THE INTERACTION OF GENETICS AND THE ENVIRONMENT

IN ALCOHOLISM

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Literature Review

Running Head: ALCOHOL INTERACTIONS

THE INTERACTION OF GENETICS AND THE ENVIRONMENT IN ALCOHOLISM

The problem of alcoholism costs society billions of dollars. The Addiction Research Foundation of Canada published alcohol related statistics for Canada, and other countries states that deaths directly attributable to alcohol in Canada number 2,882 in 1985 or 1.6% of all deaths. It estimates the deaths indirectly related to alcohol at 15,015 in Canada in 1985. Social costs such as health care reduced productivity and law enforcement cost that are alcohol related are estimated at $6.0 billion for Canada. (Adrian, Jull, Williams, 1988). The enormity of the problem is evident.

HOW CAN WE DESIGN BETTER PROGRAMS?

Alcoholism is a complex problem with many contributing, interacting factors. The key to effective treatment and prevention lies in knowledge and understanding of the interaction of the environmental and genetic factors involved in it's pathology.

DOES STRESS AFFECT ALCOHOLISM?

Stress has repeatedly been linked to alcoholism. Increased the levels of stress in society correlate with increased alcoholism rates. Researchers compared two types of social stress conceptualized and measured at the state level to various state wide indicators of alcohol problems. Findings show that stressful events and stressful conditions were correlated with all indicators of alcoholism; 19 of 20 state wide correlations were in the predicted direction. (Linsky, Straus, & Colby 1985).

DO GENETICS AFFECT ALCOHOLISM?

Recent evidence has led experts to agree that at least in some cases there is a genetic factor at work. Holden, (1991) in a review of this in the January issue of Science, page 163 states, " For example, it is now widely accepted that a vulnerability to the disorder can be partly inherited. (This certainly is based on results of adoption, and family studies that have been rolling in since the mid - 1970s, as well as the success in breeding strains of rats that prefer alcohol over water in their drinks.)".

HOW DO GENETICS AFFECT ALCOHOLISM?

Two types of alcoholics have been identified. Type 2 alcoholics have inherited from their alcoholic fathers, a high risk of developing alcoholism. They make up twenty five percent of treated alcoholics. Type 1 alcoholics do not have this proven inherited characteristic and make up the other seventy five percent (Restak 1988).

WHO ARE TYPE 2 ALCOHOLICS

Type 2 alcoholics have proven to be the most difficult and expensive group to treat due to their high level of anti social behaviour and recidivism rates. Their problems begin in the teen years and typically, are accompanied by antisocial experiences such as fighting, arrests, and other criminality. They inherit a distinctive evocted brain wave pattern and a chemical balance that is different from the norm. This abnormal balance takes the form of a lower than normal level of serotonin and elevated levels of two other natural brain chemicals known to reduce the level of serotonin. Both these characteristics can be used to identified them and their male offspring (Restak 1988).

DO ALL OFFSPRING OF TYPE 2 ALCOHOLICS BECOME ALCOHOLIC?

Daughters of Type 2 alcoholics do not appear to inherit the Type 2 characteristics. Not everyone who inherits this risk factor becomes an alcoholic. Many environmental factors are also involved (Restak 1988).

WHAT IS THE NATURE OF THEIR INHERITANCE?

A study by Ballenger, Goodwin, Major, & Brown, (1979); found that Type 2 alcoholics had an inherited brain chemical difference and that their consumption of alcohol tended to make the chemical balance normal. The different inherited chemical balance was found to contribute to the pathology of alcoholism.

Other studies showed that the administration of serotonin orally or of serotonin uptake inhibiters, chemicals that increase the availability of serotonin, reduced the number of drinks consumed and increases the number of days that chronic alcoholics remain drink free (Naranjo, Sellers & Lawrin, 1986). Serotonin has also been found to be in present in significantly different amounts in the urine of dried out alcoholics than that of a control group (Thomson & McMillen, 1987). The study does not identify these dried out

alcoholics as Type 2 but it seems that since they had the same identifying chemical abnormality that this is very likely. Serotonin and its availability seem to play a role in limiting drinking and this same chemical seems to be in shorter supply than is normal as in Type 2 alcoholics.

WHY AN ANIMAL MODEL?

Two problems with clinical studies are that it impossible to control the availability of alcohol during the developmental period and there are limitations on biochemical research (Littleton, 1975). These problems led to the search for an animal model in which alcoholism could be studied experimentally. Several such models were developed. In fact it has been amazingly easy to develop animal models that had a marked preference for alcohol.

PROBLEMS WITH ANIMAL MODELS

Proving that such preference indicated a predilection to alcoholism has been much more difficult. Alcoholism often has been seen as a strictly human behaviour, affected by social and cultural norms.

WHY STUDY THE C57BL/ MODEL

One such mouse model is the C57bl/ mouse. This mouse was not developed as a model for alcoholism but was developed as an inbreed strain for research in various fields. It is in fact the most used mouse strain in animal research and now has many branch strains all with the C57bl/ identifier in their name. Their thirst for alcohol was not breed for but rather came about by chance. Comparison studies of mouse consumption rates have produced a continuum of consumption from those resistant to drink to those who seek readily to consume. All branches of this strain C57bl/ have been rated as high consumers of alcohol. (Festing, 1979)

The C57bl/ mouse strain, under free choice conditions, has been shown to reach a stabilized level of alcohol consumption daily, of almost 10 grams per kilogram of body weight after 7 weeks (Gentry, 1985).

Two strains of this mouse were compared to three other mouse strains. Five male mice from each strain of mice were allowed only a 10% alcohol solution as fluid for five days or until consumption was stable over a 5 day period. For the next four weeks they were then

allowed free choice of alcohol. Base lines were then established individually and the brain chemical balances were then established. The two strains of C57bl/ Mice showed much higher levels of consumption than the three other strains. These two C57bl/ strains also showed a brain chemistry balance that was different from that of the non alcohol preferring strains. (Yoshimoto, Komuura, Kano & Mizohata 1985). This chemical balance difference is the same difference that is found between Type 2 alcoholics and the rest of the population.

These mice seem to have inherited the same characteristics as Type 2 alcoholics. They are therefore good subjects for us to study the interaction between stress and genetics. The Physical and behaviourial similarity to Type 2 alcoholics out weights the arguments that alcoholism is caused by strictly human factors and can only be studied in humans. Genetic factors are at work here; factors that may even be identical. The argument that environmental factors that only effect humans may have a role in human Type 2 alcoholism that is not present in the animal model must be considered in the interpretation

of the results. Their absence from the animal model is exactly what is needed to clarify the observations made on the remaining factors, and in fact is further argument for studying alcoholism in the laboratory using this mouse model.

CAN WE STUDY ENVIRONMENTAL FACTORS AFFECTING ALCOHOLISM USING THIS MODEL?

Light has been shown to be a reliable stressor for rats. Experimenters compared two strains of rats. They measured the number of squares they entered in a given time, the number of rearings and the thigmotaxic tendency. The results were not conclusive in that differences between groups were not large. They then measured these same behaviours in light and dimly light conditions and found significant differences in them all due to differences in light levels. Both strains locomoted more and reared more in dim light than in bright light. Thigmotaxic behaviour was more pronounced in light than in dark conditions. Changes in this behaviour were assumed to represent changes in levels of fearfulness (Valle, 1970). Light was also seen to be effective in causing stress on pregnant females and the

differences between the offspring of pregnant stressed females and non-stressed females was significant (Jolley and Dreesman, 1975). Light should be a good stressor for these mice provided we stay within the limits recommended for rodents by the Canadian Council on Animal Care (1984).

DOES THIS MODEL FOLLOW THE HUMAN MODEL IN NOT AFFECTING FEMALES OF THE STRAIN?

All studies of alcohol consumption levels of C57bl/ mice have used male subjects. Experimenters tend to avoid using female subjects because of the increased variability their estrus cycle may introduce to the experiment. This variability makes interpretation of the results more difficult. No study of the levels of consumption of female C57bl/ mice can be found.

IS THERE A WAY TO TEST FEMALE C57BL/ MICE THAT WOULD CONTROL THE VARIATION CAUSED BY ESTRUS?

Tracking of the estrus cycle and comparing of consumption levels to the estrus cycle would allow us to identify the variability that was attributable to

variability related to the estrus cycle. We could them statistically remove this variability from our computations and the consumption levels of the females could then be compared to males without this variable having an effect.

HOW CAN WE TRACK THEIR ESTRUS CYCLE?

During the course of the estrus cycle different cells are present at different times and in different numbers in the discharge from the vagina. Cornified cells, large cells with flat plains on then, are present in large numbers at certain times. At other times smaller epithelial cells or luekocytes are present. Daily examining of smears from the vagina of female mice that identifies the percentages of these types of cells present enables researchers to track their estrus cycle. I refer you to Snell (1941) for a complete discussion of this technique with mice.

IF WE DO THIS WHAT WILL IT BENEFIT MANKIND?

Given all of the foregoing it is clear that this strain of mice can be very useful in the study of Type 2 alcoholism. If this strain follows the human model in

that females are not affected then the argument that the same factors are at work in both models is supported. This model may then help identify the means by which females remain unaffected, and the role of gentics and enveromental factors in the pathology of the problem. Studies of enviromental factors on both sexes could show the effects with the same genetics except for this factor. Studies of the use and effectiveness of serotonin treatments should also be carried out on them. The role that serotonin plays in Type 2 alcoholism could become clear by means of as study of its role in the drinking pattern of these mice.

The United States is embarking on a massive clinical research program over the next five years in an attempt to clarify the role that genetics plays in various addictive behaviours (Holden, C. 1991). I would suggest that a study of this mouse strain at the same time would prove most useful in providing insight into human addictions.

REFERENCES

Adrian, M., Jull, P., & Williams. (1988) Statistics and drug use in Canada and other countries (vol 1). Toronto: Addiction Research Foundation.

Canadian Council on Animal Care. (1984). Guide to the care and use of experimental animals (Vol. 1) Ottawa, Ontario: Author.

Ballenger, J. C., Goodwin, F. K. Major, L., & Brown, G. L. (1979). Alcohol and central serotonin metabolism in man. Archives of General Psychiatry, 36, 244-227.

Festing, M. F. W. (1979). Inbred strains in biomedical research. New York: Oxford University Press

Gentry, R. T, (1985). An experimental model of self-intoxication in C57 mice. Alcohol, 2, 671-675.

Holden, C. (1991). Probing the Complex Genetics of Alcoholism. Science, 252, 163-164.

Jolley, A., & Dreesman, H. (1973). Increased illumination level during gestation and offspring emotionality in the rat. Animal Behaviour, 21(4) 660-664.

Linsky, A. S., Straus, M. A. & Colby, J. (1985) Stressful events, stressful conditions and alcohol problems in the United States: A partial test of Bales theory. Journal of Studies on Alcohol, 46(1), 72-80

Littleton, J. M. (1975). The experimental approach to alcoholism. British Journal of Addiction, 70(2), 99-122

Naranjo, C. A., Sellers, E. M., & Lawrin, M. O., (1986) Modulation of ethanol intake by serotonin uptake inhibitors. Journal of Clinical Psychiatry, 47 (Suppl.), 16-22.

Restak, R. M. (1988). The Mind. Toronto: Bantam, 120-122

Snell, G. D. (1941). Biology of the Laboratory Mouse. New York: Dover Publications, 55 - 88

Thomson, S. M. & McMillen, B.A.,(1987). Test for decreased serotonin/tryptophan metabolite ratios in abstinent alcoholics. Alcohol, 4, 1-5

Valle, F. P. (1970). Effects of strain, sex, and illumination on open-field behaviour of rats. American Journal of Psychology, 83(1), 103-111.

Yoshimoto, K., Komuura, S., Kano, a., & Mizohata, k. (1985). Alcohol preference and brain monoamines in five inbred strains of mice. Psychology & Psychiatry 13 1192-1193.

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Running Head: Alcohol Interactions

ABSTRACT

Male, but not female, alcoholics have been shown to inherit an abnormal brain chemical balance that increases their risk of developing alcoholism. The same inherited chemical balance and alcoholism risk has been found in male C57/ mice. Female C57/ mice have not been tested. Do stress and genetic factors interact in C57/ females? To find out twenty female C57/ mice were randomly placed in a stress or non-stress group. Light was used as a stressor. Estrus and alcohol consumption were recorded daily. The data was statistically adjusted to remove the nonsignificant variation attributed to estrus and showed significate differences in stress, non-stress consumption. Both groups of subjects consumed less than their male conspecifics have in other earlier studies.

THE INTERACTION OF GENETICS AND THE ENVIRONMENT IN ALCOHOLISM

The problem of alcoholism costs society billions of dollars (Adrian, Jull & Williams, 1988). It is a complex problem with many contributing factors. The key to effective treatment and prevention lies in knowledge and understanding of the interaction of environmental and genetic factors involved in it's pathology.

Stress has repeatedly been linked to alcoholism. Increased levels of stress in society correlate with increased alcoholism rates (Linsky, Straus, & Colby 1985). Recent evidence has led experts to agree that at least in some cases there is a genetic factor at work (Holden, 1991).

Two types of alcoholics have been identified. Type 2 alcoholics have inherited from their alcoholic fathers, a high risk of developing alcoholism. They make up twenty-five percent of treated alcoholics. Type 1 alcoholics do not have this proven inherited characteristic and make up the other seventy-five percent. Daughters of Type 2 alcoholics do not appear to inherit the Type 2 characteristics. Not everyone who

inherits this risk factor becomes an alcoholic. Many environmental factors are also involved. Type 2 alcoholics have proven to be the most difficult and expensive group to treat due to their high level of anti-social behaviour and recidivism rates (Restak, 1988).

Type 2 alcoholics inherit a distinctive evoked brain wave pattern and a chemical balance that is different from the norm. Both these characteristics can be used to identify them and their male offspring (Restak, 1988). This chemical imbalance, a low level of serotonin and a high level of two chemicals known to control serotonin levels, contributes to the pathology of alcoholism. Drinking reduces this imbalance to near normal. There is a physical need to drink (Ballenger, Goodwin, Major, & Brown, 1979; Thomson, & McMillen, 1987).

ALcoholism's complexity led to the search for an animal model in which it could be studied experimentally (Littleton, 1975). One such model, the C57bl/ mouse strain, under free choice conditions, has been shown to reach a stabilized level of alcohol consumption daily, of almost 10 grams per kilogram of

body weight after 7 weeks (Gentry, 1985; Festing, 1979).

Two different strains of these mice were found to differ from three other inbreed mouse strains not only in their readiness to consume alcohol but also in having the exact same chemical abnormalities as Type 2 alcoholics (Yoshimoto, Komuura, Kano & Mizohata 1985).

These mice seem to have inherited the same characteristics as Type 2 alcoholics. They are therefore good subjects for us to study the interaction between stress and genetics.

Light has been shown to be a reliable stressor for rats. It causes significant differences in rearing, locomotion and thigmotaxic behaviour (Valle, 1970) and it was a useful independent variable in gestational stress studies on rats (Jolley and Dreesman, 1975). Light should be a good stressor for these mice provided we stay within the limits recommended for rodents by the Canadian Council on Animal Care (1984).

All studies of alcohol consumption levels of C57bl/ mice have used male subjects. Experimenters tend to avoid using female subjects because of the increased variability their estrus cycle may introduce to the

experiment. This variability makes interpretation of the results more difficult. No study of the levels of consumption of female C57bl/ mice can be found.

If female C57bl/ show the same resistance to this genetic factor that female humans do it would support using this model to study Type 2 alcoholism in humans. The more this mouse strain can be shown to be similar to human Type 2 alcoholics the stronger the arguments for using them for studying human Type 2 alcoholism. A better understanding of the interaction between genetic and environmental factors will help us improve prevention and treatment programs. I therefore proposed this following hypothesis and experiment.

HYPOTHESES

When female C57bl/ mice are allowed free choice access to alcohol they will consume less than conspecific males. Stress will increase the rate of consumption.

METHOD

Subjects: My subjects were twenty female C57bl/ mice from High Oak Ranch Limited, R.R.#1 Goodwood Ontario.

Procedure: The mice were acquired at 5 weeks of age and individually caged, each with two gravity feed water bottles. Random selection procedures were used to assign ten subjects to either a stress or non-stress group. After one week each were offered a 10% alcohol water solution in one of their water bottles.

The stress group was housed under bright light, with 2 neon tubes on the ceiling and three on the facing wall 36 inches in front of their cages. The non-stress groups were housed on the opposite side of the cage rack and shielded from the overhead light. Their light was dim.

The experiment was run for 7 weeks. The amount of alcohol consumed by each mouse was recorded daily. Their body weight was recorded weekly. Estrus was tracked daily by wiping their vaginal area with a slide and examining the slide for various cells; the presence or absence of which indicates the various stages of estrus (Snell, 1941).

RESULTS

The effects of estrus were not found to be significant at the .05 level. The effects of estrus were from here on in treated as a covariant. I used an

analysis of covariance procedure that adjusted the consumption level to eliminate the variance that could be accounted to estrus from all subsequent computations. Over the seven weeks I had significant differences between the groups, respectively at (M 9.24) and (M 8.14), f = 4.60 p = .03. Figure 1 shows the average daily levels of alcohol consumption for both groups over the seven weeks.

Insert figure 1 about here

An examination of their weekly levels of consumption by group showed an interesting pattern (see figure 2). The stress groups consumption started off more slowly than the non-stress group. After one week their consumption levels were nearly the same. From week three to week five the stress group consumed more. During week five the consumption of the stress group peaked and then began to fall so that it was below that of the non-stress group by the end of week six. The consumption of the non-stress group remained stable between weeks five and six. It then dropped off a

little to end up the seventh week at nearly the same level as the stress group.

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insert figure 2 here

At the end of seven weeks daily alcohol solution consumption had stabilized at 8.74 mL and 8.99 mL respectively, for the two groups, $p < .05$. The differences between the groups at this point were not significant. I combined them for an overall consumption mean of 8.87 mL. Comparing this to the results reported by Genty,(1985) I found that for males with the same weight the consumption rate was 29.87 mL of solution per day.

Insert Figure 3 here

Discussion

The female C57bl/ mice did consume less than what Gentry reports for males. This supports the hypothesis that females of this strain would consume less alcohol than conspecific males.

The C57bl/ female mice consumed more alcohol in the stress group than the non-stress group. This supports the hypothesis in regard to the effects of stress. The difference in the groups was reduced during the final weeks of the experiment. This suggests that the effects of chronic stress are not simple. They wear off as the subject habituates to it or it may be that there is a ceiling effect at work here. Stress is a factor even without the genetic effect. I do not know if stress still is a factor when genetics are a factor as no males were used to make comparisons.

The results support the conclusion that this strain is a good model of Type 2 alcoholism. A greater study of this strain of mice is called for, with both males' and females' consumption levels being compared directly. Such a study would confirm the differences in consumption rates and could also show the effects of stress on both genders. Why males are affected by this genetic factor while females are not? The answer to that may provide us with the insight to improve our alcohol programs.

Understanding the interaction of genetic and environmental factors is increasingly important in

light of recent experiments. Administering serotonin uptake inhibiters orally to chronic alcoholics has been found to significantly reduce their consumption rates (Naranjo, Sellers, & Lawrin, 1986). Serotonin is the brain chemical that has been identified as being at a low level in Type 2 alcoholics. This is too much of a coincidence not to have meaning and makes it all the more important that we understand who this inherited biological difference affects and how it does so. A large section, 25 percent, of the most difficult to treat alcoholics may, in the near future, have a medical component added to their treatment program that will result in dramatically improved success rates.

REFERENCES

Adrian, M., Jull, P., & Williams. (1988) Statistics and drug use in Canada and other countries (vol 1). Toronto: Addiction Research Foundation.

Canadian Council on Animal Care. (1984). Guide to the care and use of experimental animals (Vol. 1) Ottawa, Ontario: Author.

Ballenger, J. C., Goodwin, F. K. Major, L., & Brown, G. L. (1979). Alcohol and central serotonin metabolism in man. Archives of General Psychiatry, 36, 244-227.

Festing, M. F. W. (1979). Inbred strains in biomedical research. New York: Oxford University Press

Gentry, R. T, (1985). An experimental model of self-intoxication in C57 mice. Alcohol, 2, 671-675.

Holden, C. (1991). Probing the Complex Genetics of Alcoholism. Science, 252, 163-164.

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Littleton, J. M. (1975). The experimental approach to alcoholism. British Journal of Addiction, 70(2), 99-122

Restak, R. M. (1988). The Mind. Toronto: Bantam, 120-122

Snell, G. D. (1941). Biology of the Laboratory Mouse. New York: Dover Publications, 55 - 88

Thomson, S. M. & McMillen, B.A.,(1987). Test for decreased serotonin/tryptophan metabolite ratios in abstinent alcoholics. Alcohol, 4, 1-5

Naranjo, C. A., Sellers, E. M., & Lawrin, M. O., (1986) Modulation of ethanol intake by serotonin uptake inhibitors. Journal of Clinical Psychiatry, 47 (Suppl.), 16-22.

Valle, F. P. (1970). Effects of strain, sex, and illumination on open-field behaviour of rats. American Journal of Psychology, 83(1), 103-111.

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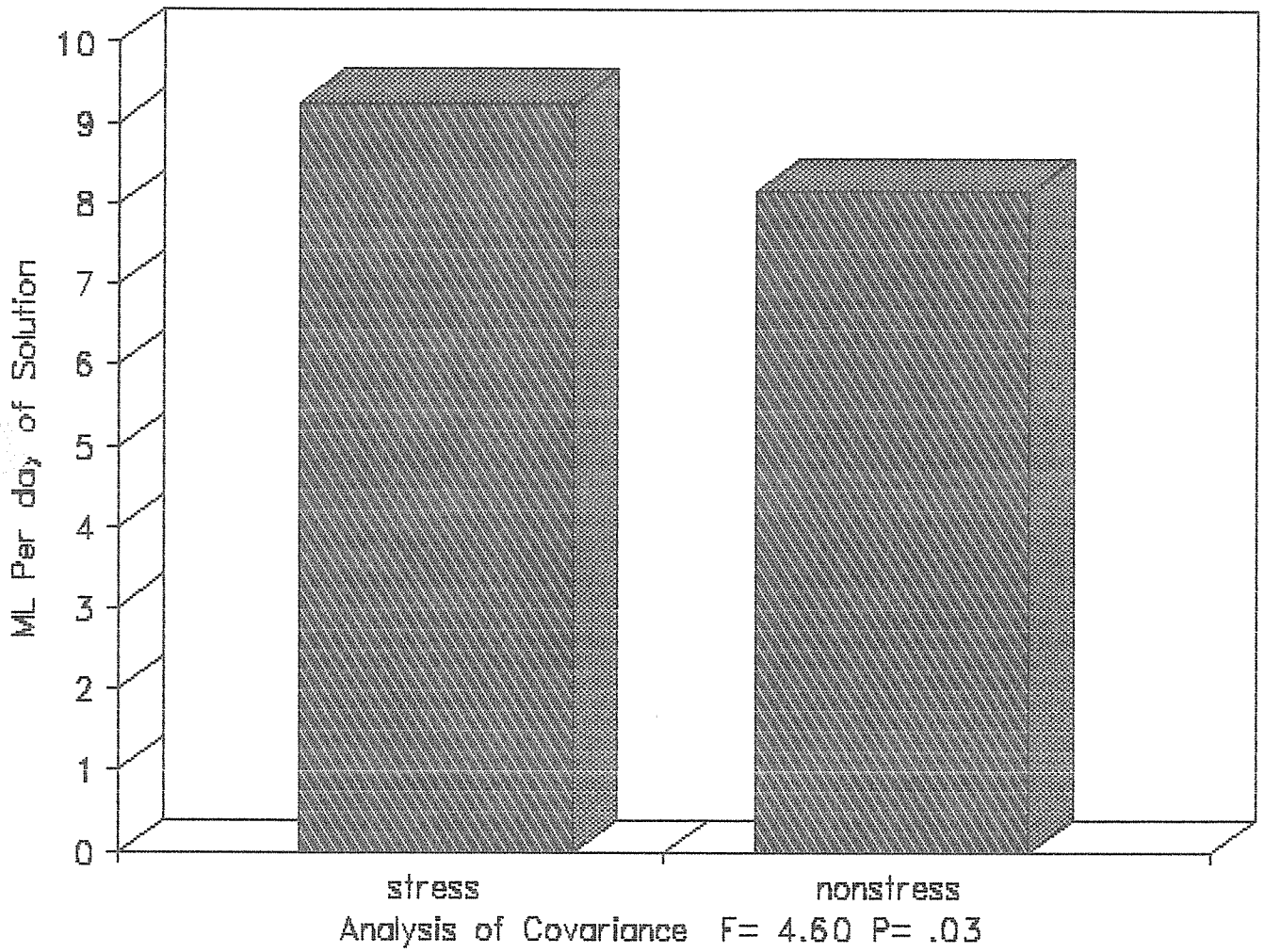
Figure Captions

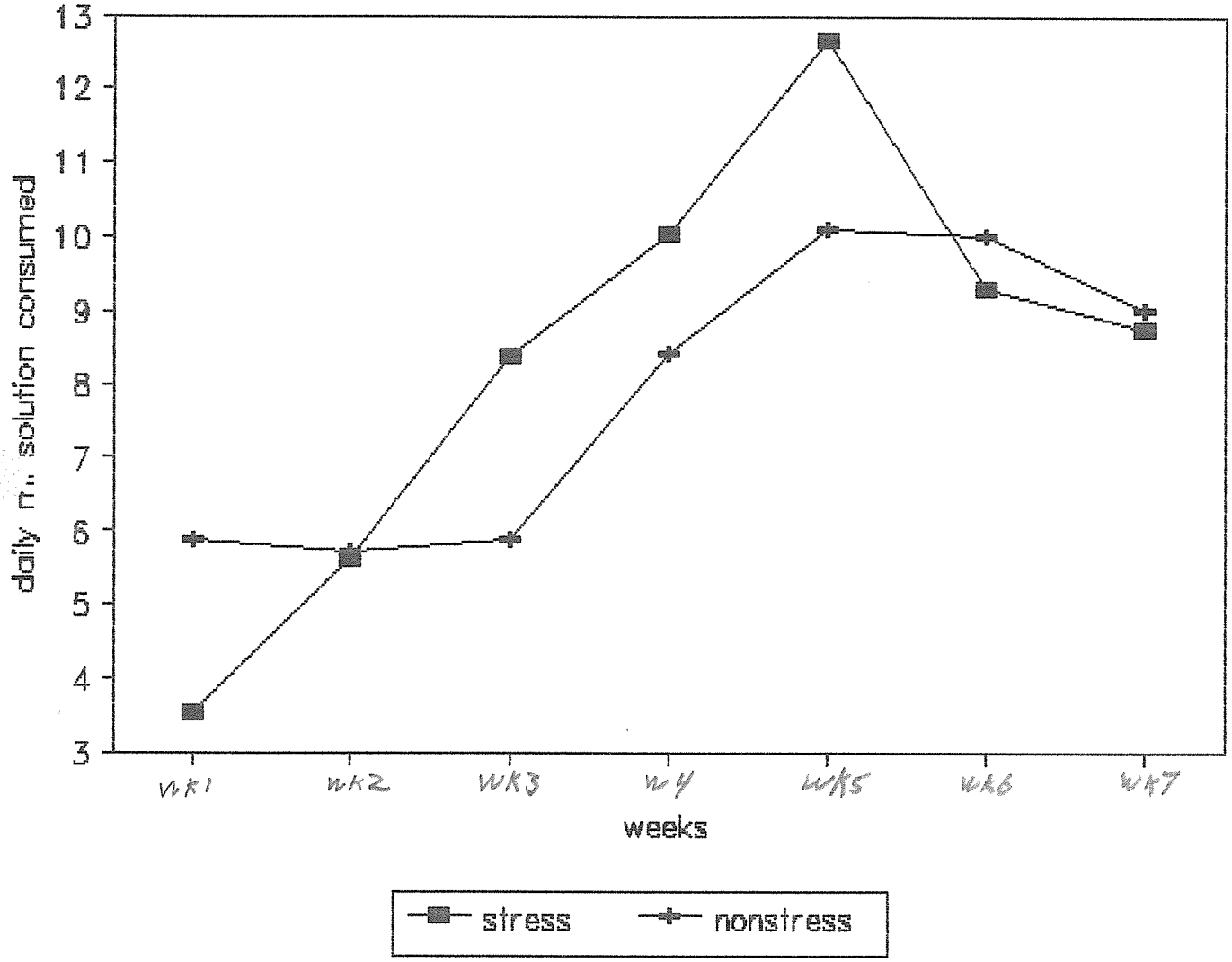
Figure 1. Consumption levels adjusted so the variance accountable to estrus has been removed, of the 10% alcohol solution for stress and non-stress groups in millilitres per day

Figure 2. Consumption levels for both groups over the seven weeks of the experiment.

Figure 3. Consumption levels of 10% alcohol solution per day with the variance for estrus removed compared to the consumption of males reported by Genty (1985). (adjusted to compensate for weight differences)



Analysis of Covariance F= 4.60 P= .03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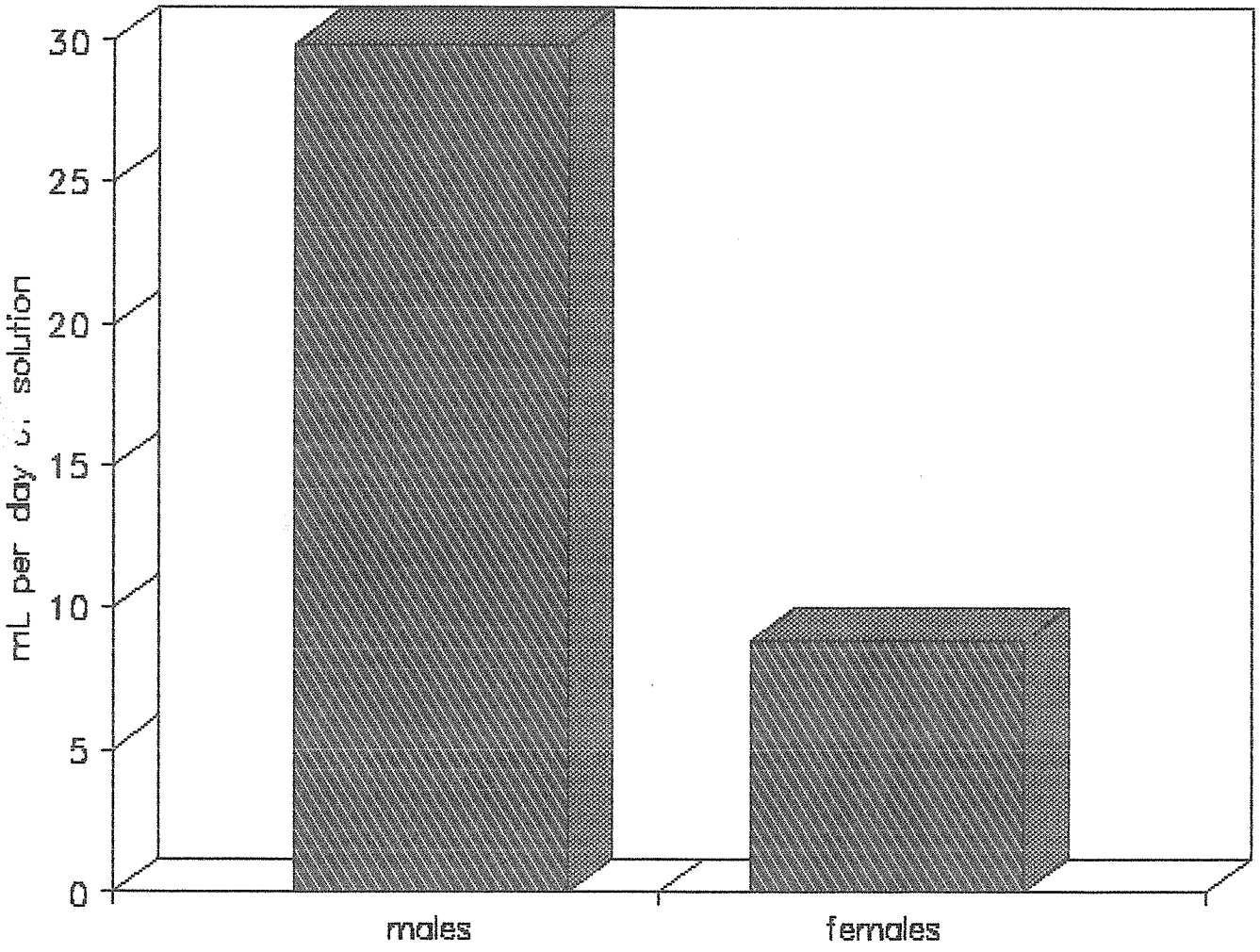
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