (i.p.) to be somewhere in excess of 500 mg/kg (i.p.). Therefore, on the basis of the known LD_{50} values a dose of chlordiazepoxide of 160 mg/kg (i.p.) should not produce the high mortality seen in ethanol withdrawn rats. Since, animals undergoing ethanol withdrawal probably suffer from other disorders (ex. electrolyte imbalance or fluid imbalance), which would normally be routinely handled in a clinical situation, these disorders may be at fault. However, in our experiments untreated controls were also subjected to administration of chlordiazepoxide (160 mg/kg, i.p.), which resulted in 66.7% mortality and was not significantly less than ethanol withdrawn rats administered the same dose of chlordiazepoxide. Male Long-Evans strain rats may be more sensitive to the toxic effects of chlordiazepoxide.

Walsh *et al.* (1970), proposed that the formation of morphine-like alkaloids from tetrahydropapaveroline formed from dopamine and its aldehyde metabolite may be responsible for the dependence liability of ethanol. This hy-

pothesis has been criticized by Goldstein and Judson (1971), when they failed to elicit naloxone-induced jumping, a morphine withdrawal sign, in ethanol dependent mice. In our experiments we have found that morphine administration during ethanol withdrawal significantly reduces the ethanol withdrawal syndrome in rats. Blockade of ethanol withdrawal signs by morphine, by itself, is not sufficient evidence to warrant the conclusion that these withdrawal signs are the result of dependence on morphine-like compounds formed during chronic ethanol administration. However, it is interesting that some of the withdrawal signs seen during ethanol withdrawal are similar to those seen during morphine withdrawal in rats. Gianutsos et al. (1975), observed the following withdrawal signs in morphine withdrawn rats: piloerection, wet shakes (with occasional headshakes, Gianutsos, personal communication), weight loss, hypothermia, ptosis, writhing and aggression, which is enhanced by apomorphine and amphetamine (Puri and Lal, 1973). In our experiments with ethanol withdrawn rats piloerection, headshakes and aggression induced by apomorphine and d-amphetamine were observed. As proposed by Walsh et al. (1970) and Davis et al. (1970), the role, if any, that morphine-like alkaloids have may be very limited and localized (ex. dopaminergic neurons), therefore, one would not expect all possible ethanol withdrawal signs to be related to these morphine-like compounds. Furthermore, non-specific effects

of morphine, such as analgesia or resporatory depression may be involved in the effectiveness of morphine in reducing the ethanol withdrawal syndrome in rats. Respiratory depression may be especially important in the light that convincing evidence for a role of respiratory alkalosis in the etiology of delirium tremens in humans (Victor, 1973).

In order to more clearly establish a role for morphine-like compounds in ethanol dependence the following conditions should be tested in the future: 1. induction of ethanol withdrawal signs similar to morphine withdrawal signs without induction of specific ethanol withdrawal signs (ex. tremors and convulsions) by a narcotic antagonist in ethanol dependent rats. 2. isolation and identification of possible morphine-like compounds from animals dependent on ethanol. 3. production of dependence on these morphine-like compounds, which upon subsequent withdrawal would result in withdrawal signs similar to morphine-ethanol withdrawal signs (ex. aggression). Although, blockade of ethanol withdrawal signs by morphine, by itself, is not sufficient evidence for a role for morphine-like compounds, failure to block ethanol withdrawal signs would have cast serious doubt on this hypothesis. However, the results of our experiments indicate that further study appears warranted.

Rats made physically dependent upon morphine show spontaneous aggression after withdrawal of morphine administration (Puri and Lal, 1973). This spontaneous aggression

is enhanced by directly (apomorphine) and indirectly (amphetamine) acting dopamine receptor stimulating agents and is blocked by the narcotic, methadone and the dopamine receptor blocking neuroleptic haloperidol (Puri and Lal, 1973). Prior administration of alpha-methyl-para-tyrosine blocks the effect of amphetamine, but not apomorphine (Puri and Lal, 1973). These data were interpreted to indicate the presence of dopamine-receptor supersensitivity in morphine dependent and withdrawn rats. Apomorphine is a known stimulator of dopamine receptors (Anden et al., 1967; Ernst, 1967) and capable of producing aggression itself, but at doses several fold higher than needed to intensify morphine withdrawal aggression (Gianutsos, 1974). It has also been shown that chronic blockade of dopamine receptors by the neuroleptic, haloperidol, renders rats supersensitive to small doses of apomorphine (Gianutsos et al., 1974a), which do not elicit aggression in normal animals (Gianutsos, 1974). As seen in Table 11 the intensity of aggression induced by apomorphine in ethanol withdrawn rats increases with the duration of ethanol withdrawal. Apomorphine-induced aggression in ethanol withdrawn rats was most intense at 72 hours of withdrawal.

Table 12 summarizes the effect of the dose of apomorphine on apomorphine-induced aggression in 72-hour ethanol withdrawn rats. However, the results were complicated by deaths occurring from injuries sustained during intense aggression after a dose of 5.0 mg/kg apomorphine. Examina-

tion of the data in the usual manner (total attacks and bites, rearing and vocalizations) shows that a dose of 2.5 and 5.0 mg/kg of apomorphine significantly increased all parameters of aggression compared to rats administered only saline after 72 hours of ethanol withdrawal. Apomorphine does of 2.5 and 5.0 mg/kg also significantly increased all parameters of aggression when compared to a dose of 1.25 mg/kg apomorphine. However, when apomorphine doses of 2.5 mg/kg and 5.0 mg/kg are compared a dose of 5.0 mg/kg significantly reduced all parameters of aggression, except attacks and bites which were not significantly different. Aggression was extremely intense after a dose of 5.0 mg/kg of apomorphine. It was observed that such intense attacking and biting occurred that few vocalizations and minimal rearing were recorded. This aggression can be described as follows: Two rats would rear briefly or fail to rear and attack immediately; one rat would usually gain a dominant position and bite the other rat; the dominant rat would hold (while continuing to bite) the succumbed rat for long periods of time; when the dominant rat released its hold both rats would fall apart, as if stunned, then resume fighting or attack or be attacked by the third rat in the aggression group. Aggression as seen here is seldom cooperative (2 against 1), but rather is on a one-to-one basis.

This intense aggression resulted in the deaths of

several of these rats during aggression meaurement as described in the results section. It is believed that the deaths and the intensity of the aggression, not the lack of it, was responsible for the decreased rearing and vocalizations seen after a dose of 5.0 mg/kg apomorphine. This is borne out in Table 12 where the data for aggression after 2.5 and 5.0 mg/kg of apomorphine in ethanol withdrawn rats is broken down into the first 30 minutes and the last 30 minutes of observation. During the first 30 minutes rats administered 5.0 mg/kg of apomorphine had significantly greater numbers of attacks and bites when compared to rats administered 2.5 mg/kg of apomorphine (40% increase), while rearing was not significantly different and vocalizations were significantly reduced. During the last 30 minutes when several animals were either severely injured, dying or dead, attacks and bites are not significantly different and rearing and vocalizations are greatly reduced. A dose of 2.5 mg/kg of apomorphine was chosen for further studies, since it produced intense aggression in ethanol withdrawn rats without the mortality following a dose of 5.0 mg/kg of apomorphine.

From this data it appears that while only very low levels of spontaneous aggression are seen in ethanol withdrawn rats, unlike morphine withdrawn rats, apomorphine in small doses produces intense aggression in ethanol withdrawn rats, similar to rats withdrawn after chronic

haloperidol. Since, aggression seen after chronic treatment of rats with morphine or haloperidol has been suggested to be due to latent supersensitivity of dopamine receptors, it would be of future interest to test the relationship between aggression in ethanol withdrawn rats and supersensitivity of dopamine receptors. Induction of aggression by apomorphine in ethanol withdrawn rats is one indication of dopamine receptor sensitivity.

Morphine withdrawal aggression is enhanced by administration of amphetamine (Puri and Lal, 1973). As seen in Table 13 administration of d-amphetamine to rats withdrawn from ethanol for 72 hours induces aggression. Interestingly, doses of d-amphetamine in non-ethanol exposed rats does not induce aggression, even when doses which were lethal were administered. These results indicate sensitivity to d-amphetamine in ethanol withdrawn rats that can not be simulated in non-ethanol dependent rats, unlike apomorphine-induced aggression.

Gianutsos et al. (1974b.), showed that morphine withdrawal aggression was still present after protracted periods of withdrawal. As seen in Table 14 ethanol withdrawn rats remain sensitive to apomorphine and d-amphetamine for at least seven days after withdrawal. These data again indicate similarities between ethanol and morphine withdrawal in rats.

As seen in Tables 15, 16 and 17 ethanol, chlordiaze-

poxide and morphine, respectively, all reduced apomorphine-induced aggression in ethanol withdrawn rats. One would expect that if increased sensitivity to apomorphine in ethanol withdrawn rats is specifically related to chronic administration of ethanol then readministration of ethanol or a drug, such as chlordiazepoxide, which is known to have cross-tolerance with ethanol, would antagonize apomorphine sensitivity. Furthermore, the effect of morphine in reducing apomorphine-induced aggression in ethanol withdrawn rats may be related to replacement of a morphine-like alkaloid formed in or near dopamine neurons during chronic administration of ethanol. This morphine-like alkaloid, through an action similar to morphine or haloperidol in blocking dopamine receptors, could result in supersensitivity of dopamine receptors.

Unfortunately, the effects of ethanol on neuroamine uptake, storage and release of catecholamines in the central nervous system is not clear (Friedhoff and Miller, 1973) and many conflicting reports are present in the literature. Many of the conflicting results may stem from the differences occurring after acute compared to chronic administration of ethanol as well as the dosage administered (Friedhoff and Miller, 1973). One of the effects of acute ethanol administration to rats is the production of narcosis to which tolerance develops (Friedhoff and Miller, 1973; this research). Administration of several biogenic amines,

including dopamine, has been shown to prolong ethanol narcosis (Rosenfeld, 1960). Therefore, if increased mobilization of dopamine or other catecholamines occurs in the mediation of ethanol-induced narcosis, then continuous administration of ethanol might result in decreased production of dopamine by either repression of biosynthetic enzymes or negative feedback (Friedhoff and Miller, 1973). This could serve to reduce levels of available dopamine and restore normal levels of arousal in the face of continued ethanol administration (Friedhoff and Miller, 1973). This could also result in dopamine receptor sensitivity that would be unmasked upon cessation of ethanol administration.

Recently, the conflicting reports on catecholamine turnover in the literature have been explained by Hunt and Majchrowicz (1974). Hunt and Majchrowicz (1974), have shown that after a single oral dose of ethanol (5.0 g/kg) to rats the turnover of brain norepinephrine is increased, while the turnover of dopamine is unaffected during the first few hours after treatment. After the first few hours after ethanol administration the turnover of both norepinephrine and dopamine are decreased (Hunt and Majchrowicz, 1974). In alcohol dependent rats, whether intoxicated or undergoing withdrawal, the turnover of norepinephrine is increased, while that of dopamine is decreased (Hunt and Majchrowicz, 1974). At this time the mechanism by which ethanol may bring about these changes in catecholamine turnover is not known. However, an hypothesis of

adrenergic subsensitivity has been proposed by French et al. (1974), based upon the sustained release of norepinephrine by ethanol, as reported by Hunt and Majchrowicz (1974), and the reduced cyclic adenosine monophosphate response to norepinephrine in brain slices of rats chronically fed ethanol (French et al., 1974). This hypothesis proposes that, since norepinephrine exerts an inhibitory effect in the brain which tends to limit the spread of audiogenic seizure discharge in the rats, then convulsions and tremors seen during ethanol withdrawal in rats may be due to the subsensitivity of adrenergic receptors in the brain. This statement has been supported by the evidence of Goldstein (1973), that drugs which deplete brain catecholamines or block alpha or beta receptors aggravates ethanol withdrawal seizures in mice. Furthermore, the aggression seen in ethanol withdrawn rats, as reported in this research, may be due to decreased turnover and release of dopamine, resulting in supersensitivity of dopamine receptors, which is unmasked during ethanol withdrawal by the administration of the dopamine receptor stimulating agent apomorphine. The lack of spontaneous aggression during ethanol withdrawal may reflect the decreased turnover of dopamine that occurs during chronic administration of ethanol, which results in increased sensitivity of dopamine receptors, but the continued decrease in turnover during withdrawal masks the sensitivity of dopamine receptors even when ethanol is

eliminated. Therefore, only by increasing dopamine release by d-amphetamine or by direct stimulation of dopamine receptors by apomorphine does the sensitivity of dopamine receptors become apparent. Whether these effects on catecholamine turnover after chronic administration of ethanol are due to direct effects of ethanol or its metabolite acetaldehyde or aberrant biogenic amine metabolites is unknown.

The model of ethanol physical dependence developed in this research provides a useful tool in the study of ethanol physical dependence by providing an animal model which is rapidly produced and responds relatively similar during ethanol administration and withdrawal as does man and also responds to drugs known to be useful in the treatment of alcohol withdrawal in man. Furthermore, aside from providing a useful model, this research has presented data which indicate that physical dependence on ethanol may have some relationship to physical dependence on morphine in rats and that drug dependencies in general may be related in ways not previously recognized.

SUMMARY

1. Rats exposed to continuous inhalation of ethanol vapors for 5 days showed an increase in the onset and a reduction in the duration of ethanol-induced narcosis.

2. Withdrawal of rats after continuous inhalation of ethanol resulted in withdrawal signs: piloerection, abnormal posture, tremors convulsions, headshakes, tail-lifts and deaths. All signs reach maximum severity by 36 hours after ethanol withdrawal and return to near normal by 72 hours after withdrawal. All signs are reduced by ethanol readministration during withdrawal. The occurrence of withdrawal signs and their reduction by ethanol represents the presence of ethanol physical dependence in these rats. All withdrawal signs follow a similar time course and were combined into an ethanol withdrawal syndrome intensity measurement.

3. The ethanol withdrawal syndrome intensity was used to study the effect of chlordiazepoxide and morphine on the acute ethanol withdrawal reaction. Both chlordiazepoxide and morphine reduced the severity of the ethanol withdrawal reaction, however, chlordiazepoxide was toxic at a dose of 160 mg/kg.

4. Aggressive behaviors were seen in ethanol withdrawn rats after small doses of apomorphine or d-amphetamine. The peak intensity of drug-induced aggression was seen at

72 hours of withdrawal. Ethanol, chlordiazepoxide and morphine reduced apomorphine-induced aggression in ethanol withdrawn rats. The mechanism underlying the sensitivity to apomorphine and amphetamine is unknown.

5. Deaths during withdrawal were associated with induced and spontaneous seizures. Deaths were increased after a dose of chlordiazepoxide (160 mg/kg). Deaths resulting from factors other than seizures were probably also significant.

APPENDIX I

SUM OF WITHDRAWAL SIGN INTENSITY RATINGS FOR PILOERECTION, ABNORMAL POSTURE, TREMORS AND CONVULSIONS

		HOURS OF ETHANOL WITHDRAWAL					
GROUP	RAT #	0	12	24	36	48	72
NAIVE	1	2	-	-	-	-	-
	2	2	-	-	-	-	-
	3	0	-	-	-	-	-
	4	1	-	-	-	-	-
	5	2	-	-	-	-	-
	6	1	-	-	-	-	-
	7	2	-	-	-	-	-
	8	1	-	-	-	-	-
	9	1	-	-	-	-	-
	10	3	-	-	-	-	-
	11	1	-	-	-	-	-
	12	1	-	-	-	-	-
	13	1	-	-	-	-	-
	14	1	-	-	-	-	-
	15	2	-	-	-	-	-
	16	1	-	-	-	-	-
	17	1	-	-	-	-	-
	18	0	-	-	-	-	-
	19	2	-	-	-	-	-
	20	1	-	-	-	-	-
	21	2	-	-	-	-	-
	22	1	-	-	-	-	-
	23	0	-	-	-	-	-
	24	2	-	-	-	-	-
	25	3	-	-	-	-	-
	26	2	-	-	-	-	-
	27	0	-	-	-	-	-
	28	1	-	-	-	-	-
	29	0	-	-	-	-	-
	30	2	-	-	-	-	-
	MEDIAN	1.0					
PYRAZOLE + AIR	1	4	3	3	0	3	0
	2	2	1	2	1	2	2
	3	1	1	1	0	1	0
	4	3	1	1	1	0	3
	5	2	0	0	4	0	2
	6	1	0	0	2	0	0
	7	4	3	3	0	3	0
	8	2	2	1	1	2	2

APPENDIX I (continued)

GROUP	RAT #	HOURS OF ETHANOL WITHDRAWAL 0	12	24	36	48	72
PYRAZOLE + AIR	9	3	3	2	1	2	2
	10	1	3	3	2	3	2
	11	3	3	3	1	3	1
	12	1	2	3	3	3	1
	13	2	1	1	0	1	3
	14	3	2	2	0	2	0
	15	3	1	0	1	0	2
	16	0	0	0	2	1	0
	17	0	0	0	2	0	1
	18	1	0	0	2	0	2
	MEDIAN	2.0	1.0	1.0	1.0	1.5	1.5
PYRAZOLE + ETHANOL 5 DAY LOW	1	0	4	5	D	-	n.d.
	2	0	2	5	5	3	n.d.
	3	0	4	5	4.	4	n.d.
	4	0	2	3	3	1	n.d.
	5	0	3	4	3	2	n.d.
	6	0	2	3	3	2	n.d.
	MEDIAN	0.0	2.5	4.5	3.0	2.0	n.d.
PYRAZOLE + ETHANOL 5 DAY HIGH	1	2	5	6	9	5	n.d.
	2	0	4	5	7	D	n.d.
	3	0	7	5	5	D	n.d.
	4	2	5	5	8	10	n.d.
	5	0	4	4	7	3	n.d.
	6	0	1	4	6	3	n.d.
	7	3	n.d.	8	n.d.	3	n.d.
	8	3	n.d.	7	n.d.	3	n.d.
	9	3	n.d.	7	n.d.	3	n.d.
	10	2	n.d.	7	n.d.	4	n.d.
	11	1	n.d.	5	n.d.	2	n.d.
	12	1	n.d.	4	n.d.	4	n.d.
	13	3	n.d.	n.d.	n.d.	6	n.d.
	14	3	n.d.	n.d.	n.d.	5	n.d.
	15	4	n.d.	n.d.	n.d.	4	n.d.
	16	3	n.d.	n.d.	n.d.	5	n.d.
	17	4	n.d.	n.d.	n.d.	5	n.d.
	18	1	n.d.	n.d.	n.d.	5	n.d.
	19	3	5	6	12	D	-
	20	5	4	5	6	5	3
	21	0	6	5	7	6	3

APPENDIX I (continued)

GROUP	RAT #	HOURS OF ETHANOL WITHDRAWAL 0	12	24	36	48	72
	22	0	1	5	8	8	D
PYRAZOLE	23	0	2	6	6	3	5
+	24	2	5	7	6	6	4
ETHANOL	25	1	3	5	10	D	-
5 DAY	26	2	3	8	11	D	-
HIGH	27	4	5	5	6	5	3
	28	3	4	4	5	4	4
	29	2	3	9	D	-	-
	30	0	2	3	5	4	3
	31	3	6	6	7	5	4
	32	1	4	4	5	8	5
	33	1	4	4	10	D	-
	34	2	3	6	6	3	5
	35	3	4	6	6	6	5
	36	2	3	3	4	4	3
	37	1	3	4	6	4	3
	38	3	3	5	7	5	4
	39	2	5	6	7	7	D
	40	0	3	4	5	4	3
	41	2	4	4	6	4	3
	42	3	3	5	5	D	-
	43	1	3	5	9	6	3
	44	0	3	4	5	5	4
	45	2	4	6	6	4	4
	46	2	3	4	6	6	4
	47	1	2	4	6	4	3
	48	1	1	5	5	4	4
	MEDIAN	2.0	3.5	5.0	6.0	4.5	4.0
PYRAZOLE	1	n.d.	n.d.	3	n.d.	5	n.d.
Z	2	n.d.	n.d.	4	n.d.	D	n.d.
ETHANOL	3	n.d.	n.d.	D	n.d.	-	n.d.
7 DAY	4	n.d.	n.d.	5	n.d.	3	n.d.
HIGH	5	n.d.	n.d.	7	n.d.	D	n.d.
	6	n.d.	n.d.	4	n.d.	7	n.d.
	7	n.d.	n.d.	3	6	D	n.d.
	8	n.d.	n.d.	5	4	n.d.	n.d.
	9	n.d.	n.d.	5	D	-	n.d.
	10	n.d.	n.d.	3	7	D	n.d.
	11	n.d.	n.d.	2	5	D	n.d.
	12	n.d.	n.d.	5	D	-	n.d.

APPENDIX I (continued)

GROUP	RAT #	HOURS OF ETHANOL WITHDRAWAL					
		0	12	24	36	48	72
PYRAZOLE	13	2	4	7	8	2	n.d.
+	14	1	5	6	8	8	n.d.
ETHANOL	15	0	4	8	10	3	n.d.
7 DAY	16	1	3	3	6	4	n.d.
HIGH	17	3	5	6	9	5	n.d.
	18	3	4	7	9	7	n.d.
MEDIAN		1.5	4.0	5.0	7.5	5.0	n.d.

D = Died at some time prior to observation.
- = Died during or prior to last observation
n.d. = not done; all groups during preliminary experiments were not observed at all time periods.

APPENDIX II

PERCENT FREQUENCY OF WITHDRAWAL SCORES FOR WITHDRAWAL SIGNS IN RATS AFTER PYRAZOLE OR ETHANOL UNDER THE 5 DAY HIGH PARADIGM

PRETREATMENT	HOURS WITHDRAWN	STATISTICS	PILOERECTION SCORE 0	1	2	3	N
PYRAZOLE	0	FREQ.	2	6	10	0	18
		%	11.1	33.3	55.6	0.0	
	12	FREQ.	7	5	6	0	18
		%	38.9	27.8	33.3	0.0	
	24	FREQ.	7	6	5	0	18
		%	38.9	33.3	27.8	0.0	
	36	FREQ.	8	6	4	0	18
		%	44.4	33.3	22.2	0.0	
	48	FREQ.	6	6	5	1	18
		%	33.3	33.3	27.8	5.6	
	72	FREQ.	7	6	5	0	18
		%	38.9	33.3	27.8	0.0	
ETHANOL + PYRAZOLE	0	FREQ.	12	15	17	4	48
		%	25.0	31.2	35.4	8.3	
	12	FREQ.	2	10	22	2	36
		%	5.6	27.8	61.1	5.6	
	24	FREQ.	0	3	31	8	42
		%	0.0	7.1	73.8	19.1	
	36	FREQ.	0	1	21	13	35
		%	0.0	2.9	60.0	37.1	
	48	FREQ.	0	4	31	5	40
		%	0.0	10.0	77.5	12.5	
	72	FREQ.	0	3	17	2	22
		%	0.0	13.6	77.3	9.1	
			ABNORMAL POSTURE SCORE				
			0	1	2	3	N
PYRAZOLE	0	FREQ.	8	6	4	0	18
		%	44.4	33.3	22.2	0.0	
	12	FREQ.	10	7	1	0	18
		%	55.6	38.9	5.6	0.0	
	24	FREQ.	11	6	1	0	18
		%	61.1	33.3	5.6	0.0	
	36	FREQ.	10	8	0	0	18
		%	55.6	44.4	0.0	0.0	
	48	FREQ.	12	5	1	0	18
		%	66.7	27.8	5.6	0.0	
	72	FREQ.	11	7	0	0	18
		%	61.1	38.9	0.0	0.0	
ETHANOL + PYRAZOLE	0	FREQ.	28	14	6	0	48
		%	58.3	29.2	12.5	0.0	
	12	FREQ.	1	14	19	2	36
		%	2.8	38.9	52.8	5.6	
	24	FREQ.	1	4	24	13	42
		%	2.4	9.5	57.1	31.0	

APPENDIX II (Continued)

PRETREATMENT	HOURS WITHDRAWN	STATISTICS	ABNORMAL POSTURE SCORE 0	1	2	3	N
ETHANOL	36	FREQ.	0	1	14	20	35
+		%	0.0	2.9	40.0	57.1	
PYRAZOLE	48	FREQ.	0	9	27	4	40
		%	0.0	22.5	67.5	10.0	
	72	FREQ.	0	8	14	0	22
		%	0.0	36.4	63.6	0.0	
			TREMOR SCORE 0	1	2	3	N
PYRAZOLE	0	FREQ.	17	1	0	0	18
		%	94.4	5.6	0.0	0.0	
	12	FREQ.	18	0	0	0	18
		%	100.0	0.0	0.0	0.0	
	24	FREQ.	17	1	0	0	18
		%	94.4	5.6	0.0	0.0	
	36	FREQ.	17	1	0	0	18
		%	94.4	5.6	0.0	0.0	
	48	FREQ.	18	0	0	0	18
		%	100.0	0.0	0.0	0.0	
	72	FREQ.	18	0	0	0	18
		%	100.0	0.0	0.0	0.0	
ETHANOL	0	FREQ.	48	0	0	0	48
+		%	100.0	0.0	0.0	0.0	
PYRAZOLE	12	FREQ.	25	10	1	0	36
		%	69.4	27.8	2.8	0.0	
	24	FREQ.	14	22	5	1	42
		%	33.3	52.4	11.9	2.4	
	36	FREQ.	5	16	8	6	35
		%	14.3	45.7	22.9	17.1	
	48	FREQ.	19	16	5	0	40
		%	47.5	40.0	12.5	0.0	
	72	FREQ.	19	3	0	0	22
		%	86.4	13.6	0.0	0.0	
			CONVULSION SCORE 0	1	2	3	N
PYRAZOLE	0	FREQ.	18	0	0	0	18
		%	100.0	0.0	0.0	0.0	
	12	FREQ.	18	0	0	0	18
		%	100.0	0.0	0.0	0.0	
	24	FREQ.	18	0	0	0	18
		%	100.0	0.0	0.0	0.0	
	36	FREQ.	18	0	0	0	18
		%	100.0	0.0	0.0	0.0	
	48	FREQ.	18	0	0	0	18
		%	100.0	0.0	0.0	0.0	
	72	FREQ.	18	0	0	0	18
		%	100.0	0.0	0.0	0.0	

APPENDIX II (continued)

PRETREATMENT	HOURS WITHDRAWN	STATISTICS	CONVULSION SCORE 0	1	2	3	N
ETHANOL	0	FREQ.	48	0	0	0	48
+		%	100.0	0.0	0.0	0.0	
PYRAZOLE	12	FREQ.	36	0	0	0	36
		%	100.0	0.0	0.0	0.0	
	24	FREQ.	40	0	1	1	42
		%	95.2	0.0	2.4	2.4	
	36	FREQ.	30	0	1	4	35
		%	85.7	0.0	2.9	11.4	
	48	FREQ.	37	0	1	2	40
		%	92.5	0.0	2.5	5.0	
	72	FREQ.	22	0	0	0	22
		%	100.0	0.0	0.0	0.0	

APPENDIX III

PERCENT FREQUENCY OF WITHDRAWAL SIGN SCORES AFTER DRUG ADMINISTRATION TO RATS WITHDRAWN 36 HOURS AFTER ETHANOL INHALATION

DRUG	DOSE	STATISTIC	PILOERECTION SCORE 0	1	2	3	N
SALINE	1.0 ml/kg	FREQ.	0	1	21	13	35
		%	0.0	2.9	60.0	37.1	
			ABNORMAL POSTURE SCORE 0	1	2	3	N
		FREQ.	0	1	14	20	35
		%	0.0	2.9	40.0	57.1	
			TREMOR SCORE 0	1	2	3	N
		FREQ.	5	16	8	6	35
		%	14.3	45.7	22.9	17.1	
			CONVULSION SCORE 0	1	2	3	N
		FREQ.	30	0	1	4	35
		%	85.7	0.0	2.9	11.4	
			PILOERECTION SCORE 0	1	2	3	N
ETHANOL	0.5 g/kg	FREQ.	0	0	5	6	11
		%	0.0	0.0	45.4	54.5	
	2.0 g/kg	FREQ.	1	0	6	3	10
		%	10.0	0.0	60.0	30.0	
	4.0 g/kg	FREQ.	1	1	5	4	11
		%	9.1	9.1	45.4	36.4	
	8.0 g/kg	FREQ.	3	4	4	0	11
		%	27.3	36.4	36.4	0.0	
			ABNORMAL POSTURE 0	1	2	3	N
	0.5 g/kg	FREQ.	0	0	8	3	11
		%	0.0	0.0	72.7	27.3	
	2.0 g/kg	FREQ.	0	3	5	2	10
		%	0.0	30.0	50.0	20.0	
	4.0 g/kg	FREQ.	3	5	3	0	11
		%	27.3	45.4	27.3	0.0	
	8.0 g/kg	FREQ.	9	2	0	0	11
		%	81.8	18.2	0.0	0.0	

APPENDIX III (continued)

DRUG	DOSE	STATISTIC	TREMOR SCORE 0	1	2	3	N
	0.5 g/kg	FREQ.	4	3	1	3	11
ETHANOL		%	36.4	27.3	9.1	27.3	
(cont.)	2.0 g/kg	FREQ.	8	1	1	0	10
		%	80.0	10.0	10.0	0.0	
	4.0 g/kg	FREQ.	10	1	0	0	11
		%	90.9	9.1	0.0	0.0	
	8.0 g/kg	FREQ.	11	0	0	0	11
		%	100.0	0.0	0.0	0.0	
			CONVULSION SCORE 0	1	2	3	N
	0.5 g/kg	FREQ.	7	0	2	2	11
		%	63.6	0.0	18.2	18.2	
	2.0 g/kg	FREQ.	10	0	0	0	10
		%	100.0	0.0	0.0	0.0	
	4.0 g/kg	FREQ.	11	0	0	0	11
		%	100.0	0.0	0.0	0.0	
	8.0 g/kg	FREQ.	11	0	0	0	11
		%	100.0	0.0	0.0	0.0	
			PILOERECTION SCORE 0	1	2	3	N
CHLORDI-	10 mg/kg	FREQ.	0	0	6	6	12
AZEPOXIDE		%	0.0	0.0	50.0	50.0	
	20 mg/kg	FREQ.	0	0	2	8	10
		%	0.0	0.0	20.0	80.0	
	40 mg/kg	FREQ.	0	2	8	2	12
		%	0.0	16.7	66.7	16.7	
	80 mg/kg	FREQ.	0	5	4	3	12
		%	0.0	41.7	33.3	25.0	
	160 mg/kg	FREQ.	5	5	2	0	12
		%	41.7	41.7	16.7	0.0	
			ABNORMAL POSTURE SCORE 0	1	2	3	N
	10 mg/kg	FREQ.	0	0	8	4	12
		%	0.0	0.0	66.7	33.3	
	20 mg/kg	FREQ.	0	0	8	2	10
		%	0.0	0.0	80.0	20.0	
	40 mg/kg	FREQ.	0	3	4	5	12
		%	0.0	25.0	33.3	41.7	
	80 mg/kg	FREQ.	3	2	7	0	12
		%	25.0	16.7	58.3	0.0	
	160 mg/kg	FREQ.	12	0	0	0	12
		%	100.0	0.0	0.0	0.0	

APPENDIX III (continued)

DRUG	DOSE	STATISTIC	TREMOR SCORE 0	1	2	3	N
	10 mg/kg	FREQ.	4	4	4	0	12
		%	33.3	33.3	33.3	0.0	
	20 mg/kg	FREQ.	4	4	2	0	10
		%	40.0	40.0	20.0	0.0	
	40 mg/kg	FREQ.	6	4	2	0	12
		%	50.0	33.3	16.7	0.0	
	8 mg/kg	FREQ.	8	2	2	0	12
		%	66.7	16.6	16.7	0.0	
	160 mg/kg	FREQ.	12	0	0	0	12
		%	100.0	0.0	0.0	0.0	
CHLORDIAZEPOXIDE (cont.)			CONVULSION SCORE 0	1	2	3	N
	10 mg/kg	FREQ.	9	0	1	2	12
		%	75.0	0.0	8.3	16.7	
	20 mg/kg	FREQ.	8	0	1	1	10
		%	80.0	0.0	10.0	10.0	
	40 mg/kg	FREQ.	12	0	0	0	12
		%	100.0	0.0	0.0	0.0	
	80 mg/kg	FREQ.	12	0	0	0	12
		%	100.0	0.0	0.0	0.0	
	160 mg/kg	FREQ.	12	0	0	0	12
		%	100.0	0.0	0.0	0.0	
			PILOERECTION SCORE 0	1	2	3	N
MORPHINE	10 mg/kg	FREQ.	0	0	11	1	12
		%	0.0	0.0	91.7	8.3	
	20 mg/kg	FREQ.	0	1	9	2	12
		%	0.0	8.3	75.0	16.7	
	40 mg/kg	FREQ.	4	5	2	0	11
		%	36.4	45.4	18.2	0.0	
			ABNORMAL POSTURE SCORE 0	1	2	3	N
	10 mg/kg	FREQ.	1	3	8	0	12
		%	8.3	25.0	66.7	0.0	
	20 mg/kg	FREQ.	3	5	3	1	12
		%	25.0	41.7	25.0	8.3	
	40 mg/kg	FREQ.	11	0	0	0	11
		%	100.0	0.0	0.0	0.0	

APPENDIX III (continued)

DRUG	DOSE	STATISTIC	TREMOR SCORE 0	1	2	3	N
MORPHINE (cont.)	10 mg/kg	FREQ.	5	3	4	0	12
		%	41.7	25.0	33.3	0.0	
	20 mg/kg	FREQ.	7	4	1	0	12
		%	58.3	33.3	8.3	0.0	
	40 mg/kg	FREQ.	11	0	0	0	11
		%	100.0	0.0	0.0	0.0	
			CONVULSION SCORE 0	1	2	3	N
	10 mg/kg	FREQ.	10	0	1	1	12
		%	83.3	0.0	8.3	8.3	
	20 mg/kg	FREQ.	12	0	0	0	12
		%	100.0	0.0	0.0	0.0	
	40 mg/kg	FREQ.	11	0	0	0	11
		%	100.0	0.0	0.0	0.0	

APPENDIX IV

SUM OF WITHDRAWAL SIGN INTENSITY RATINGS FOR PILOERECTION, ABNORMAL POSTURE, TREMORS AND CONVULSIONS MEASURED AT 36 HOURS OF ETHANOL WITHDRAWAL AFTER ETHANOL, CHLORDIAZEPOXIDE OR MORPHINE PRETREATMENT 30 MINUTES PRIOR TO OBSERVATION.

	DOSAGE							
	1 ml/kg		1 ml/kg		1 ml/kg		1 ml/kg	
PRETREATMENT	RAT	SCORE	RAT	SCORE	RAT	SCORE	RAT	SCORE
SALINE	1	9	13	n.d.	25	10	37	6
	2	7	14	n.d.	26	11	38	7
	3	5	15	n.d.	27	6	39	7
	4	8	16	n.d.	28	5	40	5
	5	7	17	n.d.	29	D	41	6
	6	6	18	n.d.	30	5	42	5
	7	n.d.	19	12	31	7	43	9
	8	n.d.	20	6	32	5	44	5
	9	n.d.	21	7	33	10	45	6
	10	n.d.	22	8	34	6	46	6
	11	n.d.	23	6	35	6	47	6
	12	n.d.	24	6	36	4	48	5
	MEDIAN							6.0

	DOSAGE							
	0.5 g/kg		2.0 g/kg		4.0 g/kg		8.0 g/kg	
ETHANOL	RAT	SCORE	RAT	SCORE	RAT	SCORE	RAT	SCORE
	1	10	13	3	25	5	37	1
	2	5	14	D	26	3	38	2
	3	D	15	5	27	3	39	0
	4	8	16	1	28	4	40	D
	5	5	17	5	29	4	41	2
	6	6	18	8	30	4	42	0
	7	7	19	5	31	3	43	2
	8	6	20	4	32	D	44	1
	9	6	21	4	33	4	45	2
	10	12	22	5	34	5	46	2
	11	8	23	3	35	1	47	0
	12	4	24	D	36	0	48	2
	MEDIAN	6.0		4.5		4.0		2.0

APPENDIX IV (continued)

CHLORDIAZEPOXIDE

DOSAGE									
10 mg/kg		20 mg/kg		40 mg/kg		80 mg/kg		160 mg/kg	
RAT	SCORE	RAT	SCORE	RAT	SCORE	RAT	SCORE	RAT	SCORE
1	5	13	7	25	5	37	5	49	0
2	8	14	4	26	6	38	4	50	1
3	8	15	6	27	5	39	3	51	2
4	7	16	5	28	3	40	2	52	0
5	7	17	D	29	7	41	3	53	1
6	5	18	10	30	2	42	5	54	0
7	4	19	D	31	3	43	3	55	1
8	10	20	6	32	6	44	5	56	0
9	5	21	6	33	5	45	3	57	1
10	8	22	8	34	7	46	4	58	1
11	7	23	7	35	5	47	1	59	2
12	4	24	4	36	4	48	6	60	0
MEDIAN	7.0		6.0		5.0		3.5		1.0

MORPHINE

DOSAGE					
10 mg/kg		20 mg/kg		40 mg/kg	
RAT	SCORE	RAT	SCORE	RAT	SCORE
1	2	13	3	25	1
2	4	14	4	26	D
3	9	15	5	27	1
4	6	16	3	28	0
5	5	17	4	29	2
6	3	18	7	30	1
7	4	19	2	31	0
8	3	20	1	32	1
9	5	21	3	33	0
10	5	22	5	34	2
11	6	23	2	35	0
12	8	24	6	36	1
MEDIAN	5.0		3.5		1.0

REFERENCES

Allan, F.F. and Swinyard, C.A.: Evaluation of tissue tolerance in ethyl alcohol by alterations in electroshock seizure thresholds in rats. Anat. Rec. 103: 419, 1949.

Anden, N.E., Rubenson, A., Fuxe, K. and Hokfet, T.: Evidence for dopamine receptor stimulation by apomorphine. J. Pharm. Pharmacol. 19: 627-629, 1967.

Axelrod, J.: In The Addictive States. ed., A. Wikler, vol. 46, pp. 247-264, Williams and Wilkins Co., Baltimore, 1968.

Barnes, C.D. and Ellerington, L.G.: Drug Dosage in Laboratory Animals: A Handbook, Univ. of Calif. Press, Berkeley, pp. 63, 1965.

Battersby, A.R.: Alkaloid biosynthesis. Quart. Rev. 15: 259-286, 1961.

Branchey, M., Rauscher, G. and Kissin, B.: Modifications in the response to alcohol following establishment of physical dependence. Psychopharmacologia (Berl.) 22: 314-322, 1971.

Bustos, G.O., Kalant, H., Khanna, J.M. and Loth, J.: Pyrazole and the induction of fatty liver by a single dose of ethanol. Sci. 168: 1598-1599, 1970.

Chambers, J.F. and Schultz, J.D.: Double-blind study of three drugs in the treatment of acute alcoholic states. Quart. J. Stud. Alc. 26: 10-18, 1965.

Cohen, G.: A role for tetrahydroisoquinoline alkaloids as false adrenergic neurotransmitters in alcoholism. In. Alcohol Intoxication and Withdrawal: Experimental Studies, ed. M.M. Gross, Advances in Experimental Medicine and Biology, vol. 35, pp. 33-44, Plenum Press, New York, 1973a.

Cohen, G.: Reactions of catecholamines with acetaldehyde to form tetrahydroisoquinoline alkaloids. In. Biological Aspects of Alcohol. eds., M.K. Roach, W.M. McIsaac and P.J. Creaven, pp. 267-284, Univ. of Texas Press, Austin, 1971.

Cohen, G.: Tetrahydroisoquinoline alkaloids: Uptake, storage and secretion by the adrenal medulla and by adrenergic nerves. In. Alcoholism and the Central Nervous System, ed., F. Seixas, Ann. N.Y. Acad. Sci. 215: 116-119, 1973b.

Collier, H.O.J.: A general theory of the genesis of drug dependence by induction of receptors. Nature (Lond.) 205: 181-182, 1965.

Davis, V.E.: Alcohol and aberrant metabolism of biogenic amines. In. Biological Aspects of Alcohol, eds., M.K. Roach, W.M. McIsaac, and P.J. Creaven, pp. 293-312, Univ. of Texas Press, Austin, 1971.

Davis, V.E.: Neuroamine-derived alkaloids: A possible common denominator in alcoholism and related drug dependence. In. Alcoholism and the Central Nervous System, ed. F. Seixas, Ann. NY. Acad. Sci. 215: 111-115, 1973.

Davis, V.E., Brown, H., Huff, J.A. and Cashaw, J.L.: Alterations of serotonin metaboism to 5-hydroxytryptophol by ethanol ingestion in man. J. Lab. Clin. Med. 69: 132-140, 1967a.

Davis, V.E., Brown, H., Huff, J.A. and Cashaw, J.L.: Ethanol-induced alterations of norepinephrine metabolism in man. J. Lab. Clin. Med. 69: 787-799, 1967b.

Davis, V.E., Cashaw, J.L., McLaughlin, B.R. and Hamlin, T.A.: Alteration of norepinephrine metabolism by barbiturates. Bio chem. Pharmacol. 23: 1877-1889, 1974.

Davis, V.E., Walsh, M.J. and Yamanaka, Y.: Augmentation of alkaloid formation from dopamine by alcohol and acetaldehyde in vitro. J. Pharmacol. Exp. Ther. 174: 401-412, 1970.

Deneau, G., Yanagita, T. and Seevers, M.H.: Self-administration of psychoactive substances by the monkey. Psychopharmacologia 16: 30-48, 1969.

Ellis, F.W. and Pick, J.R.: Ethanol-induced withdrawal reactions in rhesus monkeys. Pharmacologist 11: 256, 1969.

Ellis, F.W. and Pick, JR.: Ethanol intoxication and dependence in rhesus monkeys. In. Recent Advances in Studies of Alcoholism. eds., M.K. Mello and J.H. Mendelson, U.S. Govt. Printing Office, Publ. No. (HSM) 71-9045, pp. 401-412, Washington, D.C., 1971.

Ellis, F.W. and Pick, J.R.: Evidence of ethanol physical dependence in dogs. Fed. Proc. 29: 649, 1970a.

Ellis, F.W. and Pick, J.R.: Experimentally induced ethanol dependence in rhesus monkeys. J. Pharmacol. Exp. Ther. 175: 88-93, 1970b.

Essig, C.F. and Lam, R.C.: Convulsions and hallucinatory behavior following alcohol withdrawal in the dog. Arch. Neurol. 18: 626-632, 1968.

Essig, C.F. and Lam, R.C.: The alcohol abstinence syndrome in dogs and its treatment with pentobarbital. In. Recent Advances in Studies of Alcoholism. eds., N.K. Mello and J.H. Mendelson, U.S. Govt. Printing Office, Pub. No. (HSM) 71-9045, pp. 437-452, Washington, D.C., 1971.

Ernst, A.M.: Mode of action of apomorphine and dextroamphetamine on gnawing compulsion of rats. Psychopharmacologia (Berl.) 10: 316-323, 1967.

Falk, J.L.: Behavioral maintenance of high blood ethanol and physical dependence in the rat. Sci. 177: 811-813, 1972.

Falk, J.L.: Production of polydipsia in normal rats by an intermittent food schedule, Sci. 133: 195-196, 1961.

Favazza, A.R. and Martin, P.: Chemotherapy of delirium tremens: A survey of physician's preferences. Amer. J. Psychiat. 131: 1031-1033, 1974.

Fisher, C. and Dement, W.: Studies on the psychopathology of sleep and dreams. Amer. J. Psychiat. 119: 1160-1168, 1963.

Fleming, W.W. and Trendelenburg, V.: The development of supersensitivity to norepinephrine after pretreatment with reserpine. J. Pharmacol. Exp. Ther. 133: 41-51, 1961.

Forsander, O.A., Raiha, N., Solaspuro, M. and Moenpea, P.: Influence of ethanol on the liver metabolism of fed and starved rats. Biochem. J. 94: 259-265, 1965.

French, S.W., Reid, P.E., Palmer, D.S., Narod, M.E. and Ramey, C.W.: Adrenergic subsensitivity of the rat brain during chronic ethanol ingestion. Res. Comm. Chem. Path. Pharmacol. 9: 575-578, 1974.

Freund, G: Alcohol withdrawal syndrome in mice. Arch. Neurol. 21: 315-320, 1969.

Friedhoff, A.J. and Miller, J.: Effect of ethanol on the biosynthesis of dopamine. In. Alcoholism and the Central Nervous System, ed., F. Seixas, Ann. N.Y. Acad. Sci. 215: 183-186, 1973.

Gianutsos, G.: Personal communication, 1974.

Gianutsos, G., Drawbaugh, R.B., Hynes, M.D. and Lal, H.: Behavioral evidence for dopaminergic supersensitivity after chronic haloperidol. Life Sci. 14: 887-898, 1974a.

Gianutsos, G., Hynes, M.D., Drawbaugh, R. and Lal, H.: The narcotic withdrawal syndrome in rats. In. Drug Addiction, vol. 3, ed., J. Singh and H. Lal, pp. 293-309, 1975.

Gianutsos, G., Hynes, M.D., Puri, S.K., Drawbaugh, R.B. and Lal, H.: Effect of apomorphine and nigrostriatal lesions on aggression and striatal dopamine turnover during morphine withdrawal: Evidence for dopaminergic supersensitivity in protracted abstinence. Psychopharmacologia (Berl.) 34: 37-44, 1974b.

Gibbins, R.J., Kalant, H. and LeBlanc, A.E.: A technique for accurate measurement of moderate degrees of alcohol intoxication in small animals. J. Pharmacol. Exp. Ther. 159: 236-242, 1968.

Gibbins, R.J., Kalant, H., LeBlanc, A.E. and Clark, W.: The effects of chronic administration of ethanol on the startle threshold in rats. Psychopharmacologia (Berl.) 19: 95-104, 1971.

Golbert, T.M., Sanz, C.J., Rose, H.D. and Leitschuh, T.H.: Comparative evaluation of treatments of alcohol withdrawal syndromes. J. Amer. Med. Assoc. 201: 99-102, 1967.

Goldberg, L. and Rydberg, V.: Inhibition of ethanol metabolism in vivo by administration of pyrazole. Biochem. Pharmacol. 18: 1749-1762, 1969.

Goldberg, L., Hoelstedt, C., Neri, A. and Rydberg, V.: Synergistic action of pyrazole on ethanol incoordination: Differential metabolic and central nervous system effects. J. Pharm. Pharmacol. 24: 593-601, 1972.

Goldstein, A. and Goldstein, D.B.: Enzyme expansion theory of drug tolerance and physical dependence. In. The Addictive States. ed., A. Wikler, vol. 44, pp. 265-267, Williams and Wilkins Co., Baltimore, 1968.

Goldstein, A. and Judson, B.A.: Alcohol dependence and opiate dependence: Lack of relationship in mice. Sci. 172: 290-292, 1971.

Goldstein, D.B.: Alcohol withdrawal reactions in mice: Effects of drugs that modify neurotransmission. J. Pharmacol. Exp. Ther. 186: 1-9, 1973.

Goldstein, D.B.: An animal model for testing the effects of drugs on alcohol withdrawal reactions. J. Pharmacol. Exp. Ther. 183: 14-22, 1972b.

Goldstein, D.B.: Relationship of alcohol dose to intensity of withdrawal signs in mice. J. Pharmacol. Exp. Ther. 180: 203-215, 1972a.

Goldstein, D.B. and Pal, N.: Alcohol dependence produced in mice by inhalation of ethanol: Grading the withdrawal reaction. Sci 172: 288-290, 1971.

Greenberg, R. and Pearlman, C.: Delirium tremens and dreaming. Amer. J. Psychiat. 124: 133-142, 1967.

Gross, M.M. and Goodenough, D.R.: Sleep disturbances in the acute alcoholic psychoses. Psychiat. Res. Report. Amer. Psychiat. Assoc. 24: 132-147, 1968.

Gross, M.M., Goodenough, D.R., Tobin, M., Holpert, E., Lepore, D., Perlstein, A., Sirota, M., Dibiances, J., Fuller, R. and Kishner, I.: Sleep disturbances and hallucinations in acute alcoholic psychoses. J. Nerv. Ment. Dis. 142: 493-514, 1966.

Gross, M.M., Lewis, E. and Hostey, J.: Acute alcohol withdrawal. In. The Biology of Alcoholism. eds., B. Kissin and H. Beiglieter, vol. 3, pp. 191-263, Plenum Press, New York, 1974.

Hasumura, Y., Teschke, R. and Lieber, C.S.: Acetaldehyde oxidation by hepatic mitochondria: Decreased after chronic ethanol consumption. Sci. 189: 727-729, 1975.

Hawkins, R.D., Kalant, H. and Khanna, J.M.: Effects of chronic intake of ethanol on the rate of ethanol metabolism. Can. J. Physiol. Pharmacol. 44: 241-257, 1966.

Hoogland, D.R., Miya, T.S. and Bousquet, W.F.: Metabolism and tolerance studies with chlordiazepoxide in the rat. Tox. Appl. Pharmacol. 9: 116-123, 1966.

Hunt, W.A. and Majchrowicz, E.: Alterations in the turnover of brain norepinephrine and dopamine in alcohol-dependent rats. J. Neurochem. 23: 549-552, 1974.

Irwin, S.: Comprehensive observational assessment: In. A systematic, quantitative procedure for assessing the behavioral and physiological state of the mouse. Psychopharmacologia (Berl.) 13: 222-257, 1968.

Isbell, H., Fraser, H., Wikler, A., Belleville, B. and Eisenman, A.: An experimental study of the etiology of rum fits and delirium tremens. Quart. J. Stud. Alc. 16: 1-33, 1955.

Jaffe, J.H.: In. The Pharmacological Basis of Therapeutics. eds. L.S. Goodman and A. Gilman, 4th ed., pp. 276-313, MacMillan Co., London and Toronto, 1970.

Jaffe, J.H. and Sharpless, S.K.: Pharmacological denervation supersensitivity in the central nervous system: A theory of physical dependence. In. The Addictive States. ed., A. Wikler, vol. 46, pp. 226-243, Williams and Wilkins Co., Baltimore, 1968.

Jaffe, J.H. and Sharpless, S.K.: The rapid development of physical dependence on barbiturates. J. Pharmacol. Exp. Ther. 150: 140-145, 1965.

Jetter, W.W.: Studies in alcohol. II. Experimental feeding of alcohol to non-alcoholic individuals. Amer. J. Med. Sci. 196: 487-491, 1938.

Johnson, L.C., Burdick, J.A. and Smith, J.: Sleep during alcohol intake and withdrawal in chronic alcoholics. Arch. Gen. Psychiat. 22: 406-418, 1970.

Jones, D., Gerber, L.P. and Prell, W.: A rapid enzymatic method for estimating ethanol in body fluids. Clin. Chem. 16: 402-407, 1970.

Kaim, S.C. and Klett, C.J.: Treatment of delirium tremens - A comparative evaluation of four drugs. Quart. J. Stud. Alc. 33: 1065-1072, 1972.

Kaim, S.C., Klett, C.J. and Rothfeld, B.: Treatment of the acute alcohol withdrawal state: A comparison of four drugs. Amer. J. Psychiat. 125: 1640-1646, 1969.

Kalant, H., LeBlanc, A.E. and Gibbins, R.J.: Tolerance to, and dependence on, some non-opiate psychotropic drugs. Pharmacol. Rev. 23: 135-191, 1971.

Kissin, B., Gross, M.M. and Schutz, I.: Correlation of urinary biogenic amines with sleep stages in chronic alcoholization and withdrawal. In. Alcohol Intoxication and Withdrawal: Experimental Studies. Ed., M.M. Gross, Advances in Experimental Medicine and Biology, vol. 35, pp. 281-290, Plenum Press, New York, 1973.

Korsten, M.A., Matsuzaki, S., Feinman, L. and Lieber, C.S.: High blood acetaldehyde levels after ethanol administration: Difference between alcoholic and non-alcoholic subjects. New Eng J. Med. 292: 386-389, 1975.

Lal, H. and Shah, H.: Reduced sleeping time and increased microsomal oxidation of hexobarbital after methylchloroform (MC) inhalation in mice. Pharmacologist 10: 153, 1968.

Lal, H., O'Brien, J. and Puri, S.K.: Morphine withdrawal-aggression: Sensitization by amphetamines. Psychopharmacologia 22: 217-223, 1970.

LeBlanc, A.E., Kalant, H., Gibbins, R.J. and Berman, N.D.: Acquisition and loss of tolerance to ethanol by the rat. J. Pharmacol. Exp. Ther. 168: 244-250, 1969.

Lelbach, W.K.: Liver cell necrosis in rats after prolonged ethanol ingestion under the influence of an alcohol dehydrogenase inhibitor. Experientia (Basel) 25: 816-818, 1969.

Lester, D.: Self-selection of alcohol by animals, human variation, and the etiology of alcoholism. Quart. J. Stud. Alc. 27: 395-438, 1966.

Lester, D. and Benson, G.D.: Alcohol oxidation in rats inhibited by pyrazole, oximes and amides, Sci. 169: 282-283, 1970.

Lester, D., Keokosky, W.Z. and Felzenberg, F.: Effect of pyrazole and other compounds on alcohol metabolism. Quart. J. Stud. Alc. 29: 449-454, 1968.

Lester, B.K., Rundell, O.H., Cowder, L.C. and Williams, H.L.: Chronic alcoholism, alcohol and sleep. In. Alcohol Intoxication and Withdrawal: Experimental Studies. ed., M.M. Gross, Advances in Experimental Medicine and Biology, vol. 35, pp. 261-280, Plenum Press, N.Y., 1973.

Lieber, C.S. and DeCarli, L.M.: Ethanol oxidation by hepatic microsomes: Adaptive increase after ethanol feeding. Sci. 162: 917-918, 1968.

Lieber, C.S., Rubin, E., DeCarli, L.M., Mirsa, P. and Gang, H.: Effects of pyrazole on hepatic function and structure. Lab. Invest. 22: 615-621, 1970.

Littleton, J.M., Griffiths, P.J. and Ortiz, A.: Induction of ethanol physical dependence and the ethanol withdrawal syndrome: The effects of pyrazole. J. Pharm. Pharmacol. 26: 81-91, 1974.

Majchrowicz, E.: Induction of physical dependence on alcohol and the associated metabolic and behavioral changes in rats. Pharmacologist 15: 159, 1973.

McQuarrie, D.G. and Fingl, E.: Effect of single doses and chronic administration of ethanol on experimental seizures in mice. J. Pharmacol. Exp. Ther. 124: 264-271, 1958.

Mello, N.K.: A review of methods to induce alcohol addiction in animals. Pharmacol. Biochem. Behav. 1: 1-13, 1973.

Mello, N.K.: Behavioral studies of alcoholism. In. The Biology of Alcoholism. eds., B. Kissin and H. Beglieter, vol. 2, pp. 219-291, Plenum Press, N.Y., 1972.

Mello, N.K.: Some aspects of the behavioral pharmacology of alcohol. In. Psychopharmacology: A review of progress 1957-1967, ed., D.H. Effron, U.S. Govt. Printing Office, PHS Publ. No. 1863, pp. 787-809, Washington, D.C., 1968.

Mello, N.K. and Mendelson, J.H.: Drinking patterns during work-contingent and non-contingent alcohol acquisition. Psychosom. Med. 34: 139-164, 1972.

Mello, N.K. and Mendelson, J.H.: Evaluation of a polydipsia technique to induce alcohol consumption in monkeys. Physiol. Behav. 7: 827-836, 1971b.

Mello, N.K. and Mendelson, J.H.: Experimentally induced intoxication in alcoholics: A comparison between programmed and spontaneous drinking. J. Pharmacol. Exp. Ther. 173: 101-116, 1970.

Mello, N.K. and Mendelson, J.H.: The effects of drinking to avoid shock on alcohol intake in primates. In. Biological Aspects of Alcohol. ed., M.K. Roach, W.M. McIsaac and P.J. Creaven, pp. 313-340, Univ. of Texas Press, Austin, 1971a.

Mendelson, J.H.: Biochemical Mechanisms of Alcohol Addiction. In. The Biology of Alcoholism. eds., B. Kissin and H. Begleiter, vol. 1, pp. 513-544, Plenum Press, N.Y., 1971.

Mendelson, J.H.: Biological concomitants of alcoholism. New Eng. J. Med. 283: 24-32; 71-81, 1970.

Mendelson, J.H.: Experimentally Induced chronic intoxication and withdrawal in alcoholics. Quart. J. Stud. Acl., Suppl #2, 1964.

Mendelson, J.H., Mello, N.K., Corbett, C. and Ballard, R.: Puromycin inhibition of ethanol ingestion and liver alcohol dehydrogenase activity in the rat. J. Psychiat. Res. 3: 133-143, 1965.

Mendelson, J.H., Stein, S. and McGuire, M.T.: Comparative psychophysiological studies of alcoholic and non-alcoholic subjects undergoing experimentally induced ethanol intoxication. Psychosom. Med. 28: 13-26, 1966.

Morgan, J.C. and DiLuzio, N.R.: Inhibition of the acute ethanol induced fatty liver by pyrazole. Proc. Soc. Exp. Biol. Med. 134; 462-466, 1970.

Moskowitz, H. and Wapner, M.: Studies on the acquisition of behavioral tolerance to alcohol. Quart. J. Stud. Alc. 25: 619-626, 1964.

Myers, R.D. and Veale, W.L.: The determinants of alcohol preference in animals. In. The Biology of Alcoholism. eds., B. Kissin and H. Beglieter, vol. 2, pp. 131-168, Plenum Press, N.Y., 1972.

Nathan, P.E., O'Brien, J.S. and Lowenstein, L.M.: Operant studies of chronic alcoholism: Interaction of alcohol and alcoholics. In. The Biological Aspects of Alcohol. eds., M.K. Roach, W.M. McIsaac and P.J. Creaven, pp. 341-370, Univ. of Texas Press, Austin, 1971.

Nathan, P.E., Titler, N.A., Lowenstein, L.M., Soloman, P. and Rossi, A.M.; Behavioral analysis of chronic alcoholism. Arch. Gen. Psychiat. 22: 419-430, 1970.

Newman, H.W.: Acquired tolerance to ethyl alcohol. Quart. J. Stud. Alc. 2: 453-463, 1941.

Ogata, H., Ogata, F., Mendelson, J.H. and Mello, N.K.: A comparison of techniques to induce alcohol dependence in mice. J. Pharmacol. Exp. Ther. 180: 216-230, 1972.

Ortiz, A., Griffiths, P.J. and Littleton, J.M.: A comparison of the effects of chronic administration of ethanol and acetaldehyde to mice: Evidence for a role of acetaldehyde in ethanol dependence. J. Pharm. Pharmacol. 26: 249-260, 1974.

Owens, A.H., Jr., Marshall, E.K., Jr.: The metabolism of ethyl alcohol in the rat. J. Pharmacol. Exp. Ther. 115: 360-370, 1950.

Patel, G.J. and Lal, H.: Reduction in brain gamma-aminobutyric acid and in barbital narcosis during ethanol withdrawal. J. Pharmacol. Exp. Ther. 186: 625-629, 1973.

Pieper, W.A., Skeen, M.J., McClure, H.M. and Bourne, P.G.: The chimpanzee as an animal model for investigating alcoholism. Sci. 176: 71-73, 1972.

Puri, S.K. and Lal, H.: Effect of dopaminergic stimulation or blockade on morphine-withdrawal aggression. Psychopharmacologia (Berl.) 32: 113-120, 1973.

Puri, S.K., Reddy, C. and Lal, H.: Blockade of central dopaminergic receptors by morphine: Effect of haloperidol, apomorphine or benztropine. Res. Comm. Chem. Path. Pharmacol. 5: 389-401, 1973.

Rahwan, R.G., O'Neill, P.J. and Miller, P.D.: Differential secretion of catecholamines and tetrahydroisoquinolines from bovine adrenal medulla. Life Sci. 14: 1927-1938, 1974.

Ratcliffe, F.: Ethanol dependence in the rat: Its production and characteristics. Arch. Int. Pharmacodyn. 196: 146-156, 1972.

Reis, D.J.: A possible role of central noradrenergic neurons in withdrawal states from alcohol. In. Alcoholism and the Central Nervous System. ed., F. Seixas, Ann. N.Y. Acad. Sci. 215: 249-252, 1973.

Roach, M.K., Khan, M.M., Coffman, R., Pennington, W. and Davis, D.L.: Brain ($Na^+ + K^+$)-activator adenosine triphosphatase activity and neurotransmitter uptake in alcohol dependent rats. Brain Res. 63: 323-329, 1973.

Rosenfeld, G.: Potentiation of the narcotic action and acute toxicity of alcohol by primary aromatic monoamines. Quart. J. Stud. Alc. 21: 584-596, 1960.

Ross, D.H., Medina, M.A. and Cardenas, H.L.: Morphine and Ethanol: Selective depletion of regional brain calcium. Sci. 186: 63-65, 1974.

Rubin, E., Gang, H. and Lieber, C.S.: Interaction of ethanol and pyrazole with hepatic microsomes. Biochem. Biophy. Res. Comm. 42: 1-8, 1971.

Rydberg, V., Buijten, J. and Neri, A.: Kinetics of some pyrazole derivatives in the rat. J. Pharm. Pharmacol. 24: 651-652, 1972.

Seevers, M.H.: Morphine and ethanol physical dependence: A critique of a hypothesis. Sci. 170: 1113-1114, 1970.

Siegal, S.: Non-parametric Statistics for the Behavioral Sciences. McGraw-Hill Co., Inc., New York, 1956.

Smith, M.E. and Newman, H.W.: The rate of ethanol metabolism in fed and fasting animals. J. Biol. Chem. 234: 1544, 1959.

Snedecor, G.W. and Cochran, W.G.: Statistical Methods, 6th ed., Iowa State Univ. Press, Ames, 1967.

Spenser, I.D.: Biosynthesis of alkaloids related to norlaudosoline. Lloydia (Cinn.) 29: 71-89, 1966.

Swartz, M.H., Repke, P.I., Katz, A.M. and Rubin, E.: Effects of ethanol on calcium uptake by cardiac microsomes. Biochem. Pharmacol. 23: 2369-2376, 1974.

Tabakoff, B. and Erwin, V.G.: Purification and characterization of a reduced nicotinamide adenine dinucleotide phosphate-linked aldehyde reductase from brain. J. Biol. Chem. 245: 3263-3268, 1970.

Tabakoff, B., Ungar, F. and Alivisatos, S.G.A.: Addiction to barbiturates and ethanol: Possible biochemical mechanisms. In. Alcohol Intoxication and Withdrawal: Experimental Studies. ed., M.M. Gross, Advances in Experimental Medicine and Biology, vol. 35, pp. 45-55, 1973.

Taylor, K.M. and Snyder, S.H.: Amphetamine: Differentiation by d and 1 isomers of behavior involving brain norepinephrine or dopamine. Sci. 168: 1487-1489, 1970.

Theorell, H., Yonetani, T. and Sjoberg, B.: On the effects of some heterocyclic compounds on enzymatic activity of liver alcohol dehydrogenase. Acta. Chem. Scand. 23: 255-260, 1969.

Thomas, D.W. and Freedman, D.X.: Treatment of the alcohol withdrawal syndrome. J. Amer. Med. Assoc. 188: 244, 1964.

Truitt, E.B.: A biogenic amine hypothesis of alcohol tolerance. In. Alcoholism and the Central Nervous System, ed. F. Seixas, Ann. N.Y. Acad. Sci. 215: 177-182, 1973.

Truitt, E.B. and Walsh, M.J.: The role of acetaldehyde in the actions of ethanol. In. The Biology of Alcoholism. eds., B. Kissin and H. Beglieter, vol. 1, pp. 161-195, Plenum Press, N.Y., 1971.

Victor, M.: The role of hypomagnesemia and respiratory alkaloids in the genesis of alcohol withdrawal symptoms. In. Alcoholism and the Central Nervous System. ed., F. Seixas, Ann. N.Y. Acad. Sci. 215: 235-248, 1973.

Victor, M.: Treatment of alcohol intoxication and the withdrawal syndrome. Psychosom. Med. 28: 636-650, 1966.

Victor, M. and Adams, R.D.: The effect of alcohol on the central nervous system. Res. Publ. Assoc. Nerv. Ment. Dis. 32: 526-573, 1953.

Wallgren, H.: Neurochemical aspects of tolerance to and physical dependence on ethanol. In. Alcohol Intoxication and Withdrawal: Experimental Studies, ed. M.M. Gross, Advances in Experimental Medicine and Biology, vol. 35, pp. 15-31, 1973.

Walsh, M.J.: Biogenesis of biologically active alkaloids from amines by alcohol and acetaldehyde. In. Alcoholism and the Central Nervous System. ed., F. Seixas, Ann. N.Y. Acad. Sci. 215: 235-248, 1973.

Walsh, M.J., Davis, V.E. and Yamanaka, Y.: Tetrahydropapaveroline: An alkaloid metabolite of dopamine in vitro. J. Pharmacol. Exp. Ther. 174: 388-400, 1970.

Way, E.L., Loh, H.H. and Shen, F.: Simultaneous quantitative assessment of morphine tolerance and physical dependence. J. Pharmacol. Exp. Ther. 167: 1-8, 1969.

Williams, H. and Salamy, A.: Alcohol and Sleep. In. The Biology of Alcoholism. eds., B. Kissin and H. Beglieter, vol. 2, pp. 435-483, Plenum Press, N.Y., 1972.

Winger, G., Ikomi, F. and Woods, J.H.: Intravenous self-administration in rhesus monkeys. Proc. 32nd Ann. Conf. Comm. Prob. Drug. Dependence, NAS-NRC, pp. 6598-6605, 1970.

Woods, J.H. and Winger, G.D.: A critique of methods for inducing ethanol self-intoxication in animals. In. Recent Advances in the Studies of Alcoholism. eds., M.K. Mello and J.H. Mendelson, U.S. Govt. Printing Office, Publ. No. (HSM) 71-9045, pp. 413-436, Washington, D.C., 1971.

Woods, J.H., Ikoni, F.I. and Winger, G.: The reinforcing properties of ethanol. In. The Biological Aspects of Alcohol. eds., M.K. Roach, W.M. McIsaac and P.J. Creaven, pp. 371-388, Univ. of Texas Press, Austin, 1971.

Yamanaka, Y., Walsh, M.J. and Davis, V.E.: Salsolinol, an alkaloid derivative of dopamine formed in vitro during alcohol metabolism. Nature 227: 1143-1144, 1970.

Yanagita, T., Deneau, G.A. and Seevers, M.H.: Evaluation of pharmacological agents in the monkey by long-term intravenous self or programmed administration. Int. Cong. Physiol. Sci. 23: 66, 1965.

Yules, R.B., Freedman, D.X. and Chandler, K.A.: The effect of ethyl alcohol on man's electroencephalographic sleep cycle. Electroencephalogy. Clin. Neurophysiol. 20: 109-111, 1966.

Zbinden, G., Bagdon, R.E., Keith, E.F., Phillips, R.P. and Randall, L.O.: Experimental and clinical toxicology of chlordiazepoxide (Librium). Tox. Appl. Pharmacol. 3: 619-637, 1961.