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A COMPARISON OF CHOICE AND FORCED ALCOHOL CONSUMPTION AND ALCOHOL DEHYDROGENASE ACTIVITY

by

David Bertsch Gray

A Thesis
Submitted to the
Faculty of the School of Graduate
Studies in partial fulfillment
of the
Degree of Master of Arts

Western Michigan University
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A COMPARISON OF CHOICE AND FORCED ALCOHOL CONSUMPTION AND ALCOHOL DEHYDROGENASE ACTIVITY

David Bertsch Gray, M.A.

Western Michigan University, 1970

Earlier studies using both mice and rats have revealed a significant, positive correlation between preference of alcohol concentration and activity of the liver enzyme, alcohol dehydrogenase. In this study two groups of Sprague-Dawley rats, matched for sex, were presented alcohol by either a choice or forced condition for three twelve day periods in a choice-forced-choice or forced-choice-forced sequence. Data were recorded as the amount of alcohol consumed under each consumption condition. After the rats were sacrificed, their livers were removed and assays were made for the alcohol dehydrogenase activity. While several correlations between consumption and alcohol dehydrogenase activity were significant, it was concluded that the results did not support the hypothesis that consumption of high concentrations of alcohol is a function of the organism's metabolic capacity for alcohol exclusively.

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David Bertsch Gray

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The study of motivation has been directed toward finding some factor which would lead to an explanation of the initiation and termination of behavior. During the first half of this century, the interest focused on such motivational intervening variables as instincts, libido and drive. Recently, however, researchers have increasingly turned to the study of either the antecedent and consequent stimuli of the environment or the genetic and physiological variables in their attempts to explain behavior. While these new attempts have resulted in fewer hypothesized intervening variables, there has been a growing tendency toward incorporating both environmental and biological variables within the same experimental framework.

In the area of alcohol research, experimenters have attempted to incorporate both the stimuli and bio-genetic variables in their studies. The results and methods of these studies are described in several sections of the introduction in order to provide a basis for the experimental hypothesis.

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Environmental Variables Influencing Alcohol Consumption

The first problem confronting the student of alcohol consumption is the use of a valid method for presenting alcohol to the subject. One commonly used approach, the choice method, consists of presenting the organism with two tubes, one containing water and the other an alcohol-water mixture (McClearn, 1968). Because the presence of other variables could influence consumption, namely tube position, distance between tubes, and the concentration of alcohol used, the validity of this method has been questioned. While the technique of tube randomization has reduced the influence of the first two variables, there remains the question of which concentration of alcohol should be presented.

Fuller (1964), using the accepted pharmaceutical research practice of providing more than one dosage level in the administration of a drug, modified the

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two tube choice method of presenting alcohol.[1] Fuller's modification of the choice method consisted of the simultaneous presentation of six tubes which contained different concentrations of alcohol. By providing a wider range of alcohol concentrations, he reasoned, the experimenter can make more specific the quantification of the alcohol consumed. As a consequence, the researcher can then use the parametric correlational statistic rather than the rank-order correlations (Fuller, 1964).

A second major method of presenting alcohol to an experimental subject is forced consumption. This can be done by placing just one tube containing a given concentration of alcohol into the experimental chamber. This method measures themetabolic capacity rather than the preference of the organizm. This point is best illustrated by noting that studies using radioactive

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[1]Robert Myers wrote that "To use a single concentration for describing the alcohol preference of an animal is analogous to the pharmacologist's attempt to describe properties of a drug solely by administering one dose of that drug rather than by obtaining a dose-responsive curve." Myers, Robert D. "Ethyl Alcohol Consumption: Valid Measurement in Albino Rats", Science, 1968

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tracing of alcohol by the carbon 14 method have most often used the forced consumption condition (Segovia-Riquelme, 1962 and Schlesinger, et. al., 1967).

A second factor affecting alcohol consumption is the length of time the organism is exposed to alcohol. McClearn and Rodgers (1959) reported (1) that fourteen days of exposure was a sufficient time to determine the preference level of rats and (2) that the order of preference did not change over fourteen days. Mardones (1960) has shown that alcohol preference does not increase over long term exposures, and Rick and Wilson (1960) found that alcohol preference increased only slightly over a period of 120 days of testing with alcohol. In a more recent study McClearn (1968) learned that the preference of mice could be reliably measured if the length of exposure was limited to only three days. Thus while there is some evidence to the contrary, a measurement of preference for specific alcohol concentrations can be established within the first two weeks of exposure.

Exposing organisms to alcohol prior to testing for

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preference is a third environmental variable which has been tested for its effect on consumption. Myers and carey (1961) exposed several groups of rats to different concentrations of alcohol prior to testing them for their preference. The results revealed no significant difference in preference for concentration of alcohol between the group of rats which had been exposed to forced alcohol consumption and the group which was given no alcohol prior to testing. Rodgers et. al., (1963) state that only one strain of mice showed a significant increase in the concentration of alcohol preferred. These studies illustrate the reliability of preference methods used to test performance even when the organism is exposed to alcohol prior to testing.

Physiological And Genetic Variables Influencing The Consumption Of Alcohol

While many biological variables have been shown to have an important influence on alcohol consumption, only those crucial to a clear understanding of the

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hypothesis of this research will be reviewed. Several important variables are the metabolic pathways, site, products and enzyme(s) which determine(s) the rates of reactions which take place when alcohol is metabolized. Other equally important variables consist of the differences between sexes in alcohol consumption, the quantitative distribution of consumption and of liver enzyme, alcohol dehydrogenase, for alcohol catabolism.

An investigation into the catabolism of alcohol illustrates the important role of alcohol dehydrogenase in the break down of alcohol. The products of the first catabolic reaction of alcohol are acetaldehyde, DPNH and H^+ (White, Handler and Smith, 1954). This reaction is catalyzed by the liver enzyme, alcohol dehydrogenase (ADH), and is an important factor in determining the rate of reaction (Dajani, Danielski, and Orten, 1963).

There have been conflicting reports concerning the differences in consumption between the sexes. Zarrow et. al., (1960) found that there was no sex difference in preference for alcohol exhibited by rats. However, in his

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review article Lester (1966) states that there is a need for more research into the sex variable and its role in the consumption of alcohol.

The results of several experiments have shown the preferential consumption of alcohol to be distributed in a quantitative manner over several generations of mice. Mirone (1958) found that preference for relatively high concentrations of alcohol could be maintained for several generations through selective breeding of mice. In 1959, McClearn and Rodgers reported that different inbred strains of mice had consistently different preferences for specific concentrations of alcohol. They bred "high drinkers", mice preferring high concentrations, with extremely "low drinkers", mice consuming almost no alcohol, and found that the distribution of the consumption record of the F1 generation mice was a group preferring a relatively moderate concentration of alcohol. The differences in preference for alcohol as a function of strain differences seemed to be quantitative in nature (McClearn and Rodgers, 1959 and 1961).

Fuller (1964) used a different method of presenting the alcohol (six tubes with six different concentrations of

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alcohol) and found that by selection and back-crossing mice for their preference, the preference could be maintained for successive generations. The distributions of these F1 generations were dominated by either the genes from the "drinker" strain or the "non-drinker" strain. All these studies offer some evidence for a correlation between some enzyme and behavioral measures of alcohol consumption, but they do not specify the chemical involved.

Kurt Schlesinger (1964) found a significant relationship between the consumption of alcohol by "drinker" mice and an enzyme, alcohol dehydrogenase (ADH). By carefully breeding several generations of mice, Schlesinger separated the "drinker" mice from the "non-drinker" mice. Next, Schlesinger assayed the livers of both the "drinker" and "non-drinker" strains of mice to determine the ADH activities of the individual livers. The results of this assay confirmed Schlesinger's hypotheses that mice consuming high concentrations of alcohol by choice possessed large quantities of ADH and that mice bred for lack of preference for alcohol had the least amount of ADH. In support of Schlesinger's findings, Thiessen et.al. (1967) indicated a highly significant correlation between consumption and ADH within a strain of rats.

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This apparent discovery of the enzyme underlying the consumption-metabolism relationship was not observed by Segovia-Riquelme et.al. (1962). In an attempt to discover the differences in metabolic rates of "drinkers" and "non-drinkers", they labeled alcohol with radio-active carbon fourteen (14 Carbon). The results indicated no significant difference in the rate of alcohol metabolism between "drinker" and the "non-drinker" rats. However, using this same technique Schlesinger et.al. (1967) found that mice from a strain which typically consumed higher concentrations of alcohol by choice also metabolized alcohol more rapidly than mice from a strain which typically consumes very little of even the lowest concentrations of alcohol.

A further question must be raised about the hypothesized ADH-consumption relationship if alcohol consumption is assumed to be quantitatively inherited (McClearn and Rodgers, 1959 and 1961) and the ADH enzyme to be determined by one gene. Schlesinger et.al. (1967) acknowledge this problem when they state that while the rate of alcohol metabolism is most likely qualitatively determined, choice consumption of alcohol is probably quantitative in nature. In summary, if the activity of ADH is the only measure of metabolic capacity and the ADH levels are determined by one gene, then the

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quantitative distribution of the consumption of alcohol can not be fully explained with just the knowledge of the ADH activity. Basic to any investigation into this problem of genetic distributions of ADH and consumption of alcohol is the question of their relationship. Since the Schlesinger (1964 and 1967) and Segovia-Riquelme et.al. (1962) studies produced results which were conflicting, there is need for further investigations into the ADH activity-alcohol consumption relationship.

The Hypotheses Of The Experiment

The first question under consideration in this experiment is: What is the relationship between the ADH activity level of rats and their consumption of alcohol? The second problem which is considered is that if this relationship is shown for choice consumption, will the relationship hold for alcohol consumption under forced conditions? Following the suggestion made by Fuller (1964) a four tube choice method will be used in this study in place of the usual two tube choice method for presentation of alcohol. A second method of behavioral measurement will be made by using an increasing concentration of alcohol in just one tube. A comparison between the correlations

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obtained from both behavioral measures and the ADH assays should provide valuable data for future studies concerned with alcohol consumption and metabolic capacity. The sample population will be sub-divided by sex in an attempt to observe any sex differences in the consumption metabolism relationship.

If neither method of presenting alcohol results in significant correlations with the quantity of ADH present in the liver, then the findings of Segovia-Riquelme et.al. (1962) will be supported. If, on the other hand, the data supports the studies made by such other investigators as Schlesinger (1964 and 1967) and Theissen et.al. (1967), then there will be additional evidence for the hypothesis that the capacity for alcohol metabolism is highly correlated with consumption under the choice method of testing alcohol preference. By providing a more direct measure of the metabolic capacity of ADH, the forced method of presenting alcohol will be useful in determining the generality of the "drinker" behavioral phenotype for future genetic studies.

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METHOD

Subjects: The forty naive, Sprague-Dawley rats used in this experiment were divided into two groups called Group A and Group B. Each group was made up of ten females and ten males. At the initiation of the experiment the age range of the rats was 95 to 116 days.

Apparatus: Figure 1 illustrates the apparatus used in presenting alcohol to the rats. Fifty milliliter graduated cylinders were used to measure the daily consumption. Several tightly sealed volumetric flasks kept the different concentrations of alcohol from evaporating.

Procedure: The procedure section is divided into three parts. The first part deals with the designation of the rats into two treatment groups, the consumption of alcohol into choice or forced conditions, and the order in which the conditions were presented to the two groups. The second part deals with the measurements made for the consumption of alcohol, and the third part covers the measures made for ADH activity.

Designation of Treatment Groups, Conditions And Order of Alcohol Administration

At the beginning of the experiment the weight and sex

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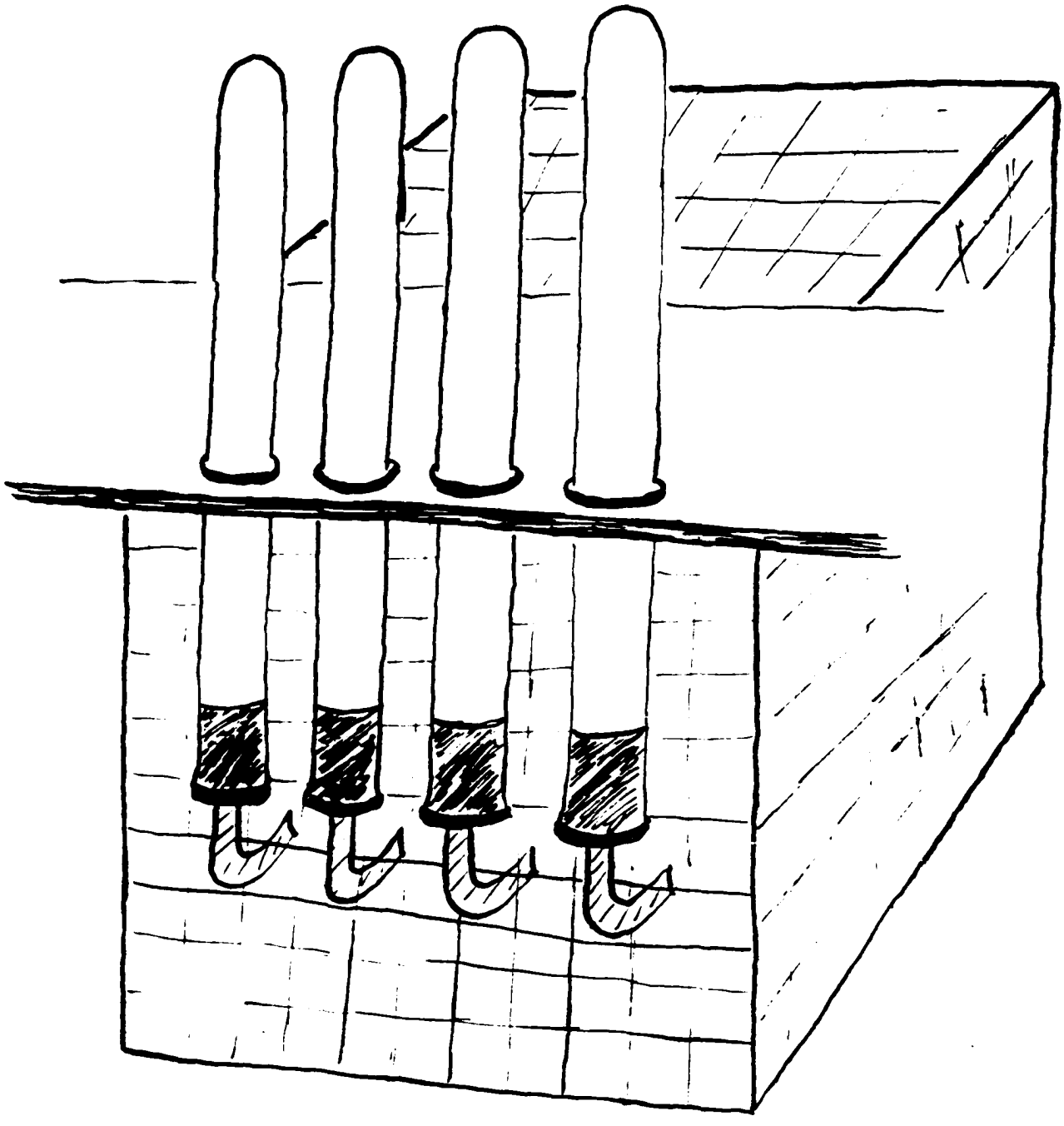

FIGURE 1: This is one of forty cages (9"x7"x11") used in this experiment. Each test tube is a six inch tube with a rubber stopper and metal stem projecting into the cage. The position of the stems and tubes was randomized each day. The liquid contained in the tubes was changed daily. All stems projected an equal distance into the cage.

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of the twenty rats in Groups A and B were determined. The ten males and ten females of Group A were matched for sex, age and weight with the rats of Group B.

Groups A and B were presented alcohol in either a choice or forced condition. The choice condition of presenting alcohol consisted of placing four test tubes with 0%, 5%, 15% and 30% alcohol v/v (Thor et.al., 1964) into each cage. This condition will be abbreviated as C, choice. The position of these tubes was randomized daily. The second method used to present alcohol was the increasing forced condition which involved presenting the rat with just one test tube per cage (abbreviated as F). The concentration of alcohol in this tube was increased each day by 4% beginning with a 1% solution (Gray, 1968).

In order to control for the influence of presentation order on consumption, the order of presenting the two conditions, C and F, differed for Groups A and B. Table I illustrates that Group A is presented the different consumption conditions in the C-F-C order while Group B was given alcohol in the F-C-F order. Because of the possible dehydration effect of alcohol, the rats were exposed to either condition for only twelve consecutive days with a two day period of free access to water interposed before resumption of the testing conditions.

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TABLE I

Procedures Used For This Experiment

Day	Method Of Presentation	Treatment Of Group A
1-12	Choice	Four tubes were presented to each rat. The tubes contained 0%, 5%, 15% and 30% alcohol (v/v).
13-14	Water	The rats were given free access to water.
15-27	Forced	Only one tube was placed into each rat cage. The percent of alcohol in that tube was increased by 4% each day.
28-29	Water	The rats were given free access to water.
30-42	Choice	Four tubes were presented to each rat. The tubes contained 0%, 5%, 15% and 30% alcohol (v/v).
43-48	Water	The rats were given free access to water.
49-55	Decapitation	The rats were decapitated and their livers removed immediately. Four homogenates were made from each liver. All homogenates were frozen. The homogenates were thawed just prior to measuring the ADH activity which was accomplished by observing the differences in spectrophotometer readings for each minute
56-90	ADH Assay	The homogenates were thawed just prior to measuring the ADH activity which was accomplished by observing the differences in spectrophotometer readings for each minute

Day	Method Of Presentation	Treatment of Group B
1-12	Forced	Only one tube was placed into each rat cage. The percent of alcohol in that tube was increased by 4% each day.
13-14	Water	The rats were given free access to water.

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TABLE I (continued)

Day	Method Of Presentation	Treatment of Group B
15-27	Choice	Four tubes were presented to each rat. The tubes contained 0%, 5%, 15% and 30% alcohol (v/v).
28-29	Water	The rats were given free access to water.
30-42	Forced	Only one tube was placed into each rat cage. The percent of alcohol in that tube was increased by 4% each day.
43-48	Water	The rats were given free access to water.
49-55	Decapitation	The rats were decapitated and their livers removed. Four homogenates were made from each liver. All the homogenates were frozen.
56-90	ADH Assay	The frozen homogenates were thawed just prior to measuring the ADH activity by observing the differences per minute in spectrophotometer readings.

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Measurements Of Alcohol Consumption

Measurements of the amounts of alcohol consumed were recorded daily. Each day the test tubes were filled to a line designating 50 ml. with the appropriate concentration of alcohol, stoppered, inverted and placed in the cage. Since a prior experiment by the author (Gray, 1968) revealed that some of the liquid from each tube was lost due to evaporation, a control was employed. Ten tubes filled with different concentrations of alcohol were placed in empty cages and the mean amount of liquid lost was subtracted from the experimental test tubes.

There were two methods used to calculate the amount of alcohol consumed. The first, absolute alcohol consumed, was determined by measuring the amount of liquid consumed from each tube each day. After subtracting the correction factor, the amount consumed from each tube was multiplied by the percent alcohol contained in that tube. This resulted in the daily amount of absolute alcohol consumed from each tube. A mean of these results for the twelve exposures was calculated and used as the mean amount of absolute alcohol consumed. A second measure, the relative amount of alcohol consumed, was computed by dividing the amount of absolute alcohol consumed per day by the total liquid consumed per day (absolute

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alcohol divided by absolute alcohol plus water). The mean of these values was used to correlate relative alcohol consumption to ADH activity.

Measurement Of Alcohol Dehydrogenase Activity

After a period ranging from six days to two weeks of free access to water the rats of Groups A and B were decapitated, livers removed and an assay made for the activity of alcohol dehydrogenase. Appendix A gives the exact prodedure followed in determining alcohol dehydrogenase activity.

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RESULTS

The data contained in this study consist of the many correlations made between several consumption conditions and several measures of ADH activities. Because the number of correlations is large, this section is divided into two parts: (1) correlational data from the choice consumption condition and (2) correlational data from the forced condition.

To measure the possible effect of sex on the consumption-metabolism correlations, the data from Groups A and B were divided into male and female sample populations. This resulted in the following six sample populations: (1) All the rats of Group A, (2) all the males of Group A, (3) all the females of Group A, (4) all the rats of Group B, (5) all the males of Group B, and (6) all the females of Group B. The two methods of presenting alcohol were called the Choice (C) and Forced (F) conditions. The differing amounts of alcohol consumed were recorded as either absolute or relative alcohol quantities. The homogenates of liver enzyme, ADH were of either a 50 mg/ml or 100 mg/ml concentration. The activity of ADH was recorded as (1) the change in concentration per minute (c/m), (2) the change in concentra-

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tion per minute per gram of liver (c/m/l) and (3) the change in concentration per minute per gram of rat (c/m/wt).

Part I: Correlational Data For Absolute And Relative Measures Of Alcohol Consumed by Groups A And B under Choice Conditions And The Activity Levels Of The Liver Enzyme Alcohol Dehydrogenase.

Group A

First Choice Presentation - absolute alcohol measure

The only significant correlations found in the first test of alcohol preference were the correlations between the absolute amount of alcohol consumed by the females of Group A and the three measures of ADH activity at the 50 mg/ml concentration, (see Table II). There were no significant correlations for the Group A rats as a whole or the males of Group A measured separately (see Table VI, Appendix B).[2]

First Choice Presentation - relative alcohol measure

The consumption by the females correlated significantly with the three measures of ADH activity at the 50 mg/ml con-

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[2]The reader will be referred several times to the appendix section of this paper for results which, in the judgment of this author, were not essential to an accurate portrayal of the outcome of the experiment.

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TABLE II

Correlation Coefficients for Absolute And Relative Alcohol Quantities Consumed By The Females Of Group A In The First Presentation Of The Choice Condition And The Reaction Rate Measures Of The 50 mg/ml Concentration Of The Liver Enzyme ADH

Measures Of ADH Activity	Coefficients Of Correlation	
	Absolute Alcohol	Relative Alcohol
Change in concentration per minute	+.701*	+.886**
Change in concentration per minute per gram liver	+.648*	+.841**
Change in concentration per minute per gram of rat	+.633*	+.851**

*Significant to the .05 level of confidence
**Significant to the .01 level of confidence

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centration when the alcohol consumption was measured relative to the total amount of liquid consumed (see Table II). The consumption of alcohol by the males and females of Group A was not significantly correlated with any of the ADH activity measures. However, the males of Group A showed a significant correlation (.05 level) between the relative measure of alcohol consumed and the 100 c/m/wt measure of ADH activity (see Table VII, Appendix B).

Second Choice Presentation - absolute alcohol measure

When the males, females or males and females combined of Group A were tested for a second time under choice conditions, there were no significant correlations between alcohol consumption measured as absolute quantities and any measure of ADH activity (see Table VIII, Appendix B).

Second Choice Presentation - relative alcohol measure

When themale rats of Group A consumed alcohol in the second choice condition, the relative amount of alcohol consumed correlated significantly but negatively with all three ADH measures at the 50 mg/ml concentration (see Table III). Neither the Group A females nor the Group A males and females combined consumed alcohol in a manner which

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TABLE III

Correlation Coefficients For The Relative Amounts Of Alcohol Consumed By Males Of Group A During The Second Presentation Of The Choice Condition And The 50 mg/ml Concentration Of ADH

Measures of ADH Activity	Coefficients Of Correlation
Change in concentration per minute	-.785**
Change in concentration per minute per gram liver	-.707*
Change in concentration per minute per gram of rat	-.791**

*Significant to the .05 level of confidence
**Significant to the .01 level of confidence

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could be correlated to any significant degree with their ADH activity (see Table IX, Appendix B).

Group B

First Choice Presentaticn- absolute alcohol measure

The first exposure to the choice condition for Group B rats was subsequent to their exposure to alcohol under the forced condition. There were no significant correlations obtained for either males and females as a group or the males and females individually where consumption was measured as absolute amounts of alcohol consumed (see Table X, Appendix B).

First Choice Presentation - relative alcohol measure

There were no significant correlations between the relative amounts of alcohol consumed and the three activity measures of either concentrations of ADH for the males and females, the male or the females of Group B (see Table XI, Appendix B).

Part II: Correlations Between Absolute And Relative Measures Of Alcohol Consumed By Groups A and B Under Forced Conditions And The Activity Level Of ADH

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Group A

First Forced Presentation - absolute alcohol measure

Group A rats had been exposed to a choice condition prior to testing under forced consumption. The only significant correlation (.05 level) obtained was for the males and females combined (see Table XII, Appendix B). When consumption was measured as absolute-alcohol consumed and activity of the 100 mg/ml concentration of ADH was measured as c/m/l and c/m/wt, a significant correlation (.05 level) was revealed (see Table IV).

First Forced Presentation - relative alcohol measure

There were no significant correlations between the relative consumption measures and ADH measures for any subpopulation of Group A when presented with the first choice condition (see Table XIII, Appendix B).

Group B

First Forced Presentation - absolute alcohol measure

There were two significant correlations (.05 level) for alcohol consumption and enzyme levels for the combined

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TABLE IV

Correlations Between Absolute Quantities Of Alcohol Consumed Under The Interposed Forced Condition By All The Rats of Group A And The Several Activity Measures Of the 100 mg/ml Concentration of ADH

Measures Of ADH Activity	Coefficients Of Correlation
Change in concentration per minute	n.s.
Change in concentration per minute per gram liver	+.4621*
Change in concentration per minute per gram of rat	+.4454*

*Significant to the .05 level of confidence

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male-female group B rats. This can be seen in Table V where the c/m and c/m/l ADH activity measures at the 100 mg/ml concentration correlated with the consumption of alcohol in the first forced presentation. A significant (.05 level) negative correlation was obtained when the consumption of Group B females was compared to the c/m/wt activity measure of the 100 mg/ml concentration of ADH (see Table XIV, Appendix B).

First Forced Presentation - relative alcohol measure

There were no significant correlations between the relative consumption measures and ADH measures for any subpopulation of Group B rats (see Table XV, Appendix B).

Second Forced Presentation - absolute alcohol measure

After Group B rats had been exposed to a choice condition, they were presented alcohol in a forced condition for a second time. Again most correlations were not significant, but two significant correlations were found for the Group of male and female rats. When the absolute amount of alcohol was correlated with the 100 mg/ml concentration of ADH for the c/m/l and c/m/wt activity measures, there is a significant correlation (see Table V). One signifi-

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TABLE V

Correlations Between Absolute Quantities Of Alcohol Consumed Under The Interposed Forced Condition By All The Rats Of Group B And The Several Activity Measures Of The 100 mg/ml Concentration Of ADH

Measures Of ADH Activity	Coefficients Of Correlation First Presentation	Second Presentation
Change in concentration per minute	+.5014*	n.s.
Change in concentration per minute per gram liver	+.5527*	+.6655*
Change in concentration per minute per gram of rat	n.s.	+.5235*

*Significant to the .05 level of confidence

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cant negative correlation (.05 level) was obtained when the females of Group B were exposed to the forced condition for the second time. This correlation was significant only for the c/m/l measure of the 100 mg/ml concentration of ADH (see Table XVI, Appendix B).

Second Forced Presentation - relative alcohol measure

There were no significant correlations obtained when relative quantities of alcohol consumed by any sub-population of Group B were correlated with any activity measure of either concentration of ADH (see Table XVII, Appendix B).

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DISCUSSION

The central focus of this experiment has been to test the hypothesis that the amount of alcohol consumed is correlated with the activity level of the liver enzyme alcohol dehydrogenase. This discussion will center on not only the major variable, presentation of alcohol in choice or forced conditions, but also on the secondary variables: exposure to alcohol sequences, the sub-sample populations, the alcohol consumption measures, the ADH activity measures and the ADH homogenate concentrations. The second part of the discussion will deal with the influence of the genetic distribution, the number of tubes, and the concentrations of alcohol on the consumption-metabolism relationship. Finally, some suggestions for further research will be proposed.

In order to make any conclusive comparisons between the correlations obtained from the choice and forced consumption conditions, two criteria must be met. The first is that a valid measure of consumption for both the choice and forced conditions be demonstrated. The twelve day exposure to alcohol with daily changes in the alcohol concentrations produced an adequate measure of alcohol

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consumption under both conditions. The second criterion for a comparison between choice and forced consumption correlations rests with the ability to demonstrate that the consumption of alcohol under both conditions remained consistent for all exposures to alcohol.

While there were several significant correlations found for the choice consumption conditions, the fact that these correlations were not consistent for Group A from the first presentation of alcohol under the choice condition to the last exposure to alcohol under choice conditions places the significance of these results in doubt. The first presentation of alcohol under choice conditions to Group A females produced significant correlations both when measured as relative to the total liquid consumed. These results agree with several earlier studies (McClearn and Rodgers, 1959 and 1961, Rodgers and McClearn, 1963, Schlesinger, 1964 and Theissen et.al., 1967) which have depicted a significant positive relationship between the consumption of alcohol in the choice condition and the metabolic capacity of the organism for alcohol. However, there are several reasons to question the validity of the significant results found in the present study. In the first place, while two measures of alcohol consumption,

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absolute and relative, were in agreement, the second presentation of choice consumption did not result in any significant correlations for the females of Group A. The three activity measures of the 50 mg/ml concentration all resulted in significant correlations for the consumption of alcohol by the females of Group A, but these relationships were not observed to be significant in the 100 mg/ml concentration of ADH. This same criticism (i.e., inconsistency) can be applied to the significant correlations found when the males of Group A were presented alcohol in the choice condition for the second time.

The second prerequisite for comparison of correlations made under choice or forced conditions is that there be a valid relationship found between consumption of alcohol under forced conditions and ADH activity measures. The forced method did produce several significant correlations when the measures were based on the absolute alcohol consumed by the total male-female sub-sample population. The validity of this relationship was supported by two findings: (1) the relationship was significant for both the first and second exposure of Group B rats and (2) the relationship was significant for the only exposure of Group A animals to forced conditions. The validity of this rela-

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tionship was also supported by the fact that for both Groups A and B, the correlations were almost always significant among the activity measures of the 100 mg/ml concentration of ADH. However, the stability of this relationship canbe questioned because neither sub-sample population of males or females showed any positive, significant correlation, and in fact, there were three examples of significant negative correlationships in the female population. The 50 c/m, 50 c/m/l and 50 c/m/wt measures showed no significance. In light of these results, it would seem that the relationship between forced consumption and ADH activity should be more thoroughly investigated.

A study by McClearn et.al. (1964) showed that mice which had been assayed for ADH activity immediately after a period of forced alcohol consumption showed an increased level of ADH activity. Those mice which had a period of water consumption following the alcohol consumption showed a decreased ADH activity. It would seem safe to state that under forced conditions the ADH activity level is at a maximum. The consumption values obtained under the forced condition should show the highest correlation with ADH activity because mice with high ADH would consume a

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relatively large amount of alcohol compared to those rats with little or no ADH enzyme. Thus the distinction between the "drinkers" and "non-drinkers" should be clear, and a comparison of the correlations made for the forced and for the choice conditions should show higher correlations for the forced consumption. However, because the consumption-metabolism relationship itself has been shown to be less than convincing by the data of this experiment, it does not seem reasonable to compare the ADH-metabolism relationships as a function of the conditions existing for the consumption of alcohol.

The order of presenting alcohol, the several subsample populations, the consumption measures, the ADH activity measures and the ADH concentrations all influenced the stability of the correlations in both the choice and forced conditions.

A comparison of the first and second presentation of the choice condition to Group A rats reveals no similarity between the correlations. The results can be explained by either the interposed treatment (forced consumption) or the length of exposure. Since the study was not designed to measure these independently, further study is necessary to find which variable, if either, produced change in

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choice consumption. Similarly, the first and second forced conditions for Group B animals produced little correspondence of correlations.

The different sub-sample populations were used in an attempt to discover if the sex of the rat was an important variable. A comparison of several correlations illustrates the lack of consistent results found for the total male-female sub-sample populations. Under the forced condition there were no significant correlations found for either the male or female sub-sample populations. The females of Group A in the choice condition consumed alcohol in a manner which correlated significantly with several ADH measures, but the male and the total male-female sub-sample population did not exhibit significant correlations. Based on these results, sex does not seem to be a major variable influencing the consumption-metabolism relationship.

The absolute and relative measures of consumption were used in this study to see which measures best depicted the hypothesized relationship between consumption and metabolism of alcohol. The data of this experiment show that the absolute measure produced more significant correlations than under the forced consumption conditions, but there

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seemed to be no difference for the choice condition correlations. When measured as absolute alcohol consumed, there were six significant correlations, but under the forced condition as measured by relative consumption there were no significant correlations. The choice condition produced significant correlations when measured as either relative or absolute alcohol consumed. In light of these results it is difficult to conclude which, if either, measure, forced or choice, represents the metabolism-consumption relationship.

Three measures of ADH activity were used to investigate the different correlations which resulted from correcting the amount of liquid consumed for weight of liver (c/m/l) or for total body weight (c/m/wt). While the data of this experiment show significant relationships in both the c/m/l and c/m/wt activity measures, the lack of consistency over the order of presentation, subsample populations, consumption conditions, concentration measures and consumption measures prevents any statement regarding the adequacy of any of the three measures.

Two concentrations of the liver homogenate were used to provide an index of validity for the spectrophotometer readings. The fact that the correlations drawn from the

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50 mg/ml or 100 mg/ml concentrations of ADH are rarely in agreement with each other presents a question to be answered by further research. Future research might attempt to find an optimal concentration for use with the spectrophotometer which would most reliably measure the reaction rate.

The discrepencies found between the results of this study and earlier studies suggests that the difference may be in the different procedures used. In this study four tubes, one with water and three with specific levels of alcohol were used while in most prior studies two tubes were used, one with some percent alcohol and the other with water (McClearn and Rodgers, 1959, and 1961, Rodgers and McClearn, 1963, Schlesinger, 1964 and Theissen et.al, 1967). The addition of several choices of relatively high concentrations of alcohol and the random positioning of the tube conceivably presented the rat with a more difficult discrimination. This might have resulted in the choice of water to avoid the aversive taste of the high levels of alcohol. This discrimination between water and alcohol may have generalized to the lower, previously accepted or preferred, level of alcohol. This would result in the aversion by the rat to

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all alcohol, and the consequent lack of the alcohol consumption would make the correlations of consumption and metabolism difficult to obtain. Future research should produce data based on the use of concentrations made in line with what the species or strain has been shown to prefer which could be used to support or reject this hypothesis.

One of the main problems with trying to correlate alcohol consumption and metabolism is that in order to sample the ADH the animal must be sacrificed. If one could develop a method of removing samples of the liver to test for ADH levels while the rat is still alive, it would add greatly to the study of the consumption-metabolism problem.

In summary, the relationship between consumption and metabolism of alcohol has been found in individual cases, but as a group, the relationship was not shown to be a stable one. A comparison between choice and forced correlations was not attempted because the correlations for each were very unstable when compared within and between consumption conditions. Presentation order, the sub-sample populations, the measures of alcohol consumption, the ADH activity measures and the ADH concentrations

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all illustrated the point that the relationship between consumption and metabolism, if it exists, is not a strong one. In future studies it would be well advised to find the genetic distribution of the sample population, take samples of the alcohol level in the blood of the rats, use more than one liver sample from each rat, analyze several different concentrations of the liver homogenate, and use several different methods of presenting alcohol to the rat.

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APPENDIX A

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ADH ASSAY

The following paragraph explains the ADH assay and follows very closely the one used by Rodgers, McClearn, Bennett and Herbert in 1963.

The livers of the rats were removed and weighed. They were divided into two sections each of which was diluted to 50 mg/ml or 100 mg/ml solutions. These samples of liver were then homogenized in a stainless steel homogenizer which was jacketed by crushed ice water at 0°C and centrifuged at 3,000 rpms. in an International refrigerated centrifuge. These homogenates were then frozen for from 40 - 100 hours before determining their activity as measured in concentration change per minute. The rather large spread in the number of hours frozen was due to the fact that the assays took four hours per animal. The ADH activity was ascertained by recording the rate of reduction of diphosphypyridine nucleotide (DPN^+ OR NAD^+), and the consequent increase in absorption at 340 millimicrons in a Beckman DU spectrophotometer. Each of the three cuvettes was filled with 2.7 ml. of a buffer solution which was made of .06M glycine, .06M NaCL, .03 NaOH and .0014M semicarbazide which resulted in a pH

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of between 9.6 and 9.8. The three cuvettes also contained 0.02 ml of DPN^+ solution containing 3.0 micromoles and 0.02 ml of DPN^+ solution containing 3.0 micromoles and 0.050 ml of 2% ethanol at 35°C. The spectrophotometer cell compartment was also at 35°C because the water jacket which surrounded the cuvettes was warmed by the 37°C water circulating through the water jacket. The frozen homogenates were thawed in a 35°C water bath for three minutes prior to measuring the optical density. Next, .1 ml of the supernant (the liver homogenate) in either the 50 mg/ml or 100 mg/ml solutions was added to the cuvettes. The mean of the differences in optical density readings[1] between zero and eighteen minutes and between two and twenty minutes was determined. The values obtained from these three tubes were corrected for evaporation by subtracting the value of the blank cuvette.[2] The net optical density difference was converted to micromoles of ethanol oxidized per minute (c/m). Although these figures are reported without correction,

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[1]These readings were taken at 340 millimicrons.

[2]The blank cuvette was prepared in exactly the same manner as the test cuvettes with the important exception that the 0.05 ml of 2% ethanol was not added to the blank cuvette.

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the data were further refined by dividing the concentration change per minute (c/m) by the weight of the liver (c/m/l) or by the weight of the rats (c/m/wt).

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APPENDIX B

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TABLE VI

Chart Of The Correlations Between Absolute Alcohol Consumption And The ADH Activity Of Group A Rats, Group A Male Rats And Group A Female Rats under the First Choice Condition

Measures of ADH Activity	Coefficients Of Correlation Group	Males	Females
Change in concentration per minute			
50 mg/ml dilution	.3221	-.005	.701*
100 mg/ml dilution	.1008	.112	-.033
Change in concentration per minute per gram liver			
50 mg/ml dilution	.2962	-.158	.633*
100 mg/ml dilution	.1464	.126	-.024
Change in concentration per minute per gram of rat			
50 mg/ml dilution	.2801	-.015	.633*
100 mg/ml dilution	.1014	.126	-.024

*Significant to the .05 level of confidence

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TABLE VII

Chart Of The Correlations Between Relative Alcohol Consumption And The ADH Activity Of Group A Rats, Group A Male Rats and Group A Female Rats Under The First Choice Condition

Measures Of ADH Activity	Coefficients Of Correlation Male-Female	Males	Females
Change in concentration per minute			
50 mg/ml dilution	.1836	.006	.886**
100 mg/ml dilution	.0987	.559	-.043
Change in concentration per minute per gram liver			
50 mg/ml dilution	.2056	.090	.841**
100 mg/ml dilution	-.1013	.580	.246
Change in concentration per minute per gram of rat			
50 mg/ml dilution	.1761	.036	.851**
100 mg/ml dilution	-.0635	.612*	-.224

*Significant to the .05 level of confidence
**Significant to the .01 level of confidence

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TABLE VIII

Chart Of The Correlation Between Absolute Alcohol Consumption And The ADH Activity Of Group A Rats, Group A Male Rats And Group A Female Rats Under The Second Choice Condition

Measures Of ADH Activity	Coefficients Of Correlation Group	Males	Females
Change in concentration per minute			
50 mg/ml dilution	.2812	.093	-.309
100 mg/ml dilution	.0648	.163	.446
Change in concentration per minute per gram liver			
50 mg/ml dilution	.3283	-.324	.370
100 mg/ml dilution	.2099	-.171	-.005
Change in concentration per minute per gram of rat			
50 mg/ml dilution	.2611	.013	.192
100 mg/ml dilution	.0646	.075	-.529

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TABLE IX

Chart Of The Correlations Between Relative Alcohol Consumption And The ADH Activity Of Group A Rats, Group A Male Rats And Group A Female Rats Under The Second Choice Condition

Measures Of ADH Activity	Coefficients Of Correlation Male-Female	Males	Females
Change in concentration per minute			
50 mg/ml dilution	-.2180	-.785**	.282
100 mg/ml dilution	-.1870	-.120	.170
Change in concentration per minute per gram liver			
50 mg/ml dilution	-.1870	-.707*	.090
100 mg/ml dilution	-.2015	-.153	-.164
Change in concentration per minute per gram of rat			
50 mg/ml dilution	-.2566	-.791**	.427
100 mg/ml dilution	-.2467	-.143	.309

*Significant to the .05 level of confidence
**Significant to the .01 level of confidence

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TABLE X

Chart Of The Correlations Between Absolute Alcohol Consumption And The ADH Activity Of Group B Rats, Group B Male Rats and Group B Female Rats Under The Interposed Choice Condition

Measures Of ADH Activity	Coefficients Of Correlation Male-Female	Males	Females
Change in concentration per minute			
50 mg/ml dilution	.0967	.211	-.036
100 mg/ml dilution	.0806	-.365	-.058
Change in concentration per minute per gram of liver			
50 mg/ml dilution	.2690	.126	.127
100 mg/ml dilution	-.1342	.232	.113
Change in concentration per minute per gram of rat			
50 mg/ml dilution	.2475	-.291	.011
100 mg/ml dilution	.2344	-.291	.032

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TABLE XI

Chart Of The Correlations Between Relative Alcohol Consumption And The ADH Activity Of Group B Rats, Group B Male Rats And Group B Female Rats Under The Interposed Choice Condition

Measures of ADH Activity	Coefficients Of Correlation Male-Female	Males	Females
Change in concentration per minute			
50 mg/ml dilution	.1575	.136	-.031
100 mg/ml dilution	.0871	-.254	.163
Change in concentration per minute per gram of liver			
50 mg/ml dilution	.1159	.177	.040
100 mg/ml dilution	.1219	.167	.248
Change in concentration per minute per gram of rat			
50 mg/ml dilution	-.0398	.100	.060
100 mg/ml dilution	.2442	-.088	.315

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TABLE XII

Chart Of The Correlations Between Absolute Alcohol Consumption And The ADH Activity Of Group A Rats, Group A Male Rats And Group A Female Rats Under The Interposed Forced Condition

Measures Of ADH Activity	Coefficients Of Correlation Male-Female	Males	Females
Changes in concentration per minute			
50 mg/ml dilution	.1329	-.328	.256
100 mg/ml dilution	.3593	-.207	.294
Change in concentration per minute per gram of liver			
50 mg/ml dilution	.4077	-.099	.281
100 mg/ml dilution	.4621*	-.150	.479
Change in concentration per minute per gram of rat			
50 mg/ml dilution	.3644	-.247	.363
100 mg/ml dilution	.4454*	-.094	.352

*Significant to the .05 level of confidence

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TABLE XIII

Chart Of The Correlations Between Relative Alcohol Consumption And The ADH Activity Of Group A Rats, Group A Male Rats and Group A Female Rats Under The Interposed Forced Condition

Measures Of ADH Activity	Coefficients Of Correlation Male-Female	Males	Females
Changes in concentration per minute			
50 mg/ml dilution	-.2167	-.317	.158
100 mg/ml dilution	-.0257	.244	-.502
Change in concentration per minute per gram of liver			
50 mg/ml dilution	-.1111	-.458	.188
100 mg/ml dilution	-.0847	.095	-.317
Change in concentration per minute per gram of rat			
50 mg/ml dilution	-.0851	-.394	.166
100 mg/ml dilution	-.0086	.173	-.562

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TABLE XIV

Chart Of The Correlations Between Absolute Alcohol Consumption And The ADH Activity Of Group B Rats, Group B Male Rats And Group B Female Rats Under The First Forced Condition

Measures Of ADH Activity	Coefficients Of Correlation Male-Female	Males	Females
Change in concentration per minute			
50 mg/ml dilution	.0230	.045	.158
100 mg/ml dilution	.5014*	.319	.287
Change in concentration per minute per gram liver			
50 mg/ml dilution	.4020	.155	.307
100 mg/ml dilution	.5527*	.332	.432
Change in concentration per minute per gram of rat			
50 mg/ml dilution	.3027	.076	.287
100 mg/ml dilution	.0575	.438	-.613*

*Significant to the .05 level of confidence

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TABLE XV

Chart Of The Correlations Between Relative Alcohol Consumption And The ADH Activity Of Group B Rats, Group B Male Rats And Group B Female Rats Under The First Forced Condition

Measures of ADH Activity	Coefficients Of Correlation Male-Female	Males	Females
Change in concentration per minute			
50 mg/ml dilution	-.1623	-.163	.286
100 mg/ml dilution	-.1221	-.213	.391
Change in concentration per minute per gram liver			
50 mg/ml dilution	-.2077	-.229	.209
100 mg/ml dilution	-.2036	-.153	.271
Change in concentration per minute per gram of rat			
50 mg/ml dilution	-.1298	-.152	.358
100 mg/ml dilution	-.0078	.064	.452

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TABLE XVI

Chart Of The Correlations Between Absolute Alcohol Consumption And The ADH Activity Of Group B Rats, Group B Male Rats And Group B Female Rats Under The Second Forced Condition

Measures Of ADH Activity	Coefficients Of Correlation Male-Female	Males	Females
Change in concentration per minute			
50 mg/ml dilution	-.0780	.045	-.122
100 mg/ml dilution	.3365	-.322	-.335
Change in concentration per minute per gram liver			
50 mg/ml dilution	.2619	-.162	-.313
100 mg/ml dilution	.6655*	-.391	-.614*
Change in concentration per minute per gram of rat			
50 mg/ml dilution	.3075	.055	-.377
100 mg/ml dilution	.5235*	-.234	-.497

*Significant to the .05 level of confidence

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TABLE XVII

Chart Of The Correlations Between Relative Alcohol Consumption And The ADH Activity Of Group B Rats, Group B Male Rats And Group B Female Rats Under The Second Forced Condition

Measures Of ADH Activity	Coefficients Of Correlation Male-Female	Males	Females
Change in concentration per minute			
50 mg/ml dilution	.3206	.328	-.199
100 mg/ml dilution	.0994	.527	-.185
Change in concentration per minute per gram liver			
50 mg/ml dilution	-.4164	.012	-.329
100 mg/ml dilution	-.2097	-.579	-.432
Change in concentration per minute per gram of rat			
50 mg/ml dilution	-.3781	.292	-.210
100 mg/ml dilution	.2582	-.523	-.329

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