PREFERENCE FOR AND TOLERANCE TO ETHANOL: ACETALDEHYDE INVOLVEMENT

by

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ABSTRACT

A study was made to investigate the physiological bases of, and the relation between initial tolerance, development of tolerance and preference for ethanol in a strain of rats originally bred to accentuate differences in learning. Two major studies were conducted using 356 male and female rats of the S_1 and S_3 Tryon strain. Strain and sex related differential preference for ethanol was demonstrated. Tolerance to ethanol, measured by latency of and sleep duration resulting from the administration of a soporific dose of ethanol, was lesser in the high-preference (S_1)than in the low-preference (S_3) strain. Metabolic factors accounted for the lesser resistance to the anaesthetic effects of ethanol in S_1 animals. In contrast, tolerance to ethanol, measured by the debilitating effect of a subhypnotic dose of ethanol on a previously learned motor task, was greater in the S_1 (high-preference) than in the S_3 (low-preference) rats. Development of behavioral tolerance on repeated exposure to ethanol occurred at a similar rate for S_1 and S_3 animals. Nonetheless S_1 animals exhibited a greater neural tolerance in the course of tolerance development than their S_3 counterparts. These findings led to the suggestion that preference for and initial tolerance to a subhypnotic dose of ethanol are related to and determined by inherent enzyme patterns that characterize the S_1 and S_3 lines of the Tryon strain. The predominant influence of brain aldehyde oxidizing capacity in the determination of preference for

ethanol has been supported by the findings of this research. A reconsideration of the role of acetaldehyde metabolism in the initiation and maintenance of alcohol drinking behavior is also suggested.

SOMMAIRE

L'éthanol et son métabolite, l'acétaldéhyde, ont été étudiés dans le but de découvrir leur rôle dans les phénomènes de préférence et de tolérance vis-à-vis l'alcool. L'expérience a porté sur 356 rats des lignées S_1 et S_3 de la souche de Tryon. Les animaux des lignées respectives manifestèrent des préférences différentes face à l'alcool. Une plus grande tolérance vis-à-vis une dose soporifique d'alcool fut remarquée chez les animaux issus de la lignée (S_3) qui démontraient peu de préférence à l'alcool. On a constaté que la faible résistance des animaux de la lignée S_1 aux effets soporifiques de l'alcool dépendait d'un métabolisme d'élimination plus lent de l'alcool. Cependant, on a remarqué que ces mêmes animaux étaient moins intoxiqués que ceux de la lignée S_3 à une dose subhypnotique. L'administration répetée de cette dose produisit une tolérance qui se développa au même rythme chez les animaux des deux lignées. Une plus grande tolérance cellulaire fut néanmoins observée chez les animaux de la lignée S_1. On en arrive a suggérer que la préférence et la tolérance en ce qui a trait à l'alcool sont possiblement reliées et façonnées par l'entremise de voies enzymatiques. De plus, les résultats de la présente recherche confirment l'importance de la capacité du cerveau pour l'oxydation de l'aldéhyde sur l'établissement d'une préférence marquée pour l'alcool. Cependant, la nature du rôle du métabolisme de l'aldéhyde dans le maintien de la toxicomanie alcoolique est à reconsidérer.

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INTRODUCTION

In humans, social and cultural factors undoubtedly play an important role in the initiation and maintenance of alcohol consumption. It is widely recognized however, that physiological factors are implicated in the etiology of alcohol dependence in both humans and animals and, among these factors, the physiological bases of, and the relations between, preference and tolerance are of particular interest for the study of the development of alcohol dependence. Acetaldehyde, the highly reactive product of ethanol oxidation, appears to play a critical role in these phenomena, but the specific mechanism whereby acetaldehyde exerts its influence on the control of ethanol consumption remains somewhat obscure.

Several investigators in animal research have examined the relation between ethanol consumption and the activities of alcohol and aldehyde dehydrogenase enzymes, the two most important enzymes involved in the metabolism of alcohol. The relation between preference and initial tolerance to ethanol in different strains of rodents has also been intensively researched. However, few investigators have shown an interest in the development of tolerance in strains characterized by their differential preference for ethanol and even fewer have investigated the impact of tolerance development on the subsequent intake of ethanol. The present research comprises two major studies attempting to clarify the relations between initial tolerance, the development of tolerance, and its

influence on the subsequent ethanol intake in two lines of rats characterized by a differential preference for ethanol. A historical introduction of the relevant literature dealing with these issues is presented. A brief description of ethanol and acetaldehyde metabolism precedes a review of the major studies on humans and animals, illustrating the predominant role acetaldehyde plays in alcohol preference. This exposition is followed by a summary of the literature on animal research covering the relation between preference for and tolerance to ethanol.

The occurrence and metabolism of acetaldehyde

The formation of acetaldehyde

The first phase in the metabolism of ethanol is the formation of acetaldehyde via the oxidation of ethanol. There is presently general agreement that the NAD+ dependent alcohol dehydrogenase enzyme (ADH,EC 1.1.1.1) plays a major role in the metabolism of ethanol. This enzyme is primarily located in the cytosolic region of the cell and found predominantly (80-90%) in the liver cells (Lundquist, 1970; Von Wartburg, 1971; Wallgren and Barry, 1970) although it is present in the kidneys, the lungs, the intestine and the blood (Hawkins and Kalant, 1972).

Alcohol dehydrogenase activity has also been reported to occur in the brain (Raskin and Sokoloff, 1968, 1970) but the brain's capacity for the oxidation of ethanol is small and considered to stand below 1nmol/min/g tissue (Tabakoff and

Gelpke, 1975). Recent developments in histochemical methods have demonstrated that ADH is unevenly distributed in many organs, such as the kidney, the endocrine organs and the brain, and is localized predominantly in certain cell types (Bühler et al, 1983). These observations suggest that, even if the overall ADH activity in a given organ is low, specialized cells within the organ may contain high amounts of the enzyme. Publication of results originating from Sippel's laboratory (Sippel, 1974; Sippel and Eriksson 1975) indicating that acetaldehyde is detected in brain tissue only when there are high levels of this substance in the arterial blood led to the belief in the unlikeliness of the occurrence of acetaldehyde in brain tissue. The findings of Bühler and his associates (1983) however, offer an alternative suggestion in that the presence of ADH in specialized neurons can lead independently of blood acetaldehyde, to acetaldehyde concentrations large enough to be measured at critical sites of the central nervous system.

Two other systems are capable of oxidizing ethanol: the H_2O_2 dependent catalase (Keilin and Hartree, 1945) and the NADPH and O_2 dependent microsomal ethanol oxidizing system (MEOS) (Lieber and De Carli, 1968). Both these systems play little or no role in the normal in vivo metabolism of ethanol as compared to ADH (for review see Hawkins and Kalant, 1972); however MEOS is considered a possible pathway in an alcohol adapted organism (Teschke et al, 1977).

Acetaldehyde catabolism

The aldehyde product of ethanol's oxidation shares the fate of the endogenous aldehydes derived from the oxidative deamination by monoamine oxidase (MAO) of catecholamines and indoleamines (Blaschko, 1952); they are either reduced to an alcoholic metabolite (Tabakoff et al, 1973) or oxidized to an acidic product (Erwin and Deitrich, 1966). Although the reductase activity in the brain is of greater importance than the oxidase activity (Deitrich and Erwin, 1975), aldehydes, with the exception of those carrying a B-hydroxyl radical such as those derived from norepinephrine (NE) synthesis, have a greater affinity for the oxidative process and are therefore degraded to their corresponding acidic product (Breese et al, 1969a,b; Rutledge and Jonason, 1967). The oxidation of acetaldehyde can be carried out by a NAD+ dependent aldehyde dehydrogenase (ALDH, EC 1.2.1.3) and two molybdenum containing flavoproteins: aldehyde oxidase (EC 1.2.3.2) and xanthine oxidase (EC 1.2.3.1) (Lundquist, 1970; Von Wartburg, 1971). The Michaelis constants are unfavorably high for these two oxidases and consequently they are of little importance in the degradation process of acetaldehyde (Akabane, 1970).

The ALDH enzyme is located in virtually every organ of the body (Weiner, 1979b) but up to 95% of the acetaldehyde is formed and degraded in the liver during the oxidation of ethanol (Weiner, 1979a). Various NAD+ dependent ALDH's can be

found which differ with respect to their molecular size, specificity, kinetic constants and subcellular localization (Deitrich, 1966). There are aldehyde dehydrogenases in the cytoplasm, mitochondrial and microsomal fraction of the cell (Goedde et al, 1982; Greenfield and Pietruszko, 1977; Koivula, 1975; Siew et al, 1976; Tottmar et al, 1973; Weiner, 1979b). Most of the acetaldehyde derived from ethanol metabolism is oxidized by the low K_m mitochondrial ALDH (Inoue and Lindros, 1982; Koivula et al, 1981; Lindros et al, 1972; Marjanen, 1972). Since the amount of ALDH is 4 to 5 times greater than ADH, the rate of removal of acetaldehyde is faster than its production from ethanol (Büttner, 1965). Consequently, the amount of acetaldehyde circulating in the blood during the oxidization of ethanol remains at a very low level.

The involvement of acetaldehyde metabolism in alcohol preference

The accumulation of acetaldehyde in blood and its impact on the consumption of alcohol; ethnic and behavioral genetic studies

Studies carried out in the early 70's clearly indicated a role for acetaldehyde in some of the biochemical and pharmacological consequences of ethanol intake. These studies prompted research on the possible action of acetaldehyde in the consumption of alcohol. Ethnic differences observed in the alcohol consumption lead to the suggestion that

physiological rather than cultural and social factors might prevent certain populations from drinking excess amounts of alcohol. Indeed, native Asiatics (Wolff, 1972) as well as an American-born population of a different ethnic background (Ewing et al, 1974) which consume very little alcohol, show a hypersensitivity to alcohol and develop a wide variety of symptoms such as flushing, heart rate accelerations, drop in blood pressure, and so on, when drinking.

These symptoms were first observed by Williams (1937) in workers who developed a hypersensitivity to alcohol when exposed to tetramethylthiuram disulfide. Hald and Jacobsen (1948) using a related compound tetraethylthiuram disulfide (disulfiram, ANTABUSE^{r}) described a similar effect. In 1956, Ferguson found that the effect of calcium cyanamide (TEMPOSIL^{r}), a substance provoking symptoms identical to the disulfiram reaction, could be related to high levels of acetaldehyde circulating in the blood. Consequently, it was suggested that acetaldehyde was the physiological substrate responsible for the hypersensitivity to alcohol and that it might induce an aversion for alcohol.

Likewise, a higher level of acetaldehyde circulating in the blood stream has been observed in individuals of ethnic groups known to exhibit low alcohol consumption and to show a facial flushing response after the ingestion of alcohol (Ijiri, 1974; Inoue et al, 1980; Mizoi et al, 1979). The heightened rate of acetaldehyde formation observed in the

alcohol sensitive population was originally thought to be due to the presence of an atypical form of ADH (Seto et al, 1978; Zeiner et al, 1977, 1979). Indeed, the occurrence of this enzyme, as it has been described by Von Wartburg and associates (1965), is found to be greater in the alcohol sensitive Japanese population (Fukui and Wakasugi, 1972; Stamatoyannopoulos et al, 1975) and relatively absent in caucasian populations (Berger et al, 1974). The finding of a similar rate of alcohol metabolism in carriers of the normal and the atypical form of ADH (Edwards and Evans, 1967) and the lack of a relation between the facial flushing response and alcohol blood concentration (Inoue et al, 1980) or rate of blood alcohol elimination (Mizoi et al, 1979) further indicates the critical role that acetaldehyde may have in the consumption of alcohol. Hence, the discovery of a deficiency in the low K_m isoenzyme of ALDH (ALDH1) in half of the liver specimens originating from a Japanese population (Goedde et al, 1979; Harada et al, 1980) stimulated subsequent research on the subject of facial flushing response in this population.

Since the frequency of the atypical form of ADH in the Japanese population (85%) was greater than the incidence of flushers found in the same population (50%), it became evident for Mizoi and his colleagues (1983) that the presence of this form of ADH alone could not explain the increased blood acetaldehyde levels observed in the flushers. In their

investigation, these authors clearly established that the highest blood acetaldehyde levels occurred only in individuals with a deficiency in the ALDH1 isoenzyme associated with the presence of the atypical form of ADH. When drinking, these individuals showed a flushing response. The suggestion that a deficiency of this isoenzyme may serve a protective role against alcoholism appeared when it was noted that the incidence of this deficiency was considerably less frequent in Japanese alcoholics than in any other subgroups of the population including drug addicts (Goedde et al, 1983; Harada et al, 1983a,b; Yoshihara et al, 1983). These observations were further substantiated by the finding of the atypical form of ADH together with a deficiency in the ALDH1 isoenzyme following liver biopsies of a Chinese population (Ricciardi et al, 1983).

While it can be concluded from the evidence cited above that high concentrations of acetaldehyde circulating in the blood stream discourage alcohol consumption in certain ethnic populations, there is evidence, that moderate levels of acetaldehyde in the blood may favor the consumption of alcohol beverages. Indeed, while the level of acetaldehyde is reported to be low or undetectable in non alcoholics of occidental origin (Lindros et al, 1982), the blood of alcoholics of oriental (Maring et al, 1983), as well as of occidental origin (Korsten et al, 1975; Lindros et al, 1982; Salaspuro et al, 1982) contains moderate levels of

acetaldehyde. The increased levels of acetaldehyde noted in the occidental alcoholics may of course be a consequence of alcohol abuse (Jenkins and Peters, 1980; Lieber and De Carli, 1972; Nuutinen et al, 1983; Salaspuro et al, 1982) but the finding of increased levels of acetaldehyde in non drinking relatives of alcoholics compared to non-drinkers with no familial history of alcoholism provides evidence of a predisposing factor in the etiology of alcoholism (Schuckit, 1980; Schuckit and Rayes, 1979). Thus, the mild discomfort that the individual at risk may experience, in contrast to the more aversive physiological reactions noted when the acetaldehyde concentration in the blood stream is very high, may serve as an initial deterrent to alcohol consumption. Yet, when other biologic and/or social factors overcome this initial effect, such individuals may engage in more serious and persistent alcohol consumption that can lead them to alcoholism.

In summary, large individual variability in the levels of acetaldehyde circulating in the blood stream is observed. This variability may result from the delicate balance between the rates of production of acetaldehyde and its removal. Experiential factors as well as genetically determined variability in the pattern of the enzymes responsible for alcohol and acetaldehyde metabolism may explain these

individual differences. Hence, the known complex isoenzyme patterns and polymorphisms of ALDH and ADH may offer a rationale not only for the observed racial differences noted in the sensitivity to the effects of alcohol but in a more subtle way also for the difference between individuals within the same population.

Behavioral genetic studies carried out in mice and rats have further substantiated the existence of genetically and environmentally determined variance of enzyme patterns associated with ethanol consumption. Thus, the mice of the low-preferring DBA strain have higher acetaldehyde levels in blood following exposure to alcohol, than the high-preferring C57 strain (Sheppard et al, 1970, Tabakoff et al, 1976). Eriksson (1980c) demonstrated that the preference for ethanol was not only related to the circulating levels of blood acetaldehyde but also to the liver ALDH enzyme activity; the importance of ALDH enzyme activity in regulating the metabolism of acetaldehyde was therefore demonstrated. And indeed, the ethanol preference of the C57Bl mouse is related to greater capacity of its hepatic ALDH and found to be more critical to preference than the ADH activity (Sheppard et al, 1968; 1970). Similarly, when rats were selected and bred on the basis of their ethanol consumption, the low-consumers also proved to have greater concentrations of acetaldehyde in the blood when intoxicated than the high-consumers (Berger and Weiner, 1977; Eriksson, 1973;

Forsander and Eriksson, 1972; Koivula et al, 1975). The AA high-drinker rats showed greater mitochondrial ALDH and lower ADH activities than their ANA low-drinker counterparts (Koivula et al, 1975).

Li and Lumeng (1977), on the contrary, failed to find any differences in the alcohol or acetaldehyde metabolism in their animals, the P and NP rats, also selected on the basis of their ethanol consumption. Meanwhile, changes in liver ADH (Dippel and Ferguson, 1977; Eriksson and Pikkarainen, 1968; Greenberger et al, 1965; Hawkins et al, 1966; Khanna et al, 1967; McClearn et al, 1964; Taberner and Unwin, 1981; Unwin and Taberner, 1982) and ALDH enzyme activities (Amir, 1977, 1978b; Eriksson, 1980c; Horton, 1971; Horton and Barrett, 1976; Koivula and Lindros, 1975) have been reported to occur following a chronic exposure to alcohol but the lack of a systematic relation between the liver ADH activity and the subsequent alcohol consumption (Dippel and Ferguson, 1977; Taberner and Unwin, 1981; Unwin and Taberner, 1982) further indicates the minimal influence of ADH enzyme activity on alcohol consumption. On the other hand, a more consistent relation has been established between liver ALDH activity and alcohol consumption in rat strains that were not selected on the basis of an alcohol related behavior (Amir, 1977; 1978b; Eriksson, 1980c). In summary, it appears that the capacity of hepatic enzymes to metabolize acetaldehyde may be a principal

physiologic parameter in the regulation of alcohol consumption in laboratory animals just as it is in humans.

Pharmacological induction of blood acetaldehyde and ethanol consumption: animal research

One approach to the manipulation of the concentration of acetaldehyde in blood is the inhibition of ALDH, the enzyme responsible for the conversion of acetaldehyde into acetic acid. In 1966, Schlesinger, Kakihana and Bennett were the first to demonstrate that a rise in blood acetaldehyde levels, following the administration of tetraethylthiuram disulfide (ANTABUSE^{r}), reduced the ethanol intake of a strain of mice (C57) which had free access to alcohol. Another inhibitor of ALDH, N-butyraldoxime also attenuates the preference for ethanol in the same strain of mice (Koe and Tenen, 1970). When 4-bromopyrazole is taken on a chronic basis, it reduces the hepatic liver ALDH activity and subsequently decreases the alcohol consumption (Koe and Tenen, 1975). The citrated calcium carbimide (cyanamide) added to a solid diet (Astra Ewos) was shown to inhibit liver ALDH activity (Tottmar et al, 1978), thereby increasing the level of blood acetaldehyde and decreasing the alcohol consumption in the AA prefering strain (Lindros et al, 1975). This diet was found to have a specific inhibiting effect on the low K_m ALDH activity of the mitochondrial fraction of the liver cell, the enzyme involved in the oxidation of acetaldehyde (Lindros et al, 1972), and to attenuate the

consumption of a low-alcohol preference strain of rats, the Sprague-Dawley. The importance of this low K_m ALDH enzyme in the consumption of alcohol was underlined by the failure of the induction of high K_m ALDH activity to change the drinking pattern of rats (Marselos and Pietikainen, 1975; Marselos et al, 1975).

Amir and Stern (1978) demonstrated that both the lesion of the ventral medial forebrain bundle (VMFB) and the administration of cyanamide (TEMPOSIL^r) decreases the alcohol consumption of Wistar rats. Therefore, the inhibition of brain ALDH enzyme activity noted by these investigators following the destruction of neurons by the VMFB lesion, is as potent in reducing alcohol preference as cyanamide, whose action is aimed at the hepatic ALDH activity. Furthermore, Amir (1977, 1978b) found that brain ALDH activity was more highly correlated with alcohol consumption than liver ALDH activity. This last finding has important theoretical implications since it suggests that the aldehyde oxidizing capacity of the brain can regulate the consumption of alcohol. Evidence supporting this assumption is provided by the observation reported by Sinclair and Lindros (1981) that cyanamide had inhibited

brain as well as liver ALDH activity and caused considerable changes in alcohol consumption of laboratory animals.

Yet, the results from an investigation using the low-consumer ANA and the high-consumer AA rats failed to show any differences in brain ALDH enzyme activity in naive rats or any concomitant changes in alcohol drinking behavior, following exposure to alcohol, that may be relevant to the changes noted in brain ALDH activity (Inoue et al, 1981). Nonetheless given the fact that ALDH inhibitors constitute some of the most efficient and most reliable tools for suppressing alcohol consumption, the particular mechanisms responsible for this suppression are yet to be identified. Liver and brain ALDH, thus must be included among the several factors affecting alcohol consumption.

Central pharmacological effects of acetaldehyde

Recent reports from subjects undergoing calcium carbimide treatment suggest that these individuals experience an increase rather than a decrease in their desire to drink alcohol when they engage in moderate drinking (Brown et al, 1983; Peachey et al, 1980). This desire may very well be related to the rise in blood acetaldehyde, following the ingestion of alcohol, caused by the inhibitory action of calcium carbimide on hepatic ALDH activity. This finding agrees with the previously quoted studies that report higher acetaldehyde levels in the blood of alcoholics, following the ingestion of alcohol, than those in non-alcoholics. The

assumption is that such individuals are capable of overcoming the mild discomfort caused by the increased levels of acetaldehyde circulating in the bloodstream. Hence, acetaldehyde must act on the central nervous system (CNS) in a way similar to that of a reinforcer in order to favor the drinking behavior. When acetaldehyde is administered in the form of intraperitoneal injection in the laboratory rat, it induces an aversion to alcohol (Brown et al, 1978; Unwin and Taberner, 1982). Laboratory rats will learn on the other hand to self-administer acetaldehyde into the brain but will not learn the task when ethanol is supplied (Amit et al, 1977a, Brown et al, 1979).

The reinforcing properties of acetaldehyde have further been demonstrated when animals that had previously received this substance through self-administration showed a corresponding preference for alcohol (Brown et al, 1980). The apparent controversy over the effects of acetaldehyde on alcohol drinking behavior, that is, acetaldehyde serving at the same time as both reinforcing and aversive, is resolved by Amir and associates (1980) in the following way. These investigators conclude that the central, but not the peripheral, administration of acetaldehyde is reinforcing and argue that it is the metabolism of acetaldehyde rather than its accumulation in the brain that is critical for mediating acetaldehyde's pharmacological effect. Indeed, since acetaldehyde is a highly toxic compound (Akabane,

1970), its accumulation in tissue or in the blood stream would be a deterrent to the further ingestion of alcohol. Thus, subjects with a greater capacity to oxidize acetaldehyde in the brain may drink more alcohol in order to compensate for the relatively short duration of the reinforcement supplied by acetaldehyde.

Preference for and tolerance to ethanol: animal research

Issues and definitions

Tolerance is a phenomenon closely related to the amount and the frequency of alcohol ingestion and represents a change in the relation between the amount of alcohol consumed and the effects it produces. Various methods are used to measure these changes and consequently the definition of tolerance may be restricted to the types of changes measured as well as to the time during which these changes are observed. The purpose of the present section is to summarize the different terms that will be used in the present thesis to define tolerance (for a review, see Kalant et al, 1971).

The first distinction to be dealt with is the one that distinguishes initial tolerance from an acquired change in tolerance. Initial tolerance describes the effect produced by a given dose of alcohol following the first exposure to the drug. This tolerance reflects a sensitivity to alcohol that is determined by congenital, as well as, by experiencial factors which contribute to the wide range of differences noted in individuals, sexes, species, age groups, ethnic

populations and so on. In addition to initial tolerance, it is possible to measure acquired changes in tolerance within the same individual as a result of repeated exposure to alcohol, such that an increase in the amount of drug is required to produce the same degree of effect, or else a lesser effect may be produced by the same dose of drug. The changes from initial sensitivity to the drug over repeated exposure may be used as an index of tolerance development.

When such a measure of tolerance is used a further distinction between physiological and psychological (learned) tolerance can be made. This distinction becomes critical when the subjects are required, under the influence of alcohol, to perform a task, previously learned in a drug free state. This psychological, or behaviorally augmented tolerance (the term suggested by Kalant et al, 1971), reflects the subject's ability to relearn the task in relation to the new constellation of drug-related stimuli. Physiological tolerance on the other hand, is defined as a compensatory or homeostatic change in the neurons affected by the drug, which renders them less sensitive to alcohol.

Several authors (de Souza Moreira et al, 1981; Wenger et al, 1980; 1981) stress the predominant role of learning in the development of tolerance. Without denying the importance it has in the development of behavioral tolerance, other investigators maintain that learning facilitates the development of cellular or neural tolerance but by no means

constitutes a separate tolerance process. In fact these authors consider that the difference between behavioral and physiological tolerance resides in the differential rates of acquisition (LeBlanc and Cappell, 1977; LeBlanc et al, 1973; 1976).

The measurement of tolerance may provide little or no information concerning its etiology. To differentiate dispositional tolerance from functional tolerance, it is necessary to relate the effects produced by alcohol to the concentration of the drug, or its metabolite, in the blood or brain. The first class of mechanisms underlying tolerance, designated dispositional tolerance,includes changes in drug absorption, distribution, excretion and metabolism which might lead to a reduction in the intensity and duration of contact between alcohol and the tissue on which it exerts its action (e.g. an increased rate of metabolism). The second type is designated as functional tolerance and describes changes in the properties and functions of the target tissue which render it less sensitive to the same degree of exposure to the drug. The investigation of the phenomenon of tolerance can better be understood when the nature of the relation between the behavioral effects and the associated pharmacological or physiological changes is assessed.

Initial tolerance to hypnotic and subhypnotic doses of ethanol

The relation between alcohol preference and initial tolerance to the soporific effects of ethanol among rodents, selected on the basis of their preference for or their initial sensitivity to ethanol, is well documented (Ahtee et al, 1980; Fuller and Church, 1977; Rusi and Eriksson, 1976; Rusi et al, 1977). A similar relation has been established in inbred mouse strains characterized by their specific degree of preference for alcohol (Belknap et al, 1972; Damjanovich and MacInnes, 1973; Kakihana et al, 1966; Lin, 1975; Randall and Lester, 1974). Agreement with the results reported for alcohol anaesthesia has been obtained with studies comparing the initial sensitivity to moderate doses of ethanol in different inbred strains of mouse (Elston et al, 1982; Damjanovitch and MacInnes, 1973; MacInnes and Uphouse, 1973; Riley et al, 1977); the low alcohol consumers which showed greater sensitivity to the soporific effects of alcohol were also more intoxicated when under the influence of a moderate dose of alcohol.

Recent investigations have produced data which cast doubt on the existence of a similar relation between alcohol preference and responses to the administration of a subhypnotic dose of ethanol in rodents selectively bred on the basis of an alcohol related behavior. Indeed, while Li and coworkers (1979) reported a greater sensitivity to the

soporific effects of ethanol in rats of the high-consumer (P) strain, Lumeng and associates (1982) found on the contrary that the performance of these animals on a jumping test was less disturbed by a moderate dose of alcohol than the performance of the low-consumer (NP) strain. Furthermore, a task-dependent difference has been observed in the LA and MA rats; the alcohol impairment of the swimming performance was greater in the LA animals (Bass and Lester, 1980), while they were shown to be less sensitive than their MA counterparts to the acute hypnotic and motor impairing effects of ethanol (Mayer et al, 1982).

Evidence supporting the hypothesis that different physiological mechanisms are reponsible for the locomotor stimulant effects of a low dose of ethanol and for the central nervous system (CNS) depression associated with high doses has been provided (Frye and Breese, 1981; Randall et al, 1975). Thus it appears that the locomotor stimulant effect of ethanol is not as predominant as its depressant action which is consistently observed in both rats and mice. A wide range of sensitivity to the stimulant effect of ethanol has been noted among rodents and mouse strains that show different degrees of preference for alcohol and these findings suggest that the mechanism responsible for these variations may be under genetic control. Hence, different conclusions may be drawn on the nature of initial tolerance to alcohol, when tolerance testing uses subhypnotic instead

of hypnotic doses of ethanol and when different motor tasks are employed. Inferences about the relation between preference for and initial tolerance to ethanol should be made cautiously.

Behavioral tolerance to ethanol

The relation between sensitivity to the initial versus repeated exposures of ethanol in strains of rodents that show different initial preference for alcohol is of great interest. Nikander and Pekkanen (1977) demonstrated that the initial sensitivity difference noted between the AA (high-consumer) and ANA (low-consumer) rats to a moderate dose of ethanol was sustained throughout a tolerance-producing treatment regimen. Meanwhile, Tampier and associates (1981) reported tolerance to the soporific effects of alcohol, following chronic ethanol treatment, in their low-consumer UChA rats while their UChB counterparts failed to demonstrate tolerance under the same conditions. Yet, when latency of sleep onset was considered, UChB rats showed increased tolerance to the soporific effects of alcohol but to a lesser degree than their UChA counterparts. Finally, no differences in initial sensitivity and tolerance acquisition were noted between the UChA and the UChB line when these animals were on a voluntary intake regimen despite the considerable differences noted in alcohol consumption.

Bass and Lester (1980) have also examined the relation between the initial and the subsequent development of

tolerance in two lines of rats selected for their different CNS sensitivities to the motor-impairing effects of ethanol (Riley et al, 1976). With the use of a design that permitted the evaluation of the rate of tolerance acquisition these authors found no difference in the development of tolerance among the MA (most affected) and LA rats (least affected) when they were tested for ethanol impairment of water escape behavior. As suggested by the findings of Nikander and Pekkanen (1977), these authors observed that the difference in initial sensitivity persisted among MA and LA animals despite chronic alcohol treatment. Thus it appears that the development of tolerance is a process that can be studied independently of the initial tolerance, and behavioral tolerance can be understood as an adaptation process which may influence subsequent alcohol consumption. A more suitable explanation of the preference for alcohol observed in different strains of rodents may be found in the study of the development of behavioral rather than physiological tolerance.

The present investigation

The evidence presented in this section emphasizes the predominant role of acetaldehyde metabolism in alcohol drinking behavior in humans as well as in animals. The intimate relation that exists between alcohol drinking and tolerance renders it difficult to establish the connection between the two phenomena. Several investigations stemming

from animal research indicate the existence of a link between initial preference and initial tolerance to ethanol. Tolerance, whether physiological or behavioral, expresses an adaptation to alcohol that may lead to changes in alcohol related behaviors. The study of the physiological and behavioral changes that occur during tolerance acquisition as a function of initial preference and subsequent alcohol consumption, is of interest to the etiology of alcoholism.

The present research explored some aspects of the relation between alcohol drinking and tolerance. Male and female rats of the S_1 and S_3 Tryon strains, originally bred to accentuate differences in their capacity to solve maze problems (Tryon, 1940), were used throughout the entire investigation. These animals have been shown to differ in their manifest preference for ethanol (Russell and Stern, 1973) and demonstrated genetic variations in their aldehyde oxidizing capacity that paralleled their differential drinking patterns (Amir, 1978a).

The first study in the present research examined the relation between the ethanol preference manifested by these two strains of rats and initial tolerance to the soporific effects of ethanol.

To clarify the importance of the dose dependent variable a second experiment was designed to evaluate initial tolerance to a subhypnotic dose of ethanol; the dose chosen caused a moderate impairment in the performance of a

previously learned task. The development of tolerance to this subhypnotic dose of ethanol was further investigated in order to evaluate its relation to initial tolerance. Treatment schedules characterized by varying exposure to alcohol and a technique known to accelerate the establishment of behavioral tolerance (Kalant, et al, 1971; LeBlanc et al, 1973, 1976) were used to investigate the development of tolerance. The influence of these treatment schedules on subsequent alcohol consumption was assessed by comparing the ethanol intake before and after applying the treatment schedules. Since chronic alcohol treatment has been shown to affect both ADH and ALDH enzyme activities, these activities were evaluated in S_1 and S_3 subjects assigned to the different schedules.

The goals of this research were to evaluate the relations of initial and/or acquired behavioral tolerance to alcohol preference and to delineate the importance of alcohol metabolizing enzymes in alcohol intake.

EXPERIMENT 1

Introduction

Of particular interest to the study of alcohol dependence is the relation between preference for and tolerance to ethanol. Research summarized in the introduction indicates that animals that exhibit high voluntary intake of ethanol are usually less sensitive than low intake animals to the soporific effects of ethanol. Several investigators have also reported a greater resistance to the anaesthetic effects of ethanol in animals that show a more efficient aldehyde oxidizing capacity (Belknap et al, 1972; Damjanovich and MacInnes, 1973; Kakihana et al, 1966; Lin, 1975; Randall and Lester, 1974; Rusi and Eriksson, 1976; Rusi et al, 1977). Also, it has been demonstrated recently that genetic variations in liver and brain aldehyde oxidizing capacity parallel the alcohol drinking patterns of the S_1 (high-preference) and S_3 (low-preference) rats of the Tryon strain. Amir (1978a) found that the S_3 animals displayed a less efficient aldehyde oxidizing capacity than their S_1 counterparts. The aim of the present study was to explore the relation between ethanol preference and initial tolerance to the soporific effects of ethanol in the high (S_1) and low-preference (S_3) lines of the Tryon strains of rats.

Since tolerance may be related to an increased rate of metabolism (Damjanovich and MacInnes, 1973) or to decreased sensitivity of the central nervous system to the effects of alcohol (Bass and Lester, 1980; Kakihana et al, 1966; Li et al, 1979; Lin, 1975; Schneider et al, 1973; Wood and Laverty, 1979) or to a combination of both factors (Elston et al, 1982; Tampier et al, 1981) both the metabolic and neural tolerance were assessed in this study.

Materials and Method

Ethanol Preference Testing

In 1973, Russell and Stern observed strain and sex differences in manifest preference for ethanol among the S_1 and S_3 rats of the Tryon strain. Since the present research aimed at an examination of the relation between preference for and initial tolerance to the soporific effects of ethanol among the same strains, a replication of Russell and Stern's data on differential preference for ethanol was carried out. Forty-eight male and female rats of the S_1 and S_3 lines of the Tryon strains, approximately 90 days old, were placed in individual cages. For the first week, the animals were provided with Purina Laboratory chow and water. The water was delivered by means of two calibrated Richter tubes.

At the start of the second week, one of the Richter tubes was filled with a 4% solution (v/v) of a 95% concentration of ethanol. On the next day and each day thereafter, the animals were weighed and the amount of fluid

consumed from each bottle (ethanol and water) during the previous 24 hours was recorded. Whenever the animal drank from the Richter tube containing the ethanol solution, the concentration of ethanol was increased by 2%; if the animal refused to drink the ethanol solution, the same concentration was maintained. After the animal had rejected a concentration more than twice, the experiment was terminated and the last concentration accepted by the rat was recorded as the Final Acceptance concentration (FAC). This concentration was used as an indication of the preference for ethanol.

In addition, the voluntary intake of a standard ethanol solution was determined using 20 male and female rats of the same strain. The animals were initially given a free choice between 5% ethanol solution (v/v prepared with 95% ethanol) and water for 10 days (phase I). Following this phase, they were then offered 10% ethanol solution and water for 40 days (phase II). In this study, as for the one previously described, the position of the tubes containing the ethanol solutions was alternated daily from side to side to compensate for the development of position preference. Profiles of ethanol intake were determined for each group from the means of ethanol consumption (for a period of 5 days) of individual animals during the 50 days of the alcohol regimen. Ethanol intake was expressed as ml of absolute ethanol consumed per kg body weight per day.

Initial Tolerance Testing

Male and female rats of the S_1 and S_3 strains were used in the experiment (106 rats). They were approximately 100 days old; females weighed an average of 156 g and males, 237 g. The animals were housed in individual cages on a 12 hour light - dark cycle (light 9 a.m. - 9 p.m.) in a temperature controlled room (22±1°C) and provided with Purina laboratory chow and water ad lib. The night before the experiment, they were food deprived for periods of 12-18 hours.

On the morning of the experiment, the rats were weighed at 7:00 a.m. They were then given by gavage 7.2 g of ethanol per kg, in the form of a 30% solution (v/v, prepared fresh from a 95% concentration). Immediately after the administration of ethanol, the animals were repeatedly placed on their backs in a v-shaped cradle. The rats were assigned to two experimental groups. In the first group, the latency of sleep onset was measured as the interval between the administration of the ethanol solution and the rats' behavioral inability to regain the upright position twice within 30 seconds. In the second group, the duration of sleep was measured as the interval between the moment the rat lost its initial ability to regain an upright position and that when it succeeded in recovering an upright position twice within 30 seconds. As soon as the animal fell asleep (group 1) or awakened from its sleep (group 2), a blood sample was drawn from the tail and the animal was sacrificed

(decapitation); a cerebral blood sample was taken from the neck, and the brain was quickly removed from the skull.

Ethanol and Acetaldehyde Metabolism

A total of 71 male and female rats of the S_1 and S_3 strains (approximately 100 days old) were assigned to 3 different groups for ethanol and acetaldehyde level assessment 2, 4, and 6 hours respectively, after the administration of ethanol (by stomach gavage). The rats weighed an average of 160 g (females) and 238 g (males). They were housed and treated similarly to the animals assigned to the tolerance testing treatment.

On the day of the experiment, the animals were weighed at 7:00 a.m. and, between 7:30 and 13:00 hour, each rat was given an oral dose of 7.2 g of ethanol per kg in the form of a 30% solution (v/v freshly prepared from a 95% concentration of ethanol). The animals were then returned to their home cages for the duration of the time until sampling.

Brain Sampling

The animals were sacrificed (decapitated) without anaesthesia. The brains were quickly removed from the skull, cut in half, weighed (brain weight ranged from 0.6 g to 1 g) and homogenized (Brinkman:polytron) in 4 ml of ice cold 0.6 M perchloric acid containing 25mM thiourea (Eriksson and Sippel, 1977). The precipitates were centrifuged at 4,000x g for 15 minutes at 4°C; 500 µl of the clear supernatant was

then pipetted into 10 ml vials, sealed and kept at 4°C for subsequent chromatographic analysis.

Cerebral and Peripheral Blood Sampling

Prior to decapitation, a 100 µl sample of blood had been taken from the tip of the animal's tail. Immediately after removal of the brain a sample of 100 µl of blood was taken from the arterial blood accumulating around the higher cervical vertebrae of the spine and diluted with 0.9 ml of 0.6 M ice cold perchloric acid containing 10 mM thiourea (Eriksson, Sippel and Forsander, 1977). The samples were centrifuged at 4,000 xg for 15 minutes at 4°C and a 500 µl sample of the clear supernatant was placed in 10 ml vials which were immediately sealed. The blood samples were kept at 4° C pending the chromatographic analysis.

Analytical Techniques

For the initial tolerance study, ethanol and acetaldehyde levels were measured with a Hewlett Packard, series 5750, gas chromatograph using a 6 foot stainless steel, 2 mm i.d. column packed with 80/100 mesh Porapak Q/R 50/50 (Chromatographic Specialties Ltd, Brockville, Ontario). The oven was maintained at 140 ° C, port 160 ° C and flame ionization detector 250 °C. The flow of nitrogen was 35 cc/min, hydrogen 30 cc/min and air 240 cc/min. Under these conditions the retention time recorded for acetaldehyde was 4.4 minutes and 8.0 minutes for ethanol.

For the assessment of ethanol and acetaldehyde metabolism, concentrations were measured with a Varian, series 1440 gas chromatograph using a 6 foot glass, 2.0 mm i.d., 80/100 mesh chromosorb 101 column (Chromatographic Specialties Ltd, Brockville, ontario). The oven was maintained at 150°C , port 150°C and flame 200°C. The flow of nitrogen was 35 cc/min, hydrogen 30 cc/min and air 240 cc/min. Under these conditions the retention time recorded for acetaldehyde was 1.9 minutes and 3.2 minutes for ethanol. The samples were incubated in a water bath for 15 minutes at 65°C. One ml of the gas filling the headspace of the flasks was injected into the chromatograph (Eriksson and Sippel, 1977). The amount of ethanol and acetaldehyde was estimated from the percentage of the area covered in reference to N-propanol (BDH laboratory chemicals division, Toronto) as the internal standard. Standards were prepared by adding known amounts of ethanol and acetaldehyde (Baker Chemicals Co., Phillipsburg, New Jersey) to plasma and brain supernatant. Standards were prepared daily , stock solutions of acetaldehyde and ethanol every 15 days, and the solution of perchloric acid and thiourea every month. Ethanol and acetaldehyde were redistilled prior to the stock preparation and thiourea (Fisher Scientific Co., Fair Lawn, New Jersey) was dried at 90°C for 12 hours before preparing the perchloric acid solution (70%, Fisher Scientific Co., Fair Lawn, New Jersey). The use of thiourea is not however

sufficient to prevent the spontaneous formation of acetaldehyde in the blood of rats (Eriksson et al, 1977); accordingly the correction suggested by Eriksson (1980b) was applied for the assessment of acetaldehyde.

Statistical Analysis

Analysis of variance was used to compare data for significant differences between groups. A trend analysis was used to compare the voluntary ethanol intake in male and female rats of the S_1 and S_3 strains during the establishment of preference and to examine the variation of ethanol and acetaldehyde concentrations with time. Pearson's correlation served to determine the relation between estimates of blood and brain with sleep onset and duration. Wherever relevant, group means were compared by using the Scheffe method of multiple comparisons or the Tukey honestly significant difference method (HSD)(Ferguson, 1980).

Results

Ethanol Preference

The final acceptance concentration recorded for male and female rats of the S_1 and S_3 strains are presented in figure 1. S_1 animals drank ethanol solutions at significantly higher concentrations than the S_3 animals ($F_{1,44} = 9.29$, $p<.005$). Females of both strains also drank solutions that contained higher concentrations of ethanol than their male counterparts ($F_{1,44} = 4.27$, $p<.05$) but no sex x strain interaction was noted ($F_{1,44} = 2.68$, $p>.05$). Figure 2 shows the profiles of

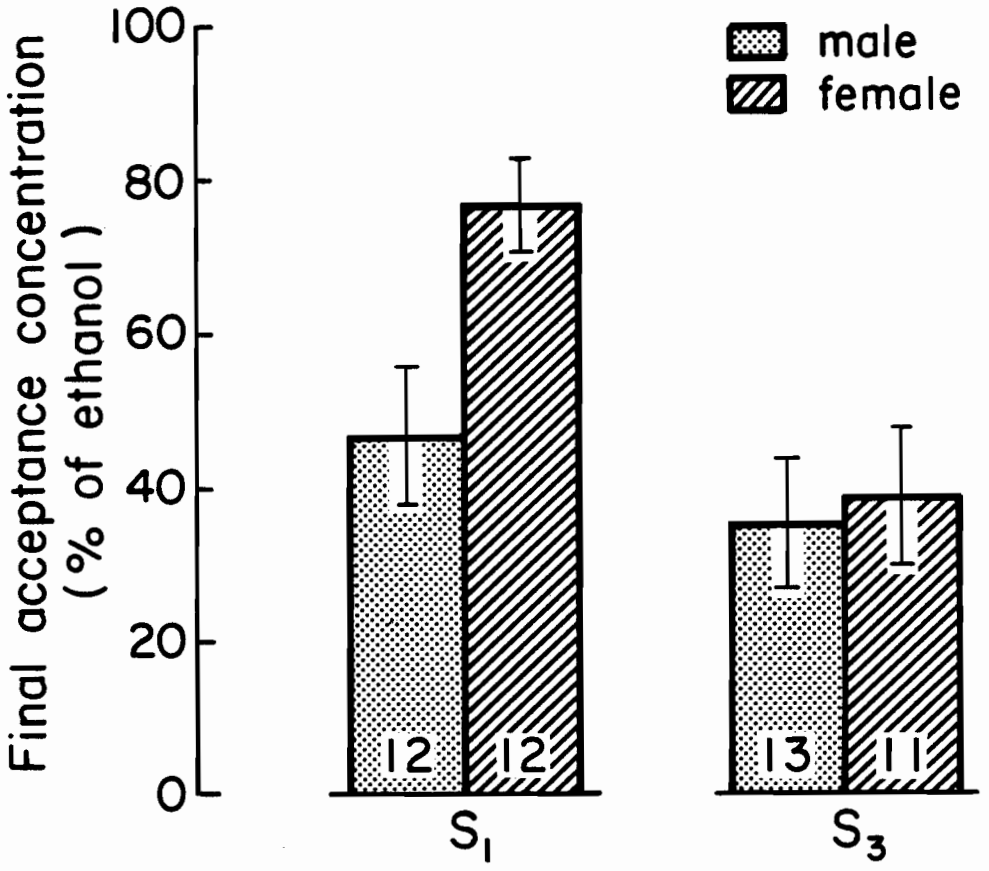

Figure 1. The final acceptance concentration (FAC) of male and female rats of the S_1 and S_3 Tryon strain. The bars and vertical lines represent group means ± SEM. The numbers inside the bars indicate the number of animals in each group.

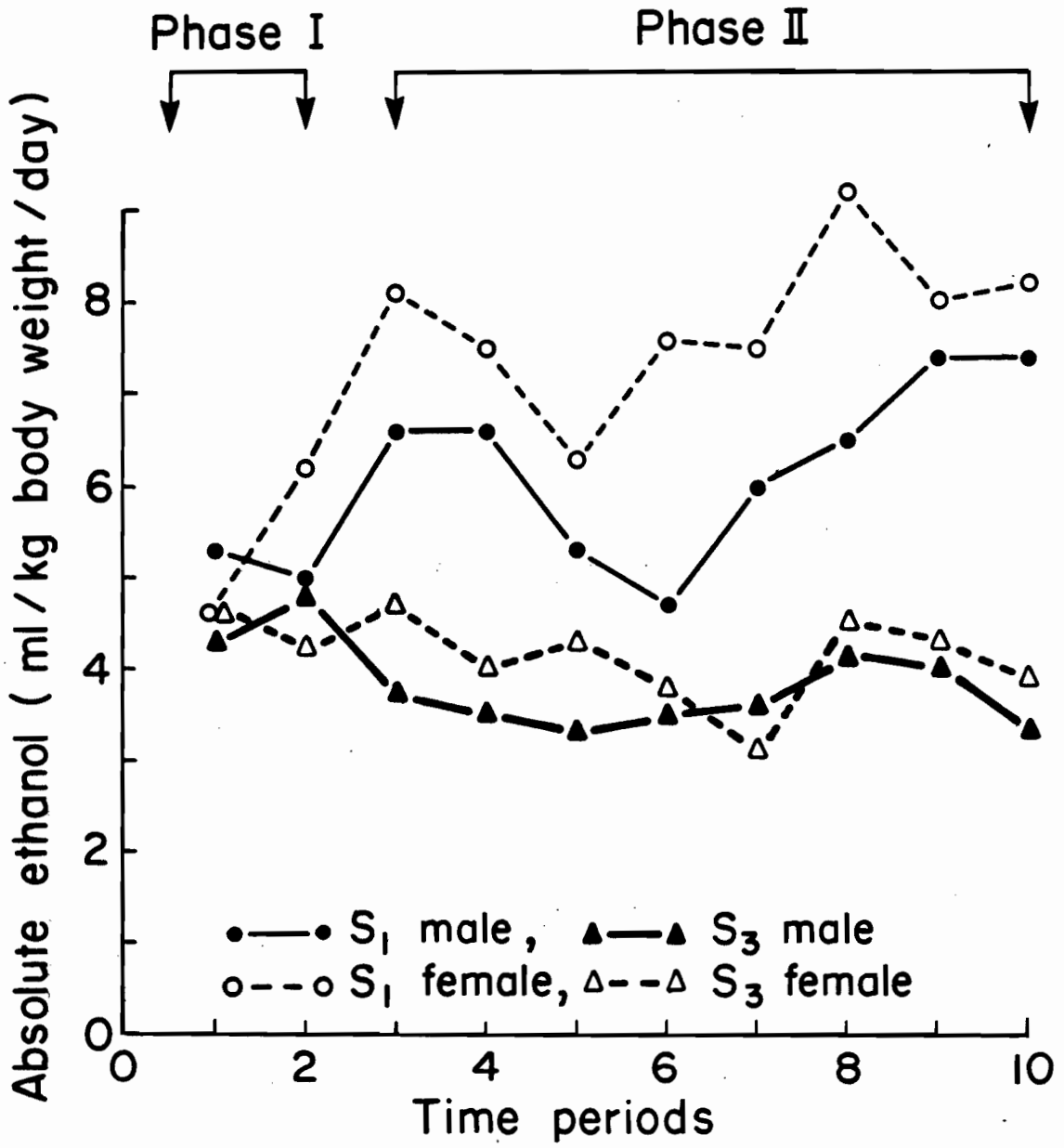

Figure 2. The profiles of ethanol intake determined for male and female rats of the S_1 and S_3 strains during the phase 1 (10 days of a 5% solution) and the phase II (40 days of a 10% solution) of the ethanol regimen. The means of ethanol intake were obtained from the means of ethanol consumption (for a period of 5 days) of individual animals.

the mean ethanol intake determined for male and female rats of the S_1 and S_3 strains used in the second study evaluating the preference for ethanol. The mean ethanol intake per day varied between the strains ($F_{1,16} = 7.79$, $p<.01$) and during the ethanol regimen schedule ($F_{9,144} = 2.62$, $p<.05$). A significant strain x time periods interaction was noted ($F_{9,144} = 3.56$, $p<.01$). Hence during the second phase of the ethanol intake regimen, when the 10% solution was offered, S_1 animals consumed significantly greater amounts of ethanol than their S_3 counterparts($p<.05$, Scheffe). During this time, a significant effect of time appeared where the S_1 animals continued to increase their drinking whereas S_3 animals maintained their consumption at a lower level ($F_{1,16} = 6.14$, $p<.05$). Finally, there was no significant main effect of sex ($F_{1,16} = 0.21$, $p>.05$) and no sex x time periods ($F_{9,144} = 0.82$, $p>.05$) or sex x strain x time periods ($F_{9,144} = 0.88$, $p>.05$) interactions noted.

Initial Tolerance

Latency of Sleep Onset

The latency of sleep onset, the ethanol and acetaldehyde concentrations recorded in the blood (peripheral and cerebral) and ethanol levels in brain tissue are reported in table 1. Female animals fell asleep significantly sooner than their male counterparts ($F_{1,54} = 6.52$, $p<.05$). At the onset of sleep, ethanol concentrations were greater for females than for males, in both cerebral blood ($F_{1,36} = 4.43$, $p<.05$)

Table 1.

Latency of sleep onset, ethanol and acetaldehyde concentrations in the blood stream (peripheral and cerebral) and ethanol levels in brain tissue for males and females of the S_1 and S_3 strains at the time of sleep onset.

		S_1		S_3	
		male	female	male	female
Sleep onset (Means ± SEM)	+	23.75 ± 3.83	14.47 ± 2.18 *	24.50 ± 4.87	16.00 ± 1.99 *
ethanol (Means ± SEM)	+ +				
peripheral		2.02 ± 0.22	2.53 ± 0.37	1.88 ± 0.40	1.60 ± 0.26
cerebral		3.16 ± 0.33	4.57 ± 0.49 *	3.43 ± 0.30	3.50 ± 0.21 *
brain		3.33 ± 0.27	4.00 ± 0.29 *	3.04 ± 0.24	3.95 ± 0.27 *
acetaldehyde (Means ± SEM)	+ +				
peripheral		2.40 ± 0.65 **	3.73 ± 0.86 **	5.98 ± 1.50	8.31 ± 1.64

+ The latency of sleep onset (minutes) was measured as the interval between the moment the rat was given the ethanol solution and that when it could not regain the upright position within 30 seconds.
+ Ethanol is expressed in mg/ml of blood or g wet wt brain. Acetaldehyde is given as ug/ml of
+ blood.

* indicates significant difference from males
** indicates significant difference from S_3 strain

and in brain tissue ($F_{1,38}$ = 13.60, $p<.001$). However, no significant sex ($F_{1,36}$ = 0.03, $p>.05$) or strain ($F_{1,36}$ = 3.16, $p>.05$) differences were noted in the peripheral blood ethanol concentrations. The S_1 animals fell asleep at a lower acetaldehyde concentration in the peripheral blood than the S_3 animals ($F_{1,26}$ = 9.84, $p<.005$). Finally, the latency of sleep correlated significantly with the weight of the animals (r =+0.3153, N=58, $p<.01$).

Duration of Sleep

The animals of the S_1 strain slept longer than those of the S_3 strain ($F_{1,43}$ = 10.88, $p<.005$). On awakening the amount of ethanol circulating in the blood stream ($F_{1,34}$ = 0.30 and $F_{1,34}$ = 0.11, $p>.05$ for peripheral and cerebral blood respectively) and in the brain ($F_{1,36}$ = 0.37, $p>.05$) did not differ for the two strains of animals. In addition, no differences were found between the S_1 and S_3 animals in the acetaldehyde concentrations circulating in the peripheral blood at the moment of recovery from sleep ($F_{1,26}$ = 0.61, $p>.05$). Although no significant sex differences were observed for sleep times, female animals did show higher brain ethanol concentrations than males upon awakening ($F_{1,36}$ = 5.83, $p<.05$) (see table 2).

The duration of sleep did not correlate with the ethanol or acetaldehyde concentrations in the blood stream or in the brain tissue. When ethanol and acetaldehyde concentrations were compared at the time of loss and recovery of the

Table 2.

Duration of sleep, ethanol and acetaldehyde concentrations in the blood stream (peripheral and cerebral) and ethanol levels in brain tissue for males and females of the S_1 and S_3 strains upon awakening.

	S_1		S_3	
	male	female	male	female
Duration of sleep+ (Means ± SEM)	660.40 ± 40.68 **	760.69 ± 37.00 **	586.58 ± 41.80	561.92 ± 44.21
ethanol + (Means ± SEM) +				
peripheral	2.57 ± 0.27	2.65 ± 0.34	2.80 ± 0.32	2.79 ± 0.35
cerebral	2.78 ± 0.20	3.09 ± 0.29	2.90 ± 0.23	3.13 ± 0.22
brain	2.44 ± 0.34	3.12 ± 0.35 *	2.19 ± 0.27	2.99 ± 0.26 *
acetaldehyde + (Means ± SEM) +				
peripheral	7.36 ± 2.50	6.65 ± 0.89	8.80 ± 1.60	8.14 ± 2.07

+ The duration of sleep (minutes) was measured as the interval between the moment the rat lost its initial ability to regain an upright position and that when it succeeded in recovering an upright position twice within 30 seconds.
+ Ethanol is expressed in mg/ml of blood or g wet wt brain. Acetaldehyde is given as ug/ml of
+ blood.

* indicates significant difference from males
** indicates significant difference from S_3 strain

righting reflex, no significant differences were found in the concentrations of acetaldehyde ($t(59) = 0.289$, $p>.05$) and ethanol ($t(76) = 0.437$ and $t(78) = 0.439$, $p>.05$ for peripheral and cerebral blood respectively) in the blood stream, nor in concentrations of ethanol in the brain tissue ($t(82) = 0.630$, $p>.05$).

Ethanol and Acetaldehyde Metabolism

The concentrations of ethanol in the brain tissue, and the cerebral and peripheral blood stream after an oral administration of 7.2 g of ethanol per kg did not differ significantly among the male and female rats of the S_1 and S_3 strains (see table 3). The values for both sexes and strains were therefore combined and compared over time periods. Ethanol concentration significantly declined with the passage of time in the brain tissue ($F_{2,62} = 7.14$, $p<.01$) as well as the cerebral ($F_{2,62} = 7.14$, $p<.01$) and peripheral blood stream ($F_{2,64} = 5.90$, $p<.01$). As observed for ethanol, the acetaldehyde concentration in the peripheral blood stream was not significantly different among males and females of the S_1 and S_3 strains (see table 4). Thus, the values were combined across sexes and strains and underwent an analysis comparing times. The amount of acetaldehyde found in the blood remained at a constant level during the times of sampling ($F_{2,63} = 2.70$, $p>.05$).

Table 3.

Ethanol concentrations in blood (peripheral and cerebral) and brain tissue for male and female rats of the S_1 and S_3 strains

Strain	Sex	Time	N	Ethanol (Means ± SEM)* Peripheral	Cerebral	Brain
S_1	Male	2	(6)	2.774± 0.510	3.187± 0.464	2.465± 0.577
		4	(6)	2.488± 0.246	2.341± 0.263	1.901± 0.266
		6	(7)	1.942± 0.286	2.236± 0.282	2.023± 0.435
	Female	2	(5)	3.114± 0.221	3.264± 0.167	2.429± 0.378
		4	(7)	2.341± 0.323	2.402± 0.244	1.955± 0.200
		6	(5)	2.207± 0.356	1.979± 0.357	2.341± 0.122
S_3	Male	2	(4)	2.673± 0.377	2.732± 0.520	2.753± 0.387
		4	(7)	2.751± 0.270	3.057± 0.277	2.400± 0.230
		6	(6)	1.803± 0.188	2.039± 0.186	2.088± 0.190
	Female	2	(4)	2.969± 0.209	3.920± 0.464	3.028± 0.658
		4	(5)	2.148± 0.268	2.286± 0.160	1.791± 0.298
		6	(6)	2.456± 0.511	2.706± 0.643	1.908± 0.132

* Ethanol is expressed in mg/ml of blood or mg/g of wet wt brain. The difference between group means are not statistically significant (analysis of variance).

Table 4.

Acetaldehyde concentrations in peripheral blood for male and female rats of the S_1 and S_3 strains

Strain	Sex	Time	N	Acetaldehyde (Means ± SEM)* Peripheral
S_1	Male	2	(6)	3.984 ± 1.504
		4	(6)	3.792 ± 1.083
		6	(7)	4.933 ± 2.099
	Female	2	(5)	3.723 ± 0.993
		4	(7)	4.186 ± 1.037
		6	(5)	7.463 ± 0.414
S_3	Male	2	(7)	4.975 ± 0.576
		4	(7)	4.429 ± 1.059
		6	(6)	6.153 ± 1.711
	Female	2	(4)	2.201 ± 0.517
		4	(5)	4.406 ± 0.699
		6	(6)	5.263 ± 1.995

* Acetaldehyde is expressed in ug/ml of blood. The difference between group means are not statistically significant (analysis of variance).

Discussion

Results from the ethanol preference tests confirmed the earlier observations of Russell and Stern (1973) that a greater preference for ethanol was exhibited by male and female rats of the S_1 strain and the lower preference by the S_3 animals. The higher ethanol preference of the S_1 animals was further noted during the voluntary intake regimen; the mean alcohol intake per day recorded from these animals clearly distinguished this group's preference from the low alcohol intake obtained from animals of the S_3 strain.

The latency of sleep onset was found to be similar among animals of the high (S_1) and the low-preference (S_3) strains. However, females of both strains fell asleep sooner than their male counterparts. The correlation noted between the latency of sleep onset with the weight of the animals suggests that the rapid intoxication of females as compared with males may be attributed to differences in the absorption rates of alcohol in body fluids and organs (Wallgren, 1970). Indeed, similar blood ethanol levels were noted among male and female subjects at the moment of sleep onset while ethanol levels in cerebral blood and brain tissue were found to be higher in females. This finding suggests that ethanol passed into the brain more rapidly in the female rats. Lower blood acetaldehyde levels were measured in male and female rats of the S_1 strain at the time of sleep onset. This finding agrees with a previous report of a more efficient

aldehyde oxidizing capacity in the high-preference (S_1) strain of animals (Amir, 1978a). However, when the animals recovered from the soporific effects of ethanol the differences noted in blood acetaldehyde vanished.

The animals of the high-preference strain (S_1) showed a low tolerance to the soporific effects of ethanol. This finding is not consistent with previous reports of a greater tolerance in high preference strains of rats and mice (Lin, 1975; Malila, 1978; Mayer et al, 1982; Randall and Lester, 1974; Rusi and Eriksson, 1976). Yet, the present results are in accord with those of Li and coworkers (1979) who reported a greater sensitivity to the soporific effects of ethanol in rats of a high consumer (P) strain. On the other hand, Tampier and Associates (1981) failed to report differences in the sleep onset and duration of their high (UchB) and low (UchA) consumer strains following the administration of a hypnotic dose of ethanol.

The inconsistent results arising from studies relating alcohol preference and initial tolerance to the soporific effects of ethanol in strains of rats raised by selective breeding methods caution us against drawing premature conclusions on the nature of the relation between preference for and tolerance to ethanol. Indeed, conflicting results on initial tolerance were obtained with the P (high-consumer) and NP (low-consumer) strains when these animals were challenged with a subhypnotic (Lumeng et al, 1982) as

compared to a hypnotic dose of ethanol (Li et al, 1979). Furthermore the measure of tolerance not only appears to be dependent on the dose of ethanol but also to the task used for its evaluation. Indeed Bass and Lester (1980), using animals from the LA (least affected) and MA (most affected) strains, found that the LA animals were more impaired in a swimming performance task when challenged with a moderate dose of ethanol whereas Mayer and associates (1982) found the LA rats to be less impaired on a different motor task. While the choice of a swimming performance as an appropriate measure for the evaluation of tolerance may be questioned, nonetheless it is reasonable to assume that the evaluation of tolerance may be dependent upon the measure and/or the drug dose used. Consequently inferences about the relation of alcohol preference and initial tolerance should be drawn cautiously.

As mentioned in the introduction to this study, tolerance to ethanol may result from an increased rate of metabolism or from a decreased sensitivity to the effects of ethanol on the CNS or a combination of both factors. No differences in the quantity of ethanol and acetaldehyde in the blood stream and brain ethanol concentration were found in male and female rats of the S_1 and S_3 strains when samples were taken at various times following the administration of ethanol.

However, it was noted that the S_1 animals slept longer than their S_3 counterparts and woke up with blood and brain ethanol levels not significantly different from the S_3 animals. This finding would suggest a slower alcohol elimination for the S_1 animals. Alternatively, while no sex difference was noted in the sleep duration, females of both S_1 and S_3 strains displayed greater ethanol concentrations than their male counterparts in brain tissue at the times of loss as well as recovery of the righting reflex. These results support the assumption of different central nervous system sensitivities to the anaesthetic effects of ethanol in male and female subjects. The possibility still remains however, that a metabolic tolerance may underlie the strain difference in resistance to the soporific effects of ethanol noted in this study; whereas a neural tolerance may underlie the sex difference observed.

To summarize, the loss and recovery of the righting reflex were used as indicators of initial tolerance to the soporific effects of ethanol in male and female rats of the high-preference (S_1) and low-preference (S_3) strains. A sex difference was noted on the measure of the latency of sleep onset; a finding that could be related to differences in rates of absorption of ethanol in body fluids and organs. A strain difference was observed when sleep duration was used

as an index of initial tolerance. The animals of the low-preference (S_3) strain were less sensitive to the soporific effects of ethanol than their S_1 counterparts. The evidence of a greater metabolic tolerance in S_3 animals and a greater neural tolerance in females of both strains could not be provided satisfactorily, but is not inconsistent with the present findings. Given that tolerance appears to be related to the drug dose as well as to the behavioral impairment measured, conclusions about the relation between alcohol preference and initial tolerance in this study would be premature. Hence it seemed pertinent to investigate further this aspect of the relation using a subhypnotic rather than a hypnotic dose of ethanol.

EXPERIMENT 2

Introduction

The previous study of the present research did not confirm the expected relation between ethanol preference and initial tolerance to the soporific effects of alcohol in male and female rats of the S_1 and S_3 Tryon strain. The strain that showed preference for ethanol (S_1) was less tolerant to the hypnotic effects of alcohol than the strain with the lower preference (S_3). Similar discrepancies between alcohol preference and initial tolerance to the soporific effects of ethanol have been reported in rodents that were selected on the basis of their preference for ethanol (Li et al, 1979; Tampier et al, 1981). Moreover, conflicting results have appeared when initial tolerance was evaluated in the same strain of rodents using a hypnotic (Li et al, 1979)as compared to a subhypnotic dose of ethanol (Lumeng et al, 1982). While Li and coworkers reported a greater sensitivity to the soporific effects of ethanol in rats of a high-consumer (P) strain, Lumeng and associates found that these animals were less intoxicated by a subhypnotic dose of ethanol than the low-consumer (NP) strain. These findings raise the possibility that the anaesthetic effect of ethanol is determined by several factors unrelated to the specificity of ethanol's pharmacological action (i.e. disturbance in the

rate of absorption, distribution and metabolism). Hence it seemed pertinent to reexamine the relation between alcohol preference and initial tolerance in animals of the S_1 and S_3 strains using a moderate dose of ethanol.

Since alcohol consumption and tolerance are closely related phenomena, the investigation of the development of tolerance as a function of initial preference and the subsequent alcohol intake is also of interest. A few studies have attempted to investigate the development of tolerance in strains of rats that show differential preference for ethanol (Nikander and Pekkanen, 1977; Tampier et al, 1981) yet even fewer investigators have attempted to relate the development of tolerance to subsequent alcohol consumption (Taberner and Unwin, 1981; Unwin and Taberner, 1982). However, in the latter studies the development of tolerance was not evaluated but assumed to be acquired following a chronic ethanol treatment. Thus the present study was aimed firstly at an evaluation of the relation between alcohol preference and initial tolerance to a subhypnotic dose of ethanol in the S_1 (high-preference) and S_3 (low-preference) rats of the Tryon strain and secondly, to determine if tolerance developed to the same extent in subjects that showed differential preference for ethanol. Finally the impact of tolerance development on the subsequent alcohol consumption was evaluated in these animals. In order to delineate further the possible influence of tolerance development on subsequent

alcohol consumption, the rates of tolerance acquisition were accelerated in animals of both S_1 and S_3 strains by an efficient gavage technique (Le Blanc et al, 1973, 1976).

Amir (1978b) measured an increase in brain aldehyde dehydrogenase activity that parallels in time the development of tolerance. It appears consequently that the metabolism of acetaldehyde may be an important parameter in the development of tolerance as well as a determinant of alcohol consumption. Since it has been demonstrated that there is an increase in alcohol dehydrogenase (ADH) activity following a chronic ethanol treatment (Taberner and Unwin, 1981; Unwin and Taberner, 1982), both liver ADH and mitochondrial ALDH as well as brain ALDH activities were measured in S_1 and S_3 subjects following termination of the behavioral studies.

Material and Methods

Ethanol Preference Testing

Male and female rats of the S_1 and S_3 Tryon strains bred in our laboratory were used in the experiment (111 rats). At 90 days of age, the animals were placed in wire mesh cages mounted with two calibrated Richter bottles. A 3 day habituation period followed for the adaptation of the animals to their new environment. During this time the animals were provided with Purina laboratory chow and water ad libitum and were trained on a rotarod apparatus. On the fourth day, the animals began the voluntary ethanol regimen previously described in study 1 (Russell and Stern, 1973). This phase

of the experiment was terminated when the animal had not ingested a concentration for more than two consecutive days and the last concentration accepted by the rat was recorded as the Final Acceptance Concentration (FAC). During the following 24 hours, both Richter tubes were filled with water. The next day, the animal was presented with a choice between water and an ethanol solution the concentration of which was 80% of the FAC, calculated individually for each subject. The animal was placed in this choice situation for 3 non consecutive days. On alternate days both tubes were filled with water. Pre-treatment alcohol consumption was estimated by calculating the average intake for these 3 days and expressed as ml of absolute ethanol/kg body weight/day.

Treatment schedules

The day following the establishment of ethanol intake, the animal was given a trial on the rotarod and then challenged with 1.8 g/kg body weight intraperitoneal (i.p.) injection of ethanol as a 30% (v/v) solution in normal saline. Exactly 2 1/2 minutes following the injection the animal was placed on the rotarod apparatus for 3 two minute periods, each separated by a three minute rest period. Following this schedule a blood sample was taken from the animal's tail for blood alcohol and acetaldehyde estimations. Performance on the rotarod following the first alcohol challenge was used as an estimate of initial tolerance (initial sensitivity). Following this test, the animals were

randomly assigned to one of the 4 following treatment schedules.

In the first group, the voluntary ethanol intake group, the animals were presented with an 80% concentration of their preferred ethanol solution on alternate days during a 40 day period. Weight and fluid intake were monitored every day and the positions of the Richter tubes were reversed. Every 10 days, the animals were challenged with an i.p. injection of ethanol at the dosage level previously indicated. The day preceding the challenge test day, animals were provided with water but had no access to food from 18:00 hour. It had been previously shown that animals under this treatment regimen would increase their ethanol intake (Russell, 1971). In the second group, the voluntary water intake group, the animals were treated in the same manner as those on the ethanol treatment schedule with the exception that water only was available in the home cages throughout the schedule. This group served as a control for animals of Group 1. The animals in the third group, the involuntary ethanol intake group, underwent gavage treatment every day with 5 g/kg body weight ethanol as a 30% (v/v) solution prepared in distilled water. During this time water was provided ad libitum and the animal's food was rationed in order to maintain its weight . The animals were always fed after the gavage. Challenge tests with ethanol were made every fifth day. On those days animals underwent gavage following their performance on the rotarod

test. The animals of the fourth group, the involuntary water intake group, were treated in the same manner as those assigned to the previous group with the exception that the gavage consisted of tap water. This group served as a control for the animals of Group III.

The choice of this experimental paradigm was directed to the control of two factors: learning and stress. The tolerance test days (i.e. the days when ethanol was given to determine development of tolerance) may be considered as a potential learning opportunity. Each group of animals was challenged with ethanol 5 times. Since the interval between the tolerance tests was either 5 or 10 days, the rate of tolerance development could be attributed to the treatment schedule. Therefore care was taken to match the schedule of each ethanol treatment group with that of its appropriate control group. The possible stress of the gavage treatment was controlled by submitting the two involuntary intake groups to gavage, one with ethanol, the other with water. Thus differences stemming from ethanol treatment could be attributed to alcohol exposure.

Post-treatment ethanol consumption

At the end of the prior phase of the experiment alcohol consumption was again assessed. Following a 24 hour rest period during which the animals had free access to food and water, they were presented with an ethanol solution which was 80% of the preferred concentration, on alternate days during

a 6 day period. Alcohol consumption was calculated as previously indicated. Following the assessment of alcohol consumption, the animals were left in their cages for 3 to 5 days with free access to water and food and then were sacrificed for alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) enzyme activity estimations.

Blood alcohol and acetaldehyde preparation and analysis

A 100 µl sample of blood was taken from the animal's tail in a cold heparinized pipette. Samples were diluted in 0.6 ml of a semicarbazide reagent preparation and centrifuged 5 minutes at a speed of 2000 RPM. A sample of 500 µl of the supernatant was then transferred into 0.2 ml of 3 M ice cold perchloric acid. This mixture was again centrifuged for 5 minutes at a speed of 10,000 RPM. A 500 µl sample of the clear supernatant was placed in 10 ml vials which were immediately sealed (Stowell et al, 1980). The blood samples were kept at -20° C pending the chromatographic analysis. Ethanol and acetaldehyde levels were measured with a Varian, series 1440 gas chromatograph under the same conditions previously cited above.

Enzyme activity determination

Animals were sacrificed without anaesthesia by decapitation. Brain and liver were quickly removed and washed in 10 ml of ice cold sucrose solution (0.25M). The liver sample used for the estimation of ADH and cytosol ALDH activities were homogenized in 9 volumes (w/v) of an ice cold

0.25 M sucrose solution in a pyrex tissue grinder with a teflon pestle attached to a power source. Samples were centrifuged for 1 hour at 40,000x g at 0° C and the clear supernatant was decanted and used as enzyme source. Brain and liver preparations used for estimation of total ALDH activity were homogenized in 9 volumes (w/v) of an ice cold 0.25 M sucrose solution containing 1% (w/v) triton X-100. Homogenates were centrifuged for 90 minutes at 100,000xg at 0° C and the clear supernatant fractions were decanted and used for enzyme assay.

The alcohol dehydrogenase activity was assayed by the method suggested by Anderson and coworkers (1979). For aldehyde dehydrogenase activity, 200 ml of a 50 mM phosphate buffer solution was prepared with pyrazole (final concentration of 1mM), quercetin (final concentration of 0.1 mM) and magnesium chloride (final concentration of 1 mM) at pH7.4. The reaction mixture for the assay contained 2.6 ml of buffer, 0.1 ml enzyme, 0.1 ml propionaldehyde (0.24M), and 0.2 ml NAD (10 mg/ml, Grade V, Sigma Chemicals Co.)

Alcohol and aldehyde dehydrogenase activities were measured as the change in optical density (OD) at 340 nm (NADH production) for 10 minutes at 20° C in a Beckman spectrophotometer, model 25, with a 1 cm light path using 3 ml quartz cuvettes. Endogenous activity as measured by blanks containing 0.1 ml pyrazole (0.03M) instead of water was subtracted from the change in 0.D observed in the reaction

mixture used for ADH determination. Endogenous activity as measured by blanks containing water in lieu of propionaldehyde was subtracted from the change in O.D. observed in the reaction mixture used for aldehyde dehydrogenase determination. Duplicate assays were performed for both the reaction mixture and blank. The initial reaction velocity was used to calculate the enzyme activity. Specific activity is defined as nanomole (nmole) of substrate converted per minute per mg protein content of tissue. Liver mitochondrial ALDH was estimated by subtracting the activity recorded in the cytosolic extracts from the activity obtained for total liver preparations. Protein content was measured by the method of Lowry et al (1951) and bovine serum albumin (Sigma Chemicals Co) was used as standard. Unless otherwise mentioned, the Chemicals used in these analyses were purchased from Fisher Scientific Co.

Statistical Analysis

The data were subjected to analysis of variance. Wherever relevant, group means were compared by using the Scheffe method of multiple comparisons or the Tukey honestly significant difference method (HSD) (Ferguson, 1980).

Results

Ethanol Consumption

Alcohol consumption measured before and following the various treatment schedules for male and female rats of S_1 and S_3 strain are presented in table 1. Animals of the S_1 strain consumed greater quantities of alcohol than those of the S_3 strain ($F_{1,103} = 8.06$, $p < .001$). The consumption by S_1 females was found to be greater than that of the males of both the S_1 and S_3 strains ($p<.05$, Tukey (HSD)) and is in great part responsible for the significant sex ($F_{1,103} = 9.20$, $p<.005$) and sex x strain ($F_{1,103} = 6.53$, $p<.01$) differences which were noted. The alcohol consumption measured before and after the application of the different treatment schedules varied significantly ($F_{1,103} = 24.86$, $p<.005$); generally, alcohol intake increased after the animal underwent a treatment schedule, regardless of the particular treatment. During the treatment phase, weight and fluid intake were recorded daily and no appreciable effect of treatment on weight gain or water intake were noted.

Animals undergoing the voluntary ethanol intake schedule were presented on alternate days with an alcohol solution (80% of their preferred concentration) during the 10 day interval between test days. Figure 1 shows the means of absolute ethanol consumed by males and females of both strains during test intervals. A trend analysis performed on these values indicated that animals of both strains

Table 1

Absolute ethanol consumption by male and female rats of the S_1 and S_3 Tryon strains measured before and after the introduction of the treatment schedule

Absolute Ethanol intake (mean ±SEM)
(ml/kg body weight/day)

		S_1		S_3	
TREATMENT SCHEDULE	Time	Male	Female	Male	Female
VOLUNTARY Water	Pre	5.85 ± 1.52	9.65 ± 3.92	4.40 ± 1.09	4.11 ± 1.95
	Post	6.36 ± 1.05	16.22 ± 2.78	5.13 ± 1.49	9.32 ± 2.38
Ethanol	Pre	5.22 ± 1.26	6.83 ± 1.87	3.56 ± 1.09	4.06 ± 0.95
	Post	7.54 ± 1.54	10.41 ± 1.91	4.05 ± 1.63	4.56 ± 0.96
INVOLUNTARY (Gavage) Water	Pre	3.50 ± 0.92	7.92 ± 2.79	4.84 ± 1.51	4.00 ± 2.31
	Post	7.98 ± 1.23	11.63 ± 1.01	7.30 ± 1.88	5.08 ± 1.29
ethanol	Pre	4.14 ± 0.66	8.07 ± 2.16	5.53 ± 1.51	5.94 ± 0.98
	Post	5.09 ± 1.86	6.99 ± 3.15	7.40 ± 1.23	9.72 ± 1.16

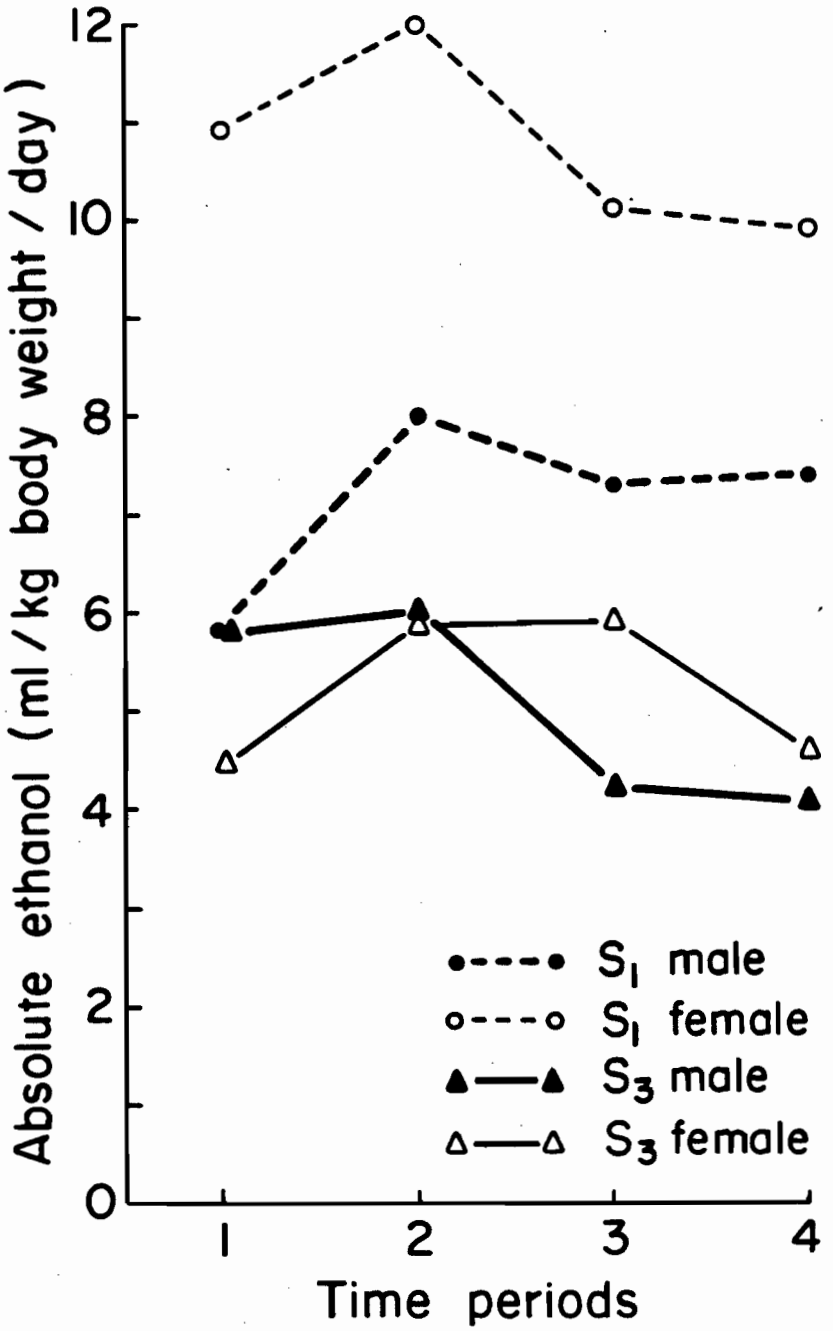

Figure 1. The profiles of ethanol intake determined for male and female rats of the S_1 and S_3 strains assigned to the voluntary ethanol intake schedule. The means of ethanol intake were obtained from the means of ethanol consumption (for a period of 10 days) of individual rats during the ethanol regimen.

exhibited steady patterns ($p>.05$ for time periods main effect and all the interactions effects) of alcohol consumption and that the overall intake of the S_1 animals was considerably greater than that of the S_3 strain ($F_{1,24} = 6.60$, $p<.05$). No sex effect ($F_{1,24} = 0.76$, $p>.05$) and sex x strain interactions were found ($F_{1,24} = 0.95$, $p>.05$), indicating that although females of the S_1 strain were drinking substantially more alcohol than their counterparts (i.e., S_1 males, male and female S_3), the difference was not statistically significant (see Appendix I for mean ethanol intake values).

Tolerance

Initial tolerance

Initial sensitivity to the disabling effect of alcohol on a previously learned behavioral task was evaluated by a comparison, across and within strains, of the mean intoxication scores on the first test day for all animals. The results from the analysis of variance are given in table 2. A strain ($F_{1,95} = 12.08$, $p<.005$) and strain x sex interaction ($F_{1,95} = 4.35$, $p<.05$) were found to be significant. S_1 females fell off the rotarod apparatus only 6.2 times on the average when challenged with an i.p. injection of ethanol for the first time; this score is significantly lower than mean scores noted for the S_1 males (10.6) and for male (13.8) and female (11.8) rats of the S_3 strain ($p<.01$, Scheffe).

TABLE 2

Results of the analysis of variance performed on the initial sensitivity measures obtained from male and female rats of the S_1 and S_3 Tryon strain of rats assigned to the various treatment schedules.

SOURCE OF VARIATION	DEGREE OF FREEDOM	VARIANCE ESTIMATE	F	P
TREATMENT	3	35.65	1.26	N.S.
STRAIN	1	341.38	12.08	.001
SEX	1	85.04	3.01	N.S.
TREATMENT X STRAIN	3	13.99	0.49	N.S.
TREATMENT X SEX	3	75.48	2.67	N.S.
STRAIN X SEX	1	122.99	4.35	.05
TREATMENT X STRAIN X SEX	3	23.30	0.82	N.S.
ERROR	95	28.27		

Behavioral tolerance

Behavioral tolerance was measured by comparing the mean intoxication score within each group with subsequent scores on each test day. Tolerance, as shown by a decrease in mean intoxication score over test days, occurred within each treatment schedule ($F_{4,380} = 34.71$, $p<.001$). Because no interaction was noted between test days and treatment schedules (see Table 3 for the results from the analysis of variance), the rate of development of tolerance was found to be similar for all treatment schedules. Figure 2 shows the scores combined across sexes and strains obtained on each test day for the various treatment schedules. Animals of the S_3 strain independently of the treatment schedules, were less tolerant to the debilitating effect of alcohol on their performance on the rotarod apparatus than were their S_1

Table 3

Results from the analysis of variance(4 way analysis with repeated measures on days) performed on the intoxication scores obtained from male and female rats of the S_1 and S_3 Tryon strain assigned to the various treatment schedules.

SOURCE OF VARIATION	DEGREES OF FREEDOM	VARIANCE ESTIMATE	F	P[a]
TREATMENT	3	239.25	4.35	.01
STRAIN	1	1781.61	32.39	.001
SEX	1	1198.14	21.79	.001
TREATMENT X STRAIN	3	119.23	2.17	N.S.
TREATMENT X SEX	3	137.02	2.49	.06
STRAIN X SEX	1	0.91	0.02	N.S.
TREATMENT X STRAIN X SEX	3	117.77	2.03	N.S.
ERROR	95	55.00		
DAYS	4	503.36	34.71	.001
DAYS X TREATMENT	12	11.55	0.80	N.S.
DAYS X STRAIN	4	18.00	1.24	N.S.
DAYS X SEX	4	4.54	0.31	N.S.
DAYS X TREATMENT X STRAIN	12	21.01	1.45	N.S.
DAYS X TREATMENT X SEX	12	11.73	0.81	N.S.
DAYS X STRAIN X SEX	4	28.38	1.96	N.S.
DAYS X TREATMENT X STRAIN X SEX	12	9.11	0.63	N.S.
ERROR	380	14.50		

a The tail probability reported for the days factor is The GREENHOUSE GEISSER probability.

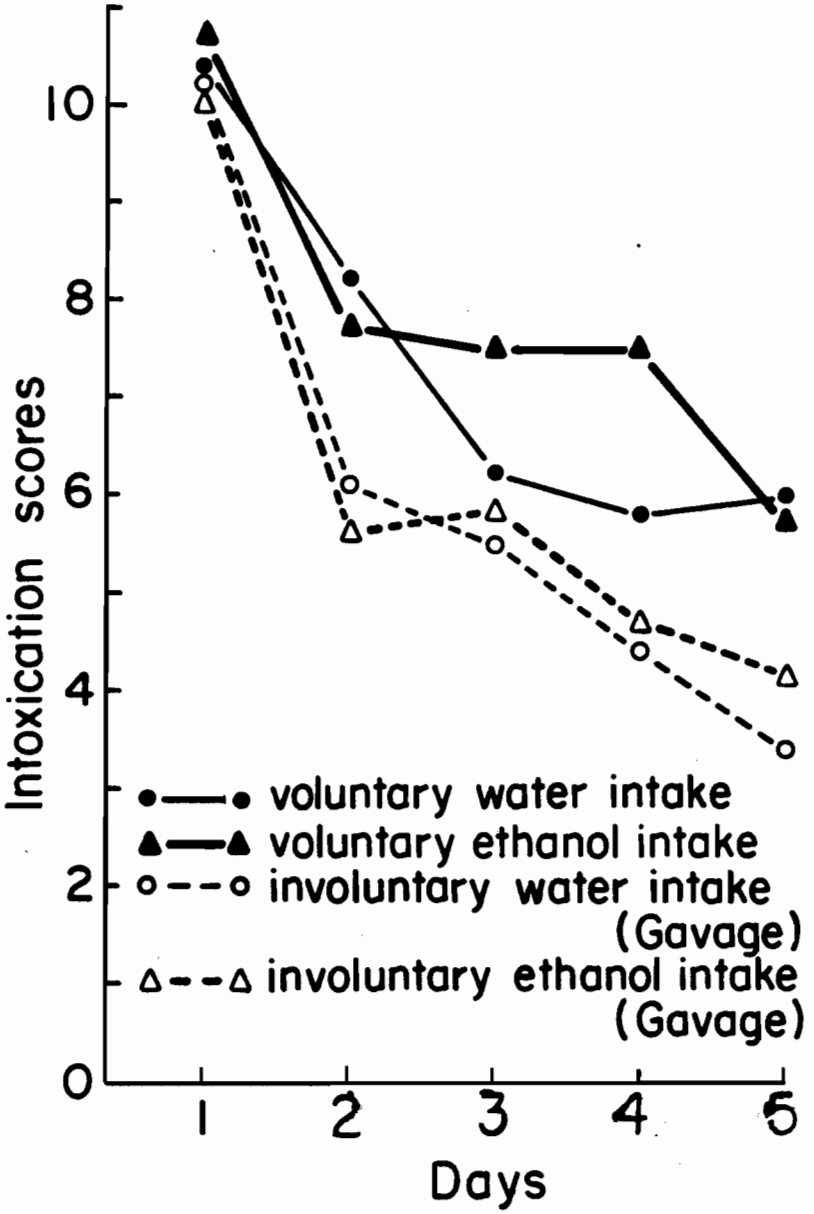

Figure 2. Mean intoxication scores combined across sexes and strains obtained on each test day for the various treatment schedules.

counterparts ($F_{1,95}$ = 32.39, p<.001) (see figure 3). Because they developed tolerance at the same rate as the S_1 animals, the S_3 rats were never able to overcome the initial disadvantage they had over S_1 animals. This statement is substantiated by the absence of a strain x test days ($F_{4,380}$ = 1.24, p>.05) or a strain x treatment x test days interaction ($F_{12,380}$ = 1.45, p>.05). Significant sex ($F_{1,95}$ = 21.79, p<.001) and a sex x treatment interaction ($F_{3,95}$ = 2.49, p<.06) were found (Figure 3).

The treatment schedules did not appreciably affect the acquisition of behavioral tolerance in female rats; however the treatment schedules which included the gavage technique accelerated the rate of development of tolerance in male rats, thereby allowing the male's performance on the rotarod apparatus to reach the levels obtained by the females (p<.05 Tukey, (HSD)). Although male subjects increased their rate of development of tolerance in these latter groups, this acceleration was not found to be significant (see figure 3).

Blood Ethanol and Acetaldehyde Levels

The blood samples taken at the end of each test day were compared for significant differences in alcohol levels by an analysis of variance (see table 4). No substantial differences in blood alcohol were noted among male and female rats of both the S_1 and S_3 strains, no matter the treatment schedule. The blood alcohol levels of the S_3 animals were slowly declining throughout the testing schedule whereas

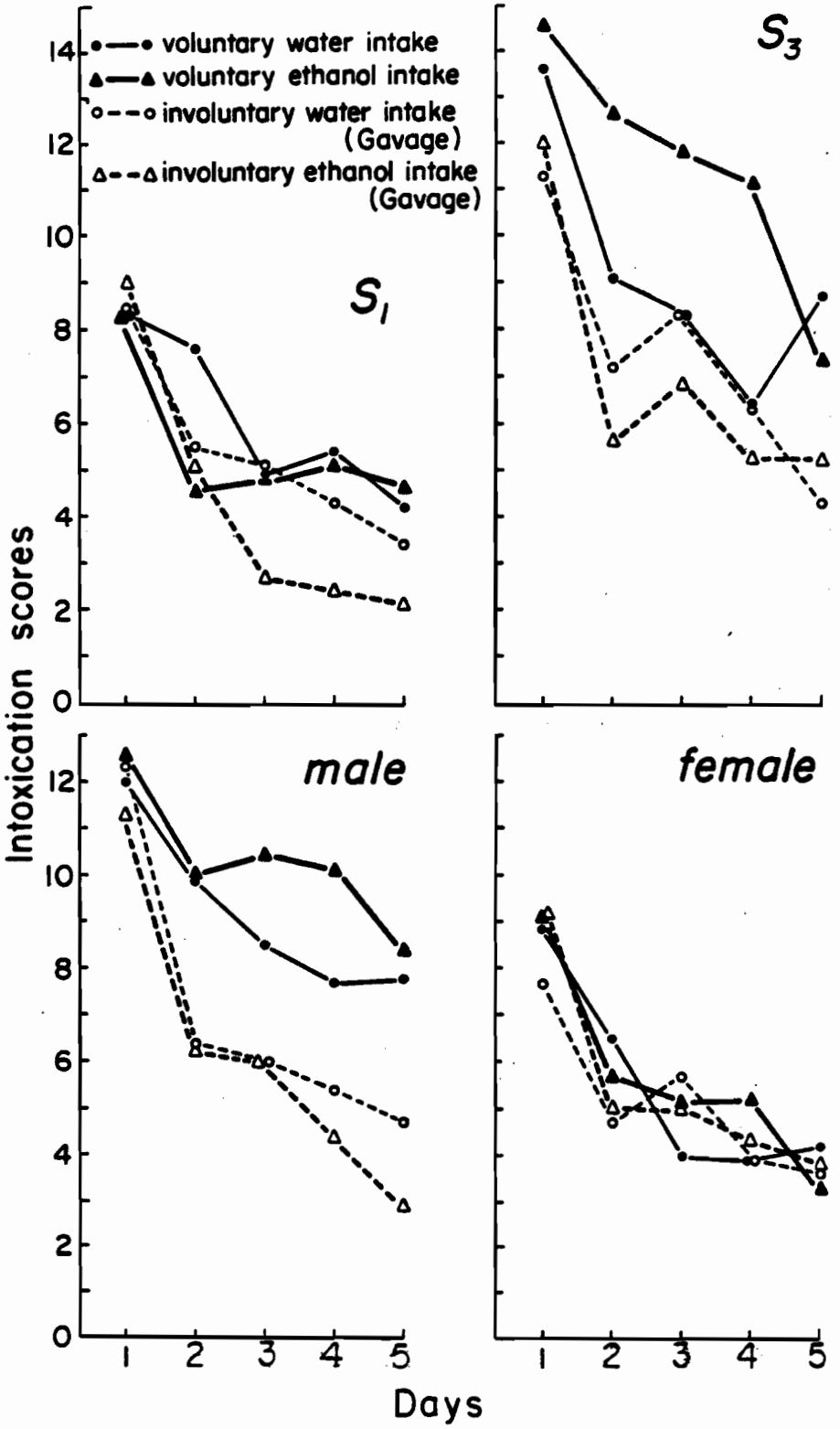

Figure 3. Mean intoxication scores combined across strains, (the two upper figures) and sexes (the two lower figures) obtained on each test day for the various treatment schedules.

Table 4

Results from the trend analysis performed on the blood alcohol estimates obtained from male and female rats of the S_1 and S_3 Tryon strains following their performance on the rotarod task.

SOURCE OF VARIATION	DEGREES OF FREEDOM	ESTIMATE OF VARIANCE	F
TREATMENT	4	0.02	0.16
STRAIN	1	0.07	0.73
SEX	1	0.00	0.03
TREATMENT X STRAIN	4	0.05	0.52
TREATMENT X SEX	4	0.03	0.34
STRAIN X SEX	1	0.02	0.16
TREATMENT X STRAIN X SEX	4	0.13	1.34
ERROR	103	0.10	
LINEAR REGRESSION			
DAYS	1	0.11	0.90
DAYS X TREATMENT	4	0.10	0.76
DAYS X STRAIN	1	0.45	3.59
DAYS X SEX	1	0.02	0.15
DAYS X TREATMENT X STRAIN	4	0.08	0.60
DAYS X TREATMENT X SEX	4	0.05	0.39
DAYS X STRAIN X SEX	1	0.11	0.86
DAYS X TREATMENT X STRAIN X SEX	4	0.21	1.64
ERROR	103	0.12	
DAYS	4	0.06	0.58
DAYS X TREATMENT	16	0.07	0.67
DAYS X STRAIN	4	0.16	1.57
DAYS X SEX	4	0.04	0.45
DAYS X TREATMENT X STRAIN	16	0.11	1.13
DAYS X TREATMENT X SEX	16	0.07	0.67
DAYS X STRAIN X SEX	4	0.04	0.38
DAYS X TREATMENT X STRAIN X SEX	16	0.11	1.03
ERROR	412	0.10	

those of the S_1 rats were slowly increasing ($F_{1,103} = 3.59$, $p<.06$) (see Appendix II for blood alcohol estimates). Acetaldehyde concentrations in these samples were not large enough to be detectable.

Enzyme activities

Brain Aldehyde Dehydrogenase Activity

Analysis of variance revealed a significant strain difference in brain ALDH activity ($F_{1,95} = 8.96$, $p<.005$). Animals of the S_1 strain had generally higher brain ALDH activity than that of the S_3 strain (table 5). A significant main effect for sex ($F_{1,95} = 0.31$, $p>.05$) was not noted. However, significant treatment ($F_{3,95} = 3.99$ $p<.01$), treatment x sex ($F_{3,95} = 4.01$, $p<.01$) and treatment x strain x sex ($F_{3,95}=5.96$, $p<.001$) effects were found. Simple effect contrasts allowed verification that the significant differences noted were entirely due to the lower brain ALDH activity recorded for female S_1 rats undergoing the treatment schedules where ethanol was presented ($p<.01$, Scheffe). The treatment x strain ($F_{3,95} = 1.45$, $p>.05$) and strain x sex ($F_{1,95} = 0.15$, $p>.05$) interactions were not found to be significant.

Mitochondrial Aldehyde Dehydrogenase Activity

Analysis of variance performed on mitochondrial ALDH activity indicated only a significant treatment x strain

Table 5

Brain aldehyde dehydrogenase activity in male and female rats of the S_1 and S_3 Tryon strains for each experimental condition.

	Brain ALDH (mean±SEM)[a]			
	S_1		S_3	
TREATMENT SCHEDULE	Male	Female	Male	Female
VOLUNTARY				
water	7.251 ± 0.479	7.069 ± 0.473	4.103 ± 0.499	6.284 ± 0.611
ethanol	6.341 ± 0.545	4.832 ± 0.616*	4.760 ± 0.909	5.461 ± 0.792
INVOLUNTARY (Gavage)				
water	5.468 ± 0.799	7.621 ± 0.340	6.470 ± 0.436	5.192 ± 0.746
ethanol	5.461 ± 0.461	5.568 ± 0.416*	4.900 ± 0.645	6.613 ± 0.359

[a] Enzyme activity is expressed in nanomoles per minute per mg protein content of the brain tissue. *Asterisks indicate significant differences from water conditions ($p<.01$, Scheffe test).

TABLE 6

Results from the analysis of variance performed on mitochondrial aldehyde dehydrogenase obtained from male and female rats of the S_1 and S_3 Tryon strain assigned to the various treatment schedules.

SOURCE OF VARIATION	DEGREE OF FREEDOM	VARIANCE ESTIMATE	F	P
TREATMENT	3	51.47	0.80	N.S.
STRAIN	1	1.36	0.02	N.S.
SEX	1	9.25	0.14	N.S.
TREATMENT X STRAIN	3	170.96	2.65	.05
TREATMENT X SEX	3	44.21	0.68	N.S.
STRAIN X SEX	1	101.01	1.56	N.S.
TREATMENT X STRAIN X SEX	3	188.16	2.91	.05
ERROR	95			

($F_{3, 95}$ = 2.65, $p<.05$) and treatment x strain x sex interactions ($F_{3,95}$ = 2.91, $p<.05$). Simple effects tests were carried out and further demonstrated that the S_1 females showed significantly lower mitochondrial ALDH activity during the treatment schedules when they were exposed to ethanol ($p<.05$) (see table 6 and 7).

Alcohol Dehydrogenase Activity

Significant differences in alcohol dehydrogenase activity occurred as a function of the treatment schedules ($F_{3,95}$ = 12.71, $p<.005$), strains ($F_{1,95}$ = 5.38, $p<.05$) and sexes ($F_{1,95}$ = 49.02, $p<.005$). Significant interactions were also noted for treatment x sex ($F_{3,95}$ = 2.89, $p<.05$) and sex x strain effects ($F_{1,95}$ = 9.65, $p<.005$) but not for the treatment x strain ($F_{3,95}$) = 1.21, $p>.05$) and the treatment x strain x sex interactions ($F_{3,95}$ = 0.69, $p>.05$). A series of

Table 7

Mitochondrial aldehyde dehydrogenase activiy in male and female rats of the S_1 and S_3 Tryon strains measured in each experimental condition.

	Mitochondrial ALDH (mean ± SEM) [a]			
	S_1		S_3	
TREATMENT SCHEDULE	Male	Female	Male	Female
VOLUNTARY				
water	28.060 ±2.532	26.637 ± 1.136	25.001 ± 5.390	31.865 ± 3.497
ethanol	29.555 ±2.927	20.717 ± 2.753 *	21.877 ± 3.985	25.773 ± 3.411
INVOLUNTARY (Gavage)				
water	24.466 ±3.200	33.263 ± 4.456	25.041 ± 4.049	21.778 ± 2.241
ethanol	24.845 ±2.434	20.875 ± 2.354 *	28.118 ± 3.028	30.771 ± 2.285

[a] Enzyme activity is expressed in nanomoles per minute per mg protein content in liver tissue.* Asterisks indicate significant differences from water conditions ($p<.05$, Scheffe test).

post hoc tests indicated lower ADH activity for females of both S_1 and S_3 strains on all treatment schedules, with the exception of the voluntary water intake schedule when the activity was compared against values obtained from males ($p<.01$, Tukey (HSD); see table 8). Furthermore, a decrease in the ADH activity of females was noted on all treatment schedules when this activity was compared to that of the voluntary water intake schedule, whereas the ADH activity of males remained constant for all treatment schedules ($p<.01$, Scheffe). When ADH activity was evaluated in order to explain the sex x strain interaction, it was evident that the higher ADH activity noted for animals of the S_3 strain could be attributed to the S_3 males ($p<.01$, Scheffe). Finally, ADH activity was also found to be greater in the S_1 males when this activity was compared to the one obtained from the S_1 and S_3 females ($p<.01$, Scheffe).

Discussion

The experimental paradigm adopted in this research aimed at the demonstration of a possible relation between the initial tolerance, the development of behavioral tolerance, and the differential preference for ethanol that characterizes male and female rats of the S_1 and S_3 Tryon strains, as well as the measurement of the effects of differential acquisition rates of behavioral tolerance on alcohol consumption.

Table 8

Alcohol dehydrogenase activity measured in male and female rats of the S_1 and S_3 Tryon strains on all experimental conditions.

	Alcohol Dehydrogenase (Mean ± SEM)			
	S_1		S_3	
TREATMENT SCHEDULE	Male	Female	Male	Female
VOLUNTARY				
water	154.933 ± 8.511	149.298 ± 3.663	183.692 ± 6.924	156.030 ± 4.555
ethanol	150.543 ± 12.331	102.247 ± 6.719 *,**	167.914 ± 9.960	102.073 ± 12.115 *,**
INVOLUNTARY (Gavage)				
water	125.593 ± 9.849	120.929 ± 9.394 *,**	150.264 ± 5.220	90.887 ± 8.664 *,**
ethanol	124.928 ± 9.954	107.050 ± 5.358 *,**	160.826 ± 6.503	114.975 ± 6.287 *,**

a Enzyme activity is expressed in nanomoles per minute per mg protein content in the liver tissue. Asterisks indicate significant differences ($P<.01$; Tukey, (HSD)) *, from males, ** the voluntary water intake schedule

The results reported here support the assumption that initial rather than acquired behavioral tolerance is associated with a high initial preference for alcohol. The first argument behind this suggestion rests on the fact that the high-ethanol consuming S_1 strain, particularly the S_1 females, was initially less affected by the debilitating effect of alcohol intoxication than the low-alcohol consuming S_3 strain. Meanwhile, the development of behavioral tolerance occurred at a similar rate in male and female rats of both S_1 and S_3 strains; however, during this period, the blood alcohol levels of the S_1 and S_3 subjects were evolving in opposite directions. These changes in alcohol metabolism cannot be attributed to the ethanol intake, since they appeared in S_1 and S_3 animals assigned to the various treatment groups. This would suggest a greater adaptation to alcohol on the part of the S_1 animals. Thus it appears that the initial tolerance to a subhypnotic dose of ethanol may be related in some way to the differential preference which characterizes the S_1 and S_3 animals of the Tryon strain and that it is independent of the development of behavioral tolerance.

Within the experimental paradigm of this study, it was expected that alcohol gavage would accelerate the acquisition of behavioral tolerance as compared to that of the water treatment group. Indeed, LeBlanc and associates (1976) had demonstrated that animals undergoing daily gavage increased

their rate of tolerance acquisition. Failure to obtain a differential rate of tolerance development in the involuntary ethanol treatment group is not likely attributable to an inefficient gavage technique, since blood samples taken 30 minutes following gavage reached alcohol levels of 60 mg%; the amount of alcohol circulating in the blood following gavage was not appreciably different from the levels found in blood samples taken after the performance on the rotarod apparatus while the animals were intoxicated.

Alternatively, one explanation of the failure to note a difference between these two treatment groups is perhaps related to the observation of acquired behavioral tolerance in the animals subjected to the voluntary and the involuntary water treatment schedule. It had previously been demonstrated that the development of behavioral tolerance did not occur when alcohol challenge tests were carried out over an interval greater than 4 days (LeBlanc et al, 1976). In the present study, the animals assigned to the water treatments were exposed to alcohol solely on test days, which occurred on every fifth or tenth day for the voluntary and involuntary treatment groups respectively. Consequently any performance improvement noted in these animals on the rotarod apparatus could be attributed to a practice effect under the influence of alcohol.

These findings agree with those of Wenger and associates (1981) who suggested that the opportunity to practice a task

under the influence of alcohol is a critical factor in the development of behavioral tolerance. Alternatively, since all the animals in the present investigation were exposed to alcohol, before they were assigned to the different treatment schedules, it could be argued that this exposure would affect the subsequent development of tolerance. However, previous exposure to alcohol by itself is unlikely to have favored an acceleration of behavioral tolerance acquisition (Khanna et al, 1967; Le Blanc et al, 1976; Wenger et al, 1981).

The possibility that the animals of both S_1 and S_3 strains had the opportunity, while exposed to alcohol in their home cages during the establishment of preference, to learn responses (such as how to balance or stand without falling despite intoxication) which could be transferred to the rotarod performance is unlikely for the two following reasons. Firstly, the alcohol consumption of the animals during this phase of the experiment, was maintained below the normal rate of ethanol elimination from the blood thereby preventing the animals from becoming chronically intoxicated (Marfaing-Jallat and Le Magnen, 1982). Secondly, during the behavioral tolerance testing phase, when the animals were assigned to the various treatment schedules, it was noted that the S_1 males and particularly the S_1 females assigned to the voluntary ethanol treatment group ingested significantly greater amounts of ethanol than their S_3 counterparts. The ethanol ingestion of the S_1 animals was maintained above the

normal rate of ethanol elimination throughout the entire schedule and still, these animals did not develop tolerance at greater rate than their S_3 counterparts. Thus the evidence presented suggests that the development of behavioral tolerance observed in the present study can be attributed to learning the task under the influence of alcohol; this finding also supports the assumption that the extent to which the development of behavioral tolerance occurs is not dependent upon the initial tolerance.

The pre and post-treatment ethanol intake of male and female rats of the S_1 Tryon strain, regardless of the treatment group to which the animals were assigned was greater than that of the male and female rats of the S_3 strain. The post-treatment consumption of ethanol was found to be greater than the pre-treatment intake measured in animals of both sexes and strains, regardless of the treatment applied to them.

These results do not support the previous finding of Russell (1971) where both S_1 and S_3 animals undergoing voluntary ethanol treatment increased their ethanol intake significantly during the course of the schedule whereas the ethanol intake of their counterparts, assigned to the voluntary water intake during that period, remained at the pre-treatment level when evaluated at the termination of the regimen. Thus it was expected that the post-treatment ethanol intake would be lower for the animals assigned to the water

treatments. The finding of a similar increase in post-treatment ethanol intake in all the subjects, independently of the treatment to which they were assigned, is of interest since the development of behavioral tolerance occurred at a similar rate in these animals. This observation raises the possibility that the acquisition of behavioral tolerance is of consequence in subsequent alcohol consumption. It further suggests that an increase in alcohol consumption occurs independently of initial preference since even low - consumers increased their intake following behavioral adaptation to alcohol.

The information about enzymatic patterns gathered from the animals placed on water treatment schedules, with respect to the lack of differences noted in mitochondrial ALDH activity between the high (S_1) and low (S_3) consumers, agrees with the general findings reported in the literature (Amir, 1978a; Koivula et al, 1975, Sheppard et al, 1968, 1970). The ADH activity was found to be greater in the S_3 animals while brain ALDH activity was greater in the S_1. No sex difference was apparent in the activities of both ADH and ALDH enzymes. However, the treatment schedules affected male and female subjects of the S_1 and S_3 strains differently. Differences in enzyme activities were noted particularly in the S_1 female rats; when the latter were exposed to the treatment schedules with ethanol (i.e., the voluntary and involuntary ethanol intake treatments) a lower ADH activity as well as a lower

liver and brain ALDH activities were noted on these animals as compared to their counterparts assigned to the water treatments. An increase rather than a decrease in liver ADH activity is more frequently reported following prolonged chronic exposure to ethanol (Dippel and Ferguson, 1977; Eriksson and Pikkarainen, 1968; Khanna et al, 1967; McClearn et al, 1964). But it has been reported recently that forced ingestion of ethanol produces changes in liver ADH activity which are unrelated to subsequent alcohol consumption (Taberner and Unwin, 1981; Unwin and Taberner, 1982).

Meanwhile brain ALDH activity (Amir and Stern, 1978; Sinclair and Lindros, 1981), rather than liver ALDH (Amir, 1977; Eriksson and Deitrich, 1980; Lindros and Sinclair, 1979), has been proposed as the principal parameter in the determination of ethanol intake in the laboratory rat. The present finding of a lower brain ALDH enzyme activity in the high consumer S_1 females following exposure to ethanol, is not in agreement with the above mentioned assumption. Indeed, contrary to expectation, the S_1 females maintained their high level of ethanol consumption while on a free choice water-ethanol regimen, rather than decreasing their intake, despite their low brain ALDH level.

In fact these findings agree with those of Inoue and coworkers (1981) who could not relate the changes in brain ALDH activity noted in the AA (high) and ANA (low) consumer strain to the changes in their drinking behavior following a chronic ethanol intake regimen. The consistent observation of

lower brain ALDH activity in the S_1 female consumers only, as compared to male S_1 and male and female S_3 rats assigned to the same treatment, suggests that brain ALDH enzyme activity is one of several factors influencing ethanol intake, but the particular role of this enzyme on alcohol drinking behavior still remains obscure. The present results seem to suggest however, that brain ALDH activities may be more important in the initiation as well as in the determination of preferential ethanol intake and less important for the maintenance of the drinking behavior.

In summary, the results of this study support the hypothesis that initial tolerance to a subhypnotic dose of alcohol is associated with ethanol preference, while acquired changes in behavioral tolerance are related to subsequent alcohol consumption. These results also support the suggestion that genetic variations in brain ALDH activities play a predominant role in the preferential intake patterns which characterize animals of the S_1 and S_3 strains as compared to liver activities of ADH and ALDH.

GENERAL DISCUSSION

The aim of the present research was to explore the relations between preference for and tolerance to ethanol, and the physiological basis of these relations.

A systematic investigation, comprising two major studies was conducted with male and female rats of the two Tryon strains. Three distinctive traits of the S_1 and S_3 rats of the Tryon strain were deemed to be significant in the search for a better understanding of the behavioral and genetic components that influence alcohol oriented behaviors.

The most salient feature of these animals is that they were originally inbred on the basis of errors made in solving maze problems (Tryon, 1940) and not, as is common to most studies in the area of alcohol research, on the basis of alcohol related behaviors. Moreover, the S_1 and S_3 rats of this strain have shown differential preference for ethanol on a voluntary ethanol-water regimen (Russell and Stern, 1973). Finally the aldehyde oxidizing capacity of these animals was shown to parallel their preferential drinking patterns (Amir, 1978a).

The first objective of the present research was to examine the relation between preference for and initial tolerance to both a hypnotic and subhypnotic dose of alcohol in S_1 and S_3 rats of the Tryon strain. The disparate findings on initial tolerance in the high (P) and low (NP) consumer strains reported by two independent research groups, one

investigating tolerance to a subhypnotic dose of ethanol (Lumeng et al, 1982) and the other, to a hypnotic dose of ethanol (Li et al, 1979), emphasized the importance of a drug dose-dependent reaction to alcohol. These results raised the possibility that the reaction to the soporific effects of ethanol was attributable to variables associated with the drug dosage rather than with the specific pharmacological action of ethanol.

The first experiment showed that the rats of the high-preference (S_1) strain were less resistant to the anaesthetic effects of ethanol than were the rats of the low-preference (S_3) strain. This finding led to the suggestion that a less efficient alcohol metabolizing system is responsible for the lesser tolerance noted in the S_1 animals. This hypothesis was strengthened by the finding, in the second study, of a lower ADH activity in male and female rats of the S_1 strain.

In contrast to their reaction to the soporific effect of ethanol, the S_1 rats were less impaired than their S_3 counterparts on the performance of a simple motor task, when challenged with a moderate dose of ethanol. In addition, when both strains were repeatedly challenged with the same dose of ethanol, the S_1 rats adapted to the disabling effect of ethanol despite their rising blood alcohol levels. Furthermore, the less efficient alcohol metabolism measured in the S_1 subjects, as compared to that of their S_3

counterparts, cannot be attributed to differential ethanol intake, since this characteristic appeared in all S_1 subjects, including the subjects assigned to one of the two water treatment groups. This distinction in alcohol metabolism between the S_1 and S_3 lines appeared to be of constitutional origin. Therefore, it can be concluded from the results of the second study that the lower alcohol metabolism observed in the S_1 subjects was not prejudicial to their ability to function under a moderate dose of ethanol. Hence, this metabolic difference between the S_1 and S_3 animals was of significance when they were challenged with a hypnotic dose of ethanol and relatively trivial when they were repeatedly challenged with subhypnotic doses. There is no evidence in the research literature to substantiate an assumption that alcohol seeking behavior in humans is predominantly oriented toward the anaesthetic properties of alcohol. It is therefore suggested that inferences concerning the relation between preference for and tolerance to ethanol would better be drawn, as in the present study, from investigations where the ethanol challenge tests are made with moderate drug doses.

The second objective was to investigate the development of behavioral tolerance to a subhypnotic dose of ethanol in order to evaluate its relation to initial tolerance. The predominant role of learning in alcohol tolerance development had been stressed by several authors (Chen, 1968; 1979; de

Souza Moreira, et al, 1981; Wenger et al, 1980; 1981). The importance of the learning factors became apparent to the present author when a complex cognitive task (Chen, 1968) was used in a pilot study to evaluate tolerance development in S_1 and S_3 animals. The learning disability of the S_3 subjects confounded the results and prevented an appropriate assessment of tolerance in these animals. Therefore, in the second experiment, the choice of the experimental paradigm and of the motor task used to evaluate behavioral tolerance was directed toward the control of learning factors. The rotarod apparatus was chosen as one that would counteract rather than emphasize the differences in learning ability that characterize the S_1 and S_3 rats. Under the present experimental conditions, the rate of acquisition of behavioral tolerance was found to be similar for the S_1 and S_3 rats. Moreover, the development of behavioral tolerance was not affected by the various treatment schedules characterized by varying exposure to alcohol or by a technique known to accelerate the establishment of behavioral tolerance (Le Blanc et al, 1973; 1976). Rather, the opportunity to practice the task under the influence of alcohol appeared to be the most important element in the development of behavioral tolerance. However, as previously mentioned, the S_1 animals exhibited a greater neural tolerance than their S_3 counterparts, since they adapted to the disabling effect of ethanol despite their rising blood

alcohol levels. Whether these results support the contention that learning merely facilitates the development of tolerance (Kalant, et al, 1971; Le Blanc, et al, 1976) rather than being a true adaptive function in alcohol tolerance remains to be investigated.

The third objective was to determine the influence of behavioral tolerance acquisition on subsequent alcohol consumption and to evaluate the importance of alcohol metabolizing enzymes in alcohol intake. Changes in ethanol consumption were observed after the acquisition of behavioral tolerance. These changes occurred independently of the inital preference manifested by the S_1 and S_3 animals and appeared to be associated with the development of behavioral tolerance rather than with the animal's initial tolerance to ethanol. This finding would suggest that the behavioral adaptation to the disabling effect of ethanol is decisive for the further ingestion of alcohol.

The changes in ethanol ingestion observed following behavioral adaptation to alcohol, could not be related systematically to differences noted in liver and brain enzymatic patterns of alcohol and aldehyde dehydrogenases. Differences in enzymatic patterns were observed in female subjects only. The alcohol dehydrogenase activity was found to be lower in female rats than in their male counterparts, wherever the former were assigned either to alcohol treatment or water gavage groups. Thus, it appears that the decrease

in ADH activity noted in females may not be related only to the ethanol exposure per se. Furthermore the finding of a lower ADH activity in the animals of the S_1 high-preference strain raises serious questions about the possibility of a significant role of alcohol dehydrogenase in the determination of ethanol preference in male and female S_1 and S_3 rats of the Tryon strain.

Differences in the enzymatic patterns of liver and brain ALDH were noted solely in females of the high-preference S_1 strain after exposure to ethanol. In agreement with Amir's findings (1978a), it was noted that the high-preference rats of the S_1 strain, assigned to the water treatment groups, exhibited a greater brain capacity to oxidize aldehyde. In contrast to his findings however, the mitochondrial liver capacity of the S_1 rats was found to be similar to that of the S_3 animals. These results validate Amir's conclusion that preference for ethanol is associated with the brain's capacity to oxidize aldehyde. However, the observation of a lower brain aldehyde oxidizing capacity in the S_1 females following chronic exposure to ethanol is in opposition to some previous findings originating from the same laboratory (Amir, 1977; 1978a). Two speculations follow from these findings. The first assumption is that the brain ALDH enzyme may be important to the specification of initial preference for ethanol but of no great significance for subsequent ethanol ingestion. Alternatively, the activity of

this enzyme may, in addition to determining preference for ethanol, sustain the level of intake in subjects that consume large amounts of alcohol. The consideration of the latter assumption raises some doubts regarding the suggestion put forward by Amir and collaborators (1980) that the metabolism of acetaldehyde is the principal parameter of the reinforcing properties attributed to acetaldehyde in the control of alcohol ingestion. The present results would instead suggest that it is the accumulation of acetaldehyde in the brain and not its metabolism which sustains high levels of alcohol intake. Hence further investigation on the brain aldehyde oxidizing capacity would be needed in order to clarify the role of acetaldehyde in the initiation and maintenance of ethanol consumption.

Summary of contributions to knowledge

Several discrepancies in the animal alcohol research literature concerning the relation between preference for and tolerance to ethanol in strains of rats showing differential preference for ethanol prompted this investigation. Moreover, few authors have shown an interest in the development of tolerance in such strains of rats and none have examined the impact of behavioral tolerance development on subsequent intake of ethanol.

The present research has provided support for the contention that preference for and initial tolerance to ethanol are related and are determined by inherent patterns

of enzyme activities. The hypothesis of a predominant involvement of brain aldehyde oxidizing capacity in the determination of initial preference was also strengthened. The results from this investigation further suggest that behavioral adaptation to alcohol may be decisive for the subsequent ingestion of alcohol. However, this adaptation is not necessarily dependent on initial preference for and/or initial tolerance to ethanol. Finally, the changes in alcohol ingestion after the development of behavioral tolerance were associated with a lower liver and brain aldehyde oxidizing capacity only in the females of the high preference strain (S_1). This finding leads to a reconsideration of the role of acetaldehyde and its possible relation to the mechanisms which regulate alcohol consumption.

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APPENDIX I

Mean Absolute ethanol intake (ml/kg body weight/day) for male and female rats of the S_1 and S_3 Tryon strain undergoing the voluntary ethanol intake schedule (experiment 2).

Mean Absolute Ethanol intake (Means ± SEM) [a]

time periods	S_1 male	S_1 female	S_3 male	S_3 female
1	5.9± 1.3	10.9± 2.0	4.6± 0.8	5.9± 1.1
2	8.0± 1.4	12.1± 2.6	5.9 ± 0.8	6.0 ± 1.0
3	7.3± 1.7	10.1± 1.9	5.9± 1.2	4.2 ± 1.3
4	7.4± 1.8	9.9± 1.4	4.6 ± 1.6	4.1 ± 0.8

[a] The means of absolute ethanol consumed during the 10 day interval (each time period represents 10 days) between test days were measured from the means of ethanol intake of individual animals.

APPENDIX II

Mean blood alcohol estimates[a] obtained from male and female rats of the S_1 and S_3 Tryon strain following their performance on the rotarod task.

Strain	Sex	Test Days	Voluntary intake		Involuntary intake	
			Water	Ethanol	Water	Ethanol
S_1	male	1	0.93 ± 0.34	0.94 ± 0.20	0.79 ± 0.26	0.80 ± 0.24
		2	0.80 ± 0.36	0.75 ± 0.28	0.96 ± 0.23	0.98 ± 0.30
		3	0.88 ± 0.41	0.71 ± 0.17	1.03 ± 0.22	0.92 ± 0.34
		4	0.99 ± 0.36	1.06 ± 0.31	0.79 ± 0.39	0.94 ± 0.31
		5	1.17 ± 0.32	1.09 ± 0.44	0.98 ± 0.13	0.99 ± 0.51
	female	1	0.75 ± 0.24	1.08 ± 0.32	0.73 ± 0.30	0.84 ± 0.35
		2	0.92 ± 0.38	0.96 ± 0.16	0.95 ± 0.29	0.82 ± 0.36
		3	0.98 ± 0.49	0.93 ± 0.42	0.96 ± 0.22	0.88 ± 0.25
		4	0.90 ± 0.33	0.93 ± 0.65	1.16 ± 0.64	0.97 ± 0.33
		5	0.75 ± 0.40	0.92 ± 0.40	1.10 ± 0.38	1.11 ± 0.23
S_3	male	1	1.01 ± 0.19	0.92 ± 0.27	0.96 ± 0.11	1.02 ± 0.24
		2	1.10 ± 0.15	1.14 ± 0.11	1.06 ± 0.10	1.00 ± 0.33
		3	0.79 ± 0.19	1.24 ± 0.40	0.99 ± 0.26	0.75 ± 0.27
		4	0.92 ± 0.11	0.99 ± 0.25	0.92 ± 0.15	0.89 ± 0.29
		5	0.79 ± 0.26	0.70 ± 0.14	1.09 ± 0.53	1.01 ± 0.22
	female	1	1.03 ± 0.34	0.72 ± 0.39	0.66 ± 0.25	1.05 ± 0.34
		2	0.99 ± 0.08	1.24 ± 0.26	0.85 ± 0.20	0.87 ± 0.28
		3	1.05 ± 0.26	1.08 ± 0.70	1.04 ± 0.28	0.93 ± 0.46
		4	1.06 ± 0.09	1.04 ± 0.41	0.86 ± 0.30	0.80 ± 0.24
		5	0.77 ± 0.28	1.10 ± 0.49	0.87 ± 0.26	0.90 ± 0.24

[a] Mean alcohol levels (means ± SD) are expressed in mg/ml of blood. The differences between group means are not statistically significant (analysis of variance).