EFFECTS OF LONG-TERM MODERATE ETHANOL INTAKE ON THE STRESS RESPONSE IN RATS

THESIS

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By

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The effect of ethanol on the stress response in rats was examined. Experimental animals were given 0.25 ml of 28 percent ethanol or 0.25 ml of water orally once a day, five days a week, for a period of twelve months and were then subjected to fifteen minute cold stress. Corticosterone levels in ethanol-treated males following stress were significantly lower (22 percent) than in the sham group. Adrenal weights in sham-treated females were significantly higher (15 percent) than in the ethanol group at the end of twelve months. Mortality in sham-treated males was significantly higher (60 percent) than in ethanol-treated males. The effects observed may be due to the sedative action of ethanol on cortical centers controlling the hypothalamus.
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CHAPTER I

INTRODUCTION

Over the centuries many different sources have claimed that moderate alcohol use is beneficial to health. However, only in recent years have scientific studies been conducted to determine the truth of this claim.

Several different studies have shown that moderate alcohol consumption is correlated with a lower incidence of coronary heart disease. The occurrence of myocardial infarction and artherosclerosis is lower in moderate drinkers than in non-drinkers (36).

Studies concerning the effects of alcohol on geriatric patients have shown that moderate alcohol intake results in an increase in social interactions, ambulation, and cognitive performance, and a decrease in the need for psychotropic medication and jacket restraint. In fact, it has been said that alcohol is the oldest tranquilizer in use (36).

The anxiety-reducing effect of alcohol is well known and may account for the persistent use of alcoholic beverages in most human societies. Various sources of stress and tension are present in both social and physical environments; therefore, the sedative action of alcohol has
often been used to moderate the effects of unavoidable stress and tension (11). Studies with human subjects using the galvanic skin conductor response to determine anxiety levels have shown that moderate amounts of alcohol are successful in reducing anxiety (21, 36). Animal studies have also demonstrated that moderate amounts of alcohol are effective in relieving fear, frustration, anxiety, and seizure behavior in rats (4, 11, 15, 30). However, anxiety and emotion are only two components of stress (36).

According to the General Adaptation Syndrome (G.A.S.) concept of Selye (31), a sudden and/or severe change in the environment induces a compensatory change in the animal mediated by what he called the pituitary-adrenal axis. The G.A.S. consists of three stages. The first stage is the Alarm Reaction which occurs upon exposure to non-adapted stimuli. During the second state, the State of Resistance, adaptation to the stressor is achieved. The third stage, the State of Exhaustion, occurs if the stressor is severe and is applied for a long period. During this phase the acquired adaptation is lost, and death may be the final result.

Response to a stressor is non-specific and occurs with any noxious stimulus. Stressors may be either physical or emotional in nature (6, 11, 31). In fact, several
researchers believe psychological stress initiates a stronger pituitary-adrenal response than does physical stress (5, 8, 37).

The pituitary-adrenal axis is activated when stimuli from the external and/or internal environments are relayed to the central nervous system. Impulses from the cerebral cortex activate the hypothalamus to release CRF, corticotropin releasing factor. CRF then induces the anterior pituitary to release ACTH, adrenocorticotropic hormone. ACTH travels via the blood to the cortex of the adrenal gland and stimulates it to produce and/or release an increased amount of corticosteroid hormones (5). These hormones have various actions that help the animal to withstand and survive stress.

For example, the mineralocorticoids, which include aldosterone, are involved with the fluid and electrolyte balance. They act upon the kidney causing an increase in sodium reabsorption and potassium excretion. Therefore, the mineralocorticoids are important regulators of blood volume and blood pressure, vital parameters to consider during stress conditions (24).

The glucocorticoids are concerned with protein, fat, and carbohydrate metabolism. They increase the breakdown of fats into fatty acids and the breakdown of proteins into amino acids. This, in turn, provides the liver with raw material for gluconeogenesis. The result is an increase in
circulating glucose, a most needed substance during and following stress (24).

The experiments described here present data concerning the effects of moderate amounts of ethanol on the pituitary-adrenal response to stress in rats. A moderate amount of ethanol for humans was defined as two ounces of eighty-five proof ethanol per day. This was translated into an equivalent amount for rats.

The effect of long-term moderate ethanol consumption on the acute cold stress response was determined by using changes in eosinophil counts and plasma corticosterone levels to indicate the involvement of the anterior pituitary and adrenal cortex respectively. The literature contains many reports stating that following stress conditions a decrease in eosinophils occurs indicating a rise in ACTH (27, 33). This is followed by an increase in plasma corticosteroids, including corticosterone, the major glucocorticoid found in rats (24).

Other parameters measured in this study included mortality and adrenal weights. The latter criterion has been used to indicate adrenal activity, but it is not as reliable as more specific biochemical criteria (6, 14, 31).

The specific aims of this study were twofold: (a) to determine if rats receiving moderate amounts of ethanol over a long period of time respond to acute cold stress more
effectively than rats not receiving ethanol and (b) to determine the role of the pituitary-adrenal axis in the acute cold stress response in ethanol-treated animals. The general aim of this study was to provide more data that might explain the reported benefits of alcohol on humans.
CHAPTER II

METHODS AND MATERIALS

Male and female Sprague-Dawley rats were used in this study. Animals were housed two per cage in an animal house with a twelve-hour light-dark cycle. All animals were fed Purina Lab Chow and tap water ad libitum.

Four groups of animals were used in this study: (a) ethanol-treated (twelve males, sixteen females); (b) sham-treated (twelve males, fourteen females); (c) control (six males, six females); and (d) ethanol determination (twelve males, twelve females). Two litters of ethanol-treated animals, two litters of sham-treated animals, and two litters of animals for ethanol level determinations were used. Control animals were littermates.

The ethanol-treated animals received 0.25 ml of 28 percent ethanol orally once a day, five days a week. The sham-treated animals received 0.25 ml of water orally once a day, five days a week. Ethanol and water were given between 9:00 and 10:00 am. A glass syringe with no needle was used for oral administration. Treatment began when the animals were two months old and ended when they were fourteen months old.
Control animals received no ethanol treatment, water treatment, or cold stress; these animals were used to determine non-stress corticosterone, eosinophil, and adrenal weight data.

The ethanol determination animals received 0.25 ml of 28% ethanol orally at the time of ethanol blood level determinations. They then had a tail blood sample taken at 0.5 hours post-ingestion and every hour thereafter until ethanol could no longer be found in the sample.

When the ethanol- and sham-treated animals reached fourteen months of age they were subjected to acute cold stress at four degrees Celsius for fifteen minutes. Cold stress experiments were conducted on four to six animals per day and were carried out between 9:00 and 11:00 am. Ethanol and water treatment ended twenty-four hours preceding cold stress. Immediately after cold stress the animals were injected with sodium pentobarbital; due to size differences, males were injected with 60 mg and females with 30 mg.

Fifteen minutes after cold stress a tail blood sample was taken for an eosinophil count. A standard white cell blood pipette was filled to the "0.5" line with blood and diluted to the "11" line with Discombe's diluting fluid. The pipette was then shaken for two minutes by hand and allowed to stand for fifteen minutes. After discarding the
first five drops from the stem of the pipette, the grid of a standard hemacytometer was filled with sample and allowed to stand for three minutes to permit settling of the cells. When Discombe's diluting fluid is used, all visible cells are eosinophils. The eosinophils in the four corner squares of the grid were counted and an average taken. The average number of cells was multiplied by a dilution factor of twenty and also multiplied by ten in order to determine the average number of eosinophils per cubic millimeter of blood (10).

Thirty minutes after cold stress a blood sample for corticosterone determination was taken from the dorsal aorta. Adrenal glands were then removed, stripped of fat, and weighed as a pair to the nearest ten-thousandth of a gram. A visual examination was conducted at autopsy to determine the presence of tumors or other pathological conditions.

Corticosterone levels were determined using the fluorometric method of Givner and Rochefort (9). The procedure included the following steps.

1. 6 ml of iso-octane (2,2,4-trimethylpentane), 2 ml of deionized water, and 2 ml of sample plasma were placed into a glass test tube.

2. This mixture was shaken by hand for one minute and centrifuged at 3000 rpm for five minutes.
3. The iso-octane layer was aspirated and discarded.
4. 5 ml of deionized water and 15 ml of reagent grade chloroform were added to the test tube.
5. The mixture was again shaken for one minute and centrifuged for five minutes.
6. The aqueous layer was aspirated and discarded.
7. 1 ml of 0.1 N sodium hydroxide was added to the chloroform layer.
8. The mixture was shaken for one minute and centrifuged for five minutes.
9. The sodium hydroxide layer was aspirated and discarded.
10. 5 ml of a 36 N sulfuric acid-95% ethanol solution (7:3 ratio) was placed into a second glass test tube.
11. 10 ml of the chloroform layer was added to the sulfuric acid-ethanol solution and the time was noted.
12. The mixture was shaken for fifteen seconds and centrifuged for five minutes.
13. The contents of the test tube were then allowed to incubate at room temperature for thirty minutes from the time of addition to the sulfuric acid-ethanol solution.
14. 4.5 ml of the acidic layer was then placed into a quartz cuvette for fluorometer reading.

Fluorometer readings were carried out with a Model 110 Turner Fluorometer. The primary filter was a Corning No.
47-B, and the secondary filter was a Corning No. 2A-12.

Figure 1 is the corticosterone calibration curve used to determine sample corticosterone levels. A recovery range of 83-106% was obtained using this method. Friedman et al. (8) obtained a 70-100% recovery using the Guillemin-Glick fluorometric method.

In order to ascertain the amount of ingested alcohol reaching the peripheral circulation and the length of time it remained in the circulation, the quantitative ultraviolet enzymatic method (Sigma Multi-test Kit No. 332-UV) was used to determine blood ethanol levels. The procedure was as follows.

1. 0.1 ml of sample plasma was added to 0.4 ml of glycine buffer reagent (Sigma Stock No. 332-9).
2. 16 ml of glycine buffer reagent was added to NAD-ADH (Sigma Stock No. 332-5).
3. The NAD-ADH solution was capped and inverted gently several times to dissolve the contents.
4. 3 ml of NAD-ADH solution was placed into a test tube.
5. 0.1 ml of diluted plasma from step one was added to the NAD-ADH solution.
6. The test tube was capped immediately, and the contents were mixed gently by inversion.
Figure 1. Corticosterone calibration curve using the fluorometric method of Givner and Rochefort.
7. The test tube was allowed to incubate capped at room temperature for ten minutes.

8. Test tube contents were transferred to a glass cuvette and covered with Parafilm.

9. Absorbance at 340 nm was read using a Bausch & Lomb Spectronic 20 spectrophotometer. Figure 2 is the ethanol calibration curve used to determine sample blood ethanol percent.

Student's $t$ test was the statistical method used for determining the statistical significance of differences in corticosterone, eosinophil, and adrenal weight means. By the end of the experimental period the effect of ethanol on male mortality became apparent. The statistical method used for determining the statistical significance of mortality data was chi-square (38).
Figure 2. Ethanol calibration curve using the quantitative ultraviolet enzymatic method.
CHAPTER III

RESULTS

The data reported here came from 78 male and female Sprague-Dawley rats maintained in a controlled light environment with ad libitum access to food and water. The data are presented in the form of graphs that depict plasma corticosterone levels (µg/100 ml), eosinophil counts (/mm$^3$ blood), adrenal weights (mg/pair), and blood ethanol levels (per cent). The data are summarized in Tables I, II, and III. The data in Table I were statistically analyzed using the Student's $t$ test, and the data in Table II were statistically analyzed using chi-square.

Figure 3 shows the effect of ethanol on plasma corticosterone levels in 10 male rats following a fifteen minute cold stress. There was a statistically significant decrease (22%) in the response to cold in the ethanol-treated animals (mean of 55.0 µg/100 ml) when compared to the sham-treated animals (mean of 70.0 µg/100 ml).

Similar findings were not observed in the 14 ethanol-treated females (Figure 4). Although these animals showed a lower corticosterone mean (62.9 µg/100 ml) than the mean of the sham-treated animals (72.8 µg/100 ml), the difference was not statistically significant.
TABLE I

A SUMMARY OF SOME EFFECTS OF ETHANOL ON THE STRESS RESPONSE IN RATS
(ETHANOL ANIMALS: 0.25 ML of 28% ETHANOL; SHAM ANIMALS: 0.25
ML OF WATER; TREATMENT: ONCE A DAY, 5 DAYS A WEEK
FOR 12 MONTHS; ANALYSIS: STUDENT'S T TEST)

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th></th>
<th>Female</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x} \pm S.D.$</td>
<td></td>
<td>$\bar{x} \pm S.D.$</td>
<td></td>
</tr>
<tr>
<td>A. Corticosterone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>($\mu g/100$ ml plasma)</td>
<td>70.9 ± 11</td>
<td>55.0 ± 11*</td>
<td>72.8 ± 20</td>
<td>62.9 ± 15</td>
</tr>
<tr>
<td>B. Eosinophils</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(/mm$^3$ blood)</td>
<td>171.4 ± 64</td>
<td>170.0 ± 42</td>
<td>116.7 ± 83</td>
<td>114.3 ± 82</td>
</tr>
<tr>
<td>C. Adrenal Weights</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg/pair)</td>
<td>15.5 ± 2</td>
<td>15.5 ± 3</td>
<td>19.3 ± 3</td>
<td>16.5 ± 4**</td>
</tr>
</tbody>
</table>

*P < 0.010
**P < 0.025
TABLE II

EFFECT OF ETHANOL ON MORTALITY OF RATS (ETHANOL ANIMALS: 0.25 ML OF 28% ETHANOL; SHAM ANIMALS: 0.25 ML OF WATER; TREATMENT: ONCE A DAY, 5 DAYS A WEEK FOR 12 MONTHS; ANALYSIS: CHI-SQUARE)

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th></th>
<th>Female</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>Ethanol</td>
<td>Sham</td>
<td>Ethanol</td>
</tr>
<tr>
<td>A. Initial number of animals</td>
<td>12</td>
<td>12</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>B. Expected number dying before reaching 14 months of age</td>
<td>1.68</td>
<td>1.68</td>
<td>0.70</td>
<td>0.80</td>
</tr>
<tr>
<td>C. Observed number dying before reaching 14 months of age</td>
<td>5*</td>
<td>2</td>
<td>2</td>
<td>2</td>
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*P < 0.05
### TABLE III

**BLOOD ETHANOL CLEARANCE IN RATS (ETHANOL DOSAGE: 0.25 ML OF 28% ETHANOL; EACH MEAN REPRESENTS THE AVERAGE OF 6 ANIMALS)**

<table>
<thead>
<tr>
<th>Hrs. Post-Ethanol</th>
<th>Males</th>
<th>Females</th>
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<tr>
<td></td>
<td>3 month</td>
<td>12 month</td>
</tr>
<tr>
<td>0.5</td>
<td>0.055</td>
<td>0.033</td>
</tr>
<tr>
<td>1.5</td>
<td>0.042</td>
<td>0.028</td>
</tr>
<tr>
<td>2.5</td>
<td>0.028</td>
<td>0.012</td>
</tr>
<tr>
<td>3.5</td>
<td>0.007</td>
<td>. . .</td>
</tr>
<tr>
<td>4.5</td>
<td>. . .</td>
<td>. . .</td>
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</table>
Figure 3. Effect of ethanol on plasma corticosterone levels in male rats following cold stress. (Blood samples taken 30 minutes post-stress; fluorometric method used; age of rats: 14 months).
Figure 4. Effect of ethanol on plasma corticosterone levels in female rats following cold stress. (Blood samples taken 30 minutes post-stress; fluorometric method used; age of rats: 14 months).
Figure 5 shows the effect of ethanol on eosinophil counts in male rats following cold stress. There was not a significant difference in eosinophil count between the means of the ethanol-treated (171.4) and sham-treated (170.0) groups.

Figure 6 shows the effect of ethanol on eosinophil counts in female rats following cold stress. There was not a significant difference in eosinophil count between the means of the ethanol-treated (114.3) and sham-treated (116.7) groups.

Figure 7 depicts the effect of ethanol on male adrenal weights. There was no difference in the adrenal weights of the ethanol- and sham-treated groups; both groups had a mean of 15.5 mg/pair.

Figure 8 depicts the effect of ethanol on female adrenal weights. There was a statistically significant difference (15%) in the mean adrenal weight of the ethanol-treated animals (16.5 mg/pair) as compared to the mean of the sham-treated animals (19.3 mg/pair).

When male and female adrenal weights were treated as mg/100 gm body weight the same results were obtained: (a) no significant difference between the means of the male ethanol- and sham-treated groups and (b) a statistically significant increase (18%) in the mean of the female sham-treated group.
Figure 5. Effect of ethanol on eosinophil counts in male rats following cold stress. (Tail blood samples taken 15 minutes post-stress; direct chamber counting method used; age of rats: 14 months).
Figure 6. Effect of ethanol on eosinophil counts in female rats following cold stress. (Tail blood samples taken 15 minutes post-stress; direct chamber counting method used; age of rats: 14 months).
Figure 7. Effect of ethanol on adrenal weights in male rats. (Age of rats: 14 months).
Figure 8. Effect of ethanol on adrenal weights in female rats. (Age of rats: 14 months).
Table I is a summary of corticosterone, eosinophil, and adrenal weight data from Figures 3 through 8.

Table II shows the effect of ethanol on mortality. Mortality for the female ethanol and sham groups was approximately the same, 12.5% and 14.0% respectively. In each group 2 rats died during the twelve month experimental period. Two rats in the male ethanol group also died, producing a 16.0% mortality. However, in the male sham group 5 rats died during the experimental period, yielding a 42.0% mortality. Ten of the 11 dead animals appear to have died of pneumonia. There was not a statistically significant difference in female mortality; however, the difference in male mortality was statistically significant.

Figure 9 depicts blood ethanol clearance in male rats after receiving 0.25 ml of 28% ethanol. Three month old males had a mean blood ethanol percent of 0.055 at 0.5 hours post-ethanol ingestion. Blood ethanol percent fell to a mean of 0.007 at 3.5 hours post-ethanol ingestion. At 0.5 hours post-ethanol ingestion, twelve month old males had a mean blood ethanol percent of 0.033, which then dropped to 0.012 by 2.5 hours post-ingestion.

Figure 10 illustrates blood ethanol clearance in female rats after receiving 0.25 ml of 28% ethanol. Mean blood ethanol percent for three month old females was 0.077 at 0.5 hours post-ethanol ingestion and decreased to 0.011 at
3 month old males
average weight = 387.4 gm
n = 6

12 month old males
average weight = 587.4 gm
n = 6

Figure 9. Blood ethanol levels in male rats at varying times post-ethanol ingestion. (Dosage: 0.25 ml of 28% ethanol).
Figure 10. Blood ethanol levels in female rats at varying times post-ethanol ingestion. (Dosage: 0.25 ml of 28% ethanol).

3 month old females
average weight = 238.0 gm
n = 6

12 month old females
average weight = 432.9 gm
n = 6
4.5 hours post-ingestion. Twelve month old females had a mean blood ethanol percent of 0.050 at 0.5 hours post-ethanol, which then decreased to 0.019 at 2.5 hours post-ingestion.

Table III summarizes the data from Figures 9 and 10. Half an hour after ethanol ingestion blood ethanol percent ranged from 0.077 to 0.033, depending upon the size of the animal. Ethanol remained in the blood for 2.5 to 4.5 hours post-ingestion, once again, depending upon animal size.

Autopsy of the cold-stressed animals revealed mammary gland tumors on three female rats, and tumors of the pinna on two male rats. Data from these animals were within the range of data from the other experimental animals and were included in the reported results. No abnormalities of the internal organs were noted on any of the cold-stressed animals.
CHAPTER IV

DISCUSSION

The purpose of this study was to examine the effects of moderate amounts of ethanol given over a long period of time on rats and their ability to respond to cold stress. Therefore, the appropriate ethanol dosage and route of administration which would not produce alcoholism needed to be determined. When rats are allowed to drink an ethanol solution ad libitum, some will consume significantly more than others (13). In order to circumvent this problem, it was decided to administer a measured amount of ethanol once a day.

Before a moderate ethanol dosage for rats could be calculated it was first necessary to define moderate drinking in humans. Therefore, for this study the following definition was formulated: 2 ounces of 85 proof alcoholic beverage per day for a 72 kg man. At the beginning of this study the average weight of both male and female rats was 200 gm. The equivalent dosage for a 200 gm rat is 0.073 ml of 190 proof ethanol per day. Turner et al. (36) have defined the upper limit of moderate drinking in humans as being 0.8 gm ethanol/kg body weight/day. Experimental animals in this study received approximately 0.1 to 0.3 gm ethanol/kg body.
weight/day, well within the range of moderate drinking. Greenberg and Lester (11) found that intoxication in rats begins at a blood ethanol level of 0.14%. Blood ethanol levels in this study were well below Greenberg and Lester's level of intoxication.

Dosage was kept constant throughout the experimental period. Ethanol dosage was not based upon changes in weight or the sex of the animal. For the human social drinker, the amount of alcohol consumed remains fairly constant throughout life despite changes in the individual's weight. Furthermore, in a human social drinking situation females are served the same amount of alcohol per drink as males, despite differences in body size (36). However, in rats there are differences between the sexes in corticosterone levels, eosinophil counts, adrenal weights, and mortality (3, 13, 16). Consequently, findings were compared only between the members of the same sex.

Corticosterone levels from this study were difficult to compare with those found in the literature. Corticosterone levels of Sprague-Dawley rats vary according to the type of stress, time of day, and method of corticosterone determination used (1). Corticosterone samples in this study were taken during the mid-morning trough in the circadian rhythm of corticosterone release (17). Nichols and Chevins (26) have found that the corticosterone response
to a stressor is greater during the trough period of the cycle than at the peak.

Differences between male and female corticosterone levels are also found in rats. Under the same conditions, females have higher corticosterone levels than those of males (3). In this study the female ethanol- and sham-treated groups have corticosterone levels only slightly higher than those of their male counterparts. Similarity between the sexes may be due to the effect of sodium pentobarbital anesthesia. Critchlow et al. (3) found that male and female plasma corticosterone concentrations are similar when rats are anesthetized with sodium pentobarbital.

The lower corticosterone levels of the male and female ethanol-treated rats are in agreement with the findings of Ratcliffe (29). Although several researchers have shown that rats treated with 1.0 gm ethanol/kg body weight or more have higher corticosterone levels than those of control animals (18, 19, 32, 34), the ethanol-treated rats in this study received a much lower dosage, 0.1 to 0.3 gm ethanol/kg body weight.

The lower corticosterone levels of the ethanol-treated animals may be due to the sedative effect of low ethanol dosages upon the higher brain centers (23). Ordinarily at the onset of the stress response the cerebral cortex stimulates the hypothalamus to release corticotropin releasing
factor (5). However, Nestoros (25) found that in animals treated with ethanol (intravenous infusion of 4 mg/kg) the inhibitory action of gamma-amino-butyric acid on cortical neurons is potentiated. Ratcliffe (29) has also found that moderate ethanol consumption depresses the sensitivity of the hypothalamus to stimulation.

The animals in this study were subjected to cold stress approximately twenty-four hours post-ethanol. However, the lower corticosterone levels of the ethanol-treated animals suggest that ethanol was still exerting its effect upon the brain. Scarborough (30) has found that the anxiety reducing effects of ethanol will last at least seventy-two hours post-ingestion. In addition, Freund (7) has reported that in the brain "... electrophysiological changes persist after relatively modest alcohol exposure . . . ."

In addition to differences in corticosterone levels, male and female rats also differ in the number of leukocytes present in the circulation. Males have higher levels than females (3). However, only eosinophils were looked at in this study. A decrease in the number of circulating eosinophils is often used as an indicator of the stress response (20, 22, 33, 35). Specifically, the eosinophil count is used as an indicator of ACTH activity. High levels of ACTH are associated with low levels of eosinophils; conversely, low levels of ACTH are associated with high levels of eosinophils (27).
Cold stress did produce eosinopenia in the experimental animals, but ethanol apparently had no detectable effect on the eosinophil counts of the cold-stressed rats. However, the differences between ethanol- and sham-treated animals in corticosterone levels imply that there were also differences in ACTH levels. The eosinopenia index is probably not sufficiently sensitive to detect such slight differences in ACTH levels (20).

The general finding that female rats have larger adrenal glands than male rats, especially in relation to body size, is evident in this study. Another general finding is that stress leads to an increase in adrenal gland size (13). Indeed, hypertrophy of the adrenal cortex is one of the characteristic indicators of Selye's General Adaptation Syndrome (31). Adrenal hypertrophy is due to the effect of increased levels of ACTH on the adrenal cortex (2). The following sequence has been proposed by Ottenweller et al. (28) to account for the circadian rhythm of ACTH release:

1. An increase in locomotor activity with the onset of darkness;
2. An increase in plasma corticosteroid binding proteins via increased lymphatic return;
3. A decrease in unbound corticosteroids;
4. An increase in ACTH.
Both the male and female ethanol-treated groups did not show any adrenal enlargement. The daily moderate amounts of ethanol received by these animals may account for their lack of adrenal hypertrophy. There are several reports in the literature on the effectiveness of low ethanol dosages in diminishing or preventing the activation of the pituitary-adrenal axis (11, 23, 36).

The male sham-treated group also did not have enlarged adrenal glands; only the female sham-treated group showed adrenal hypertrophy. Overt observation revealed that sham-treated animals displayed more locomotor activity after oral administration than did ethanol-treated animals. Among the sham-treated animals, females were active longer than males, approximately five to ten minutes for females and one to two minutes for males. Perhaps the increased locomotor activity of the female sham-treated group, occurring at a time when rats are usually not very active, led to adrenal hypertrophy via the previously mentioned mechanism of Ottenweller et al. (28).

Studies dealing with death rates in rats are usually conducted on large populations. Therefore, it should be kept in mind that mortality data from this study came from a relatively small population. The long-term effects of stress on the body are variable and determined by both the type of
stress and the individual. In general, individuals subjected to a high degree of stress are at an increased risk of developing physical disorders (6). Gussek (12) states that mental stress can actually shorten the lifespan of rats. According to Hoffman (16), mortality in 14-month old Sprague-Dawley male rats is 14.0 percent, and female mortality is 5.0 percent. Mortality figures from this study were as follows:

1. Male ethanol-treated, 16.0 percent
2. Male sham-treated, 42.0 percent
3. Female ethanol-treated, 12.5 percent
4. Female sham-treated, 14.0 percent

Experimental animals in this study were stressed via oral administration of either ethanol or water. Daily oral administration aptly fits the definition of fear given by Hodgson et al. (15), "The expected occurrence of an unpleasant event." Regarding the effect of ethanol on fear, they state that, "... a shot of ethanol does help the rat to cope with fear and frustration." Presumably ethanol enabled the male ethanol-treated animals to withstand stress more effectively than the male sham-treated animals.

The finding of increased mortality in the male sham-treated group but not in the female sham-treated group is similar to that reported by Elliott and Eis dorfer (6). In
their study, chronic stress increased mortality in male rats, but female mortality actually decreased. The adrenal hypertrophy exhibited by the female sham-treated animals may have been a factor in their resistance to the effects of daily stress. But both Hannon and Bolter (13) and Hester et al. (14) have reported that in females subjected to prolonged stress, corticosterone levels returned to control levels despite continued adrenal enlargement.

It is possible that the high mortality of the male sham-treated group is a genetic trait, although inbred rat strains exhibit a great degree of genetic homogeneity (16). Perhaps the increased male mortality could be explained on the basis of the General Adaptation Syndrome. According to Selye (31), adaptation to the stressor may be achieved after the initial exposure. However, if the stressor is severe or is applied for a long period, adaptation is lost and death may occur.

Selye (31) states that emotional excitement of the cerebral cortex can initiate activation of the adrenal cortex. Furthermore, Warburton (37) reports that emotional stress is a more powerful activator of the pituitary-adrenal axis than is physical stress. Stimulation of the adrenal cortex generally serves to mobilize the organism's defenses against the stressor; however, corticosteroid hormones currently inhibit the immune system (31). By depressing
the stress response, ethanol may lessen inhibition of the immune system and improve the stressed animal's longevity.

Evidence from this study indicates that the moderate intake of ethanol over a relatively long period of time depresses the adrenocortical response to acute stress. This finding is in agreement with those of Greenberg and Lester (11), Ratcliffe (29), and Marks and Chakroborty (23). However, the response to cold stress of the ethanol-treated animals was not totally suppressed. Post-cold stress corticosterone levels of the ethanol-treated animals were approximately 48 percent higher than the corticosterone levels of non-stressed control animals.

Another finding from this study is that in male rats long-term ethanol consumption appears to shield the animal from the ill effects of stress. Ethanol not only raises the threshold for initiation of the stress response (29) but "... may actually decrease the activity of a previously aroused hypothalamus-pituitary-adrenal system, probably through a sedative action on the CNS and by removal of anxiety" (23). In humans, moderate ethanol consumption is also correlated with a decrease in mortality from myocardial infarction and coronary occlusion (36).

Summarily, the data in this study indicate that 1. Moderate ethanol intake lessens, but does not eliminate, the response to acute cold stress in rats, and
2. Depression of the stress response via ethanol's potentiation of cortical neuron inhibition may be beneficial to human health in relation to longevity.


