EFFECTS OF PREGNANCY, ESTRADIOL BENZOATE AND MER-25 ON VOLUNTARY ETHANOL CONSUMPTION IN THE RAT

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ABSTRACT

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Concordia Univeristy, 1982

The present experiments were designed to study the reported decrease in voluntary ethanol intake during pregnancy and to determine whether estrogens were responsible for the effect. The experiments were carried out in rats given access to food, water and a 10% ethanol solution. In Experiment 1, pregnant animals drank significantly less ethanol during the third trimester than did non-mated animals, but did not differ from them during the first two trimesters. The specificity of the effect of pregnancy on ethanol consumption was investigated in Experiment 2 by giving pregnant animals free access to food and water and to either a sucrose solution isocaloric with the ethanol solution or to a non-nutritive saccharin solution. Consumption of sucrose decreased during the third trimester, whereas consumption of the saccharin solution remained stable.

Since increases in the the level of estrogens during

pregnancy coincide with the suppression of ethanol consumption, it was decided to determine directly the effect of estrogens on ethanol consumption. In Experiment 3, daily injections of estradiol benzoate (EB) were administered to ovariectomized rats. EB led to marked decreases in ethanol consumption that were, however, transient. This pattern of change paralleled previously reported effects of EB on food intake. The antiestrogenic compound, ethamoxytriphetol (MER-25), which antagonizes many estrogen-dependent effects but which mimics the action of EB on food intake, also led to decreases in ethanol consumption (Experiment 4). In Experiments 5 and 6 these behavioral effects of EB and MER-25 were shown not to be due to altered ethanol metabolism or to result from malaise developing out of an interaction between EB and ethanol. In the final experiment (Experiment 7), the effect of daily injections of EB on the pattern of food and ethanol consumption was studied in ovariectomized rats. EB suppressed both food and ethanol intake. Gradual increases in the frequency of meals and of ethanol-drinking bouts allowed total food and ethanol intake to return to baseline.

The results of the experiments are discussed in terms of the possible effects produced by estradiol on taste and olfactory reactivity, on ethanol metabolism, and on the energy balance of the rat.

Acknowledgments

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I dedicate this thesis to Malla. Her companionship over the years has added much meaning to all my work.

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Introduction

Considerable evidence exists that ethanol consumption, across a wide range of species, declines during pregnancy. Interest in this topic has grown as a result of reports that an adverse physiological reaction may be responsible for the observation of decreased ethanol consumption by women during pregnancy (Hook, 1976; Little, Schultz, & Mandell, 1976). This finding; together with the knowledge that ethanol is a potent teratogen (Abel, 1981; Streissguth, 1978), has led to speculation concerning the existence of mechanisms that lead various species to reject during pregnancy substances that are potentially toxic to the fetus (Hook, 1976; Little, Schultz, & Mandell, 1976). Unfortunately, the human studies suffer from the common weakness that the information concerning ethanol drinking habits was based upon retrospective self-reports which are not easily interpreted because of the nature of the information being tapped -- health habits during pregnancy. The social desirability of answering in a predetermined fashion could introduce a source of systematic bias into the results.

A study in non-human primates has revealed a similar tendency for ethanol to be avoided during pregnancy (Elton & Wilson, 1977). This work while countering some of the criticisms levelled against the human studies, reported results from only four animals. Moreover, the study concentrated on ethanol consumption during relatively short periods prior and subsequent to conception and delivery,

while ignoring the intervening six months of gestation. Of those four monkeys tested, three showed an attenuation in ethanol intake, post-conception.

Hamsters also decrease ethanol intake during pregnancy in a situation in which both a 10% ethanol solution and water are freely available (Carver, Nash, Emerson, & Moore, 1953; Emerson, Brown, Nash, & Moore, 1952). The suppression of ethanol intake was shown to be most marked during the later stages of pregnancy. A species difference might be indicated by the report that C57BL/Crgl mice increase their ethanol consumption slightly during pregnancy (Thiessen, Whitworth, & Rodgers, 1966). This apparent inconsistency notwithstanding, these findings indicate that pregnancy, across a wide range of species, is associated with alterations in voluntary ethanol consumption.

Pregnancy is accompanied by diverse changes in pituitary, ovarian, and placental function (Heap, Perry, & Challis, 1973). It is thus plausible that alterations in hormone secretion are responsible for the diminished ethanol consumption observed during gestation. A number of studies have provided evidence indicating a role for ovarian hormones in the determination of differences in ethanol consumption. Women taking various oral contraceptive preparations have been reported to drink significantly less ethanol than women not taking them (Jones & Jones, 1976, 1977). In addition, female rats treated with estradiol, with the synthetic estrogen diethylstilbestrol, or with a

commercially available contraceptive hormone preparation, exhibit decreases in ethanol consumption in a paradigm in which the rat has a choice between water and an ethanol solution (Aschkenasy-Lelu, 1960a,b; K. Eriksson, 1969). Furthermore, and possibly most convincing, it has been demonstrated that the voluntary ethanol consumption of female rats and pigs varies at different stages of the estrous cycle, with less ethanol being consumed during the estrous than during the diestrous phase of the cycle (Aschkenasy-Lelu, 1960b, Van Cleve, Tumbleson, Dexter, Tinsley, & Middleton, 1980). Estrus is characterized hormonally in the female rat as a period of heightened estrogen secretion and is associated with various behavioral changes including decreased feeding (Tarttelin & Gorski, 1971; Ter Haar, 1972). Diestrus, in contrast, is characterized by a relatively low level of estrogen secretion.

These studies suggest that ovarian hormones, and in particular estrogens, have an inhibitory influence on ethanol consumption. But while there appears to be some consensus about the phenomenon itself, little attention has been given to determining the mechanism mediating these inhibitory effects.

The Present Investigation

A series of experiments was designed to study reports of decreased ethanol consumption during pregnancy and to evaluate the role that estrogens might play in this effect. In the first study, changes in consumption of a 10% ethanol solution and of tap water by pregnant rats were investigated. In the second study, intake of solutions other than ethanol, was studied. A calorically-rich solution (14% sucrose) and a non-nutritively sweetened solution (.75% sodium saccharin) were used for this purpose. This study was conducted in order to determine the degree of specificity of the changes observed in ethanol intake by the pregnant rats in the first experiment. In the third and fourth experiments, daily injections of estradiol benzoate (EB) and of the antiestrogen, ethamoxytriphetol (MER-25), were administered to ovariectomized rats given continuous access to a 10% ethanol solution, water, and lab chow. These studies were conducted in view of reports that estrogen administration suppresses ethanol consumption and the observation that estradiol levels increase dramatically during pregnancy (Shaikh, 1971; Soloff, Alexandrova, & Fernstrom, 1979). The fifth experiment was designed to determine whether the hormonal manipulations made in the previous two experiments led to changes in either ethanol or acetaldehyde elimination -- changes that might serve as the basis for the development of a toxic reaction. Using a similar rationale but a different methodological approach,

the sixth experiment examined the possibility that the changes observed in ethanol consumption subsequent to estrogen treatment might be attributable to a malaise resulting from an interaction between ethanol and estradiol. In the seventh and final experiment, the effects of EB on the patterns of both food and ethanol intake were assessed in the same animal. The purpose of this study was to evaluate the degree of symmetry between the effects produced by EB on food intake and ethanol consumption.

The description of the separate experiments will be followed by a discussion of the various action of estrogens that might account for the effects observed on ethanol consumption.

Experiment 1

The first experiment was carried out to determine whether changes in ethanol consumption observed during pregnancy in other species (Carver et al., 1953; Elton & Wilson, 1977; Emerson et al., 1952; Hook, 1976; Little, et al., 1976; Little & Streissguth, 1978; Thiessen et al., 1966) take place during gestation in the albino rat.

Method

Three separate replications of Experiment 1 were conducted over a one-year period. In this and all subsequent studies 60-70 day-old Wistar rats weighing 175-200 g and purchased from Canadian Breeding Farms Laboratories, served as subjects. Animal rooms were illuminated 10 h per day. Animals were housed individually and were given continuous access to food, an ethanol solution, and to tap water. The fluids were dispensed from Richter tubes attached to the front of the cage and positioned immediately adjacent to the source of food. Tube position was alternated daily to counteract the effects of a position preference. Fluid consumption from each tube was measured to the nearest ml and the tubes were refilled with fresh solutions daily. The rats were also weighed daily. The quantity of ethanol consumed was then computed in terms

of grams of absolute ethanol and in terms of the body weight. Water intake was also calculated in terms of body weight. For statistical analyses, the daily consumption of water and ethanol was summed over two-day periods. This was necessary because the amount of ethanol consumed depended on the position of the ethanol tube. Such a finding has been reported previously by Gillespie and Lucas (1958). Ethanol concentration was increased from 6% (v/v, 95% ethanol) during the first week to 8% in the second; at the outset of the third week the concentration was raised to 10% and remained at this level for the remainder of the study (Veale & Myers, 1969).

At the end of the third week, one group of females was housed for six days with stud males of the same strain; two females with one male. A second group of females was housed with gonadectomized males for the same period. A choice of 10% ethanol and tap water was available during this period, but the amount drunk was not measured. At the end of the mating period the females were rehoused in individual cages, and daily measurements of fluid consumption and body weight were resumed. Parturition of the pregnant females marked the end of the study. The number of offspring born in each litter was recorded at that time.

For data analyses, the date of conception was estimated by assuming the mean length of gestation to be 22 days. By this method it was determined that almost all the animals conceived on the fifth or sixth day of cohabitation.

Therefore, changes in drinking patterns could be analyzed only from the third day of gestation. The data from those animals that were housed with stud males but that did not become pregnant were added to those from the group that were not mated.

Results

Separate statistical analyses of the data from each of the three replications of Experiment 1 revealed very similar results and thus the data were combined for presentation. Figure 1 illustrates a comparison of ethanol consumption by pregnant and nonpregnant rats over consecutive 2-day periods during gestation.

A repeated measures analysis of variance revealed a highly significant interaction between groups by blocks of days, $F(9;621) = 4.18$, $p<.001$. The pregnant group consumed progressively less ethanol over days while the nonpregnant females maintained a stable pattern of consumption. Neither the group effect, $F(1,69) = 0.84$, $p>.35$ nor the blocks-of-days effect, $F(9,621) = 1.70$, $p>.05$ was significant in itself. Post hoc analyses were done to determine when during pregnancy the groups began to differ.

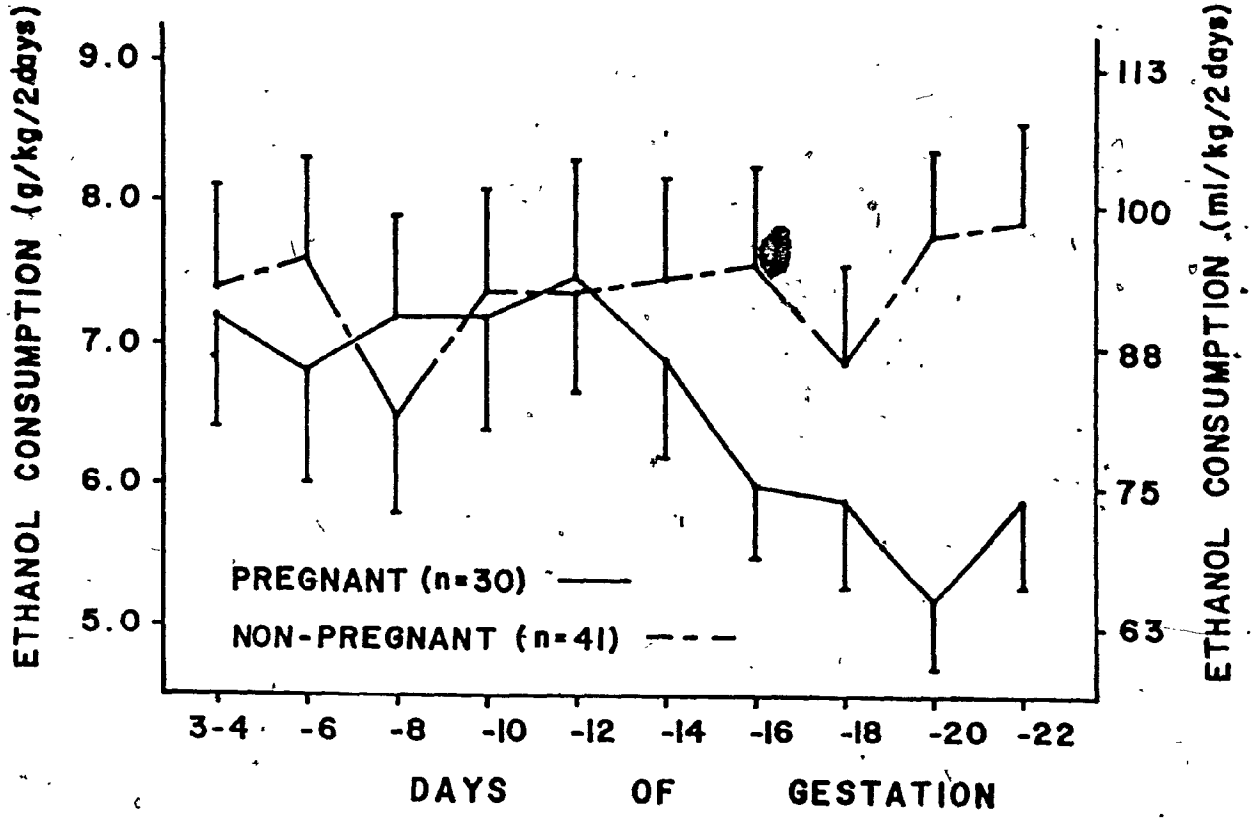

Figure 1. Mean ethanol consumption by pregnant and non-pregnant rats over the course of gestation. Each point represents the total consumption for two days. Vertical bars represent the standard error of the mean (SEM).

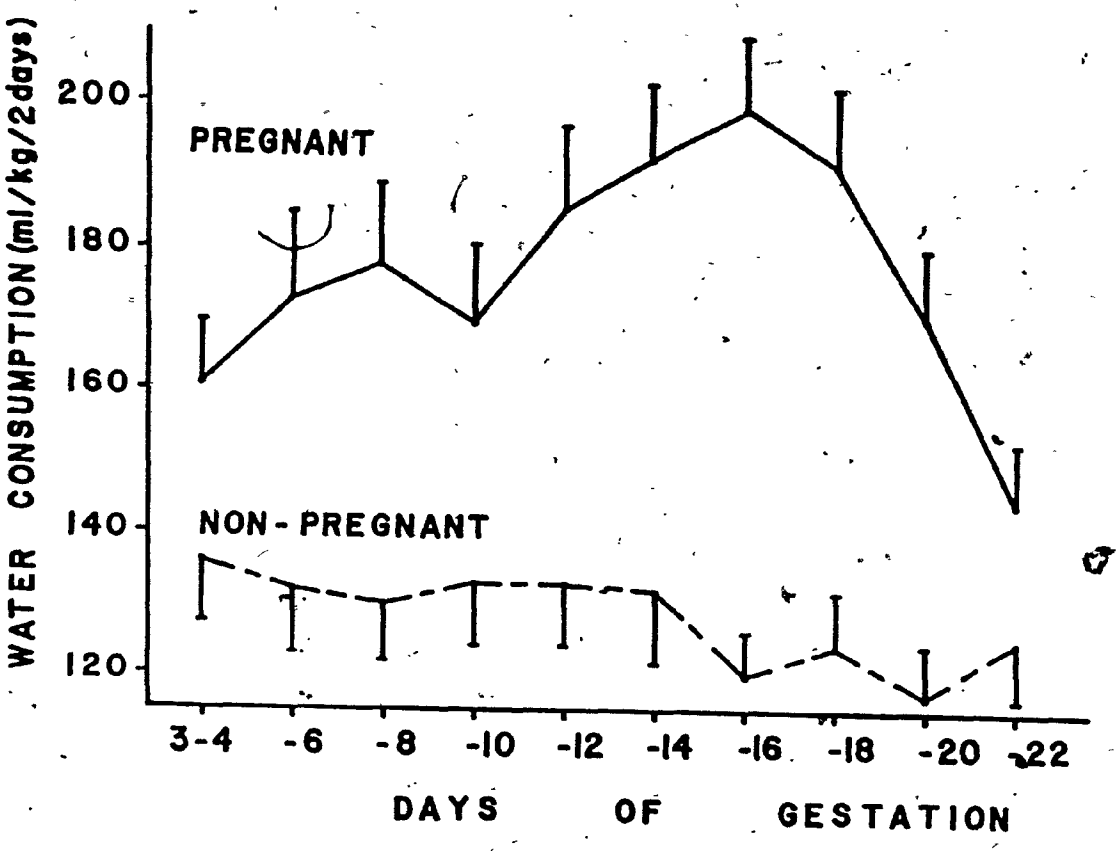

Figure 2. Mean (±SEM) water consumption by pregnant and non-pregnant rats over the course of gestation. Each point represents the total consumption for two days. (N's as in Figure 1).

Analyses of the individual blocks of days revealed that the pregnant rats were consuming significantly less ethanol by days 19-22 of gestation than the nonpregnant rats ($p<.01$, F-test for simple main effects). The trend towards diminished ethanol intake, however, appears to have commenced as early as day 14 of gestation. In contrast, as mentioned, the nonpregnant females maintained a stable intake throughout.

Water intake during the same period is illustrated in Figure 2. Consumption by the two groups did not differ significantly during the six days prior to the mating period ($F(1,69) = 0.61$, $p>.40$)(not illustrated in figure). During gestation, however, the pregnant group consumed significamtly more water as pregnancy progressed; the analysis revealed highly significant Group ($F(1,69) = 17.81$, $p<.001$) and Blocks of Days ($F(9,621) = 3.97$, $p<.001$) effects. In addition, the intake of the pregnant females varied as a function of the period of gestation ($F(9,621) = 6.14$, $p<.001$). There were no significant changes in consumption by the nonpregnant females. Post hoc analyses revealed that the water consumption of the pregnant females was significantly elevated above that of the nonpregnant group beginning on Days 5-6 of gestation ($p<.001$, F-test) and declined to levels not significantly different by Days 21-22.

The weight gain of the pregnant females over the course of gestation appeared to be normal and the mean litter size

was 11.3 pups, well within the normal range for this strain.

Discussion

The results of Experiment 1 are in agreement with those of others who have shown that mice (Thiessen et al., 1966), hamsters (Carver et al., 1953; Emerson et al., 1952), monkeys (Elton & Wilson, 1977), and humans (Hook, 1976; Little et al., 1976; Little & Streissguth, 1978) decrease their ethanol consumption over the course of gestation. Since conducting the present study, there has been an additional report of an experiment that investigated the effect of pregnancy on ethanol consumption in mice (Randall, Lochry, Hughes, & Boggan, 1980). In that study, pregnant mice were given a choice between water and a 10% ethanol solution, beginning on the fifth day of pregnancy. Ethanol consumption declined during pregnancy relative to a non-mated control group. The effect was most marked during the second half of pregnancy; a finding similar to the present results. It is noteworthy that estradiol levels during pregnancy in the rat and mouse are negligible except during the last trimester of pregnancy when they rise sharply (Shaikh, 1971; Soloff et al.,1979; McCormack & Greenwald, 1974). In contrast, the ovary secretes large amounts of progesterone throughout pregnancy (Pepe & Rothchild, 1974; McCormack & Greenwald, 1974). It is thus

plausible that increasing estradiol levels during the last trimester of pregnancy are responsible for the present finding of reduced ethanol consumption during this same period.

In conclusion, there is considerable evidence, including the present data, indicating that pregnancy, across a wide range of species, results in a reduced intake of ethanol. Moreover, there is reason to believe that an increase in the level of estradiol is responsible for this effect. It is impossible, however, to determine from these reports whether pregnancy has an effect on consummatory behaviors that is restricted to the suppression of ethanol consumption. It was thus considered necessary to test for the specificity of this apparent avoidance response. In Experiment 2 the intake by pregnant rats of other solutions was studied.

Experiment 2

Investigators that have documented the phenomenon of reduced ethanol consumption during pregnancy have, until now, discussed their findings in terms of an adaptive behavioral response designed to guard the developing fetus against the feto-toxic actions of ethanol (Hook, 1976; Little et al., 1976; Little & Streissguth, 1978). An alternative interpretation of these findings might be that pregnant animals, reduce their ethanol intake as part of a general shift in diet selection away from food-stuffs that provide rapidly metabolizable calories, and towards proteins and fats that will better provide for the needs of the growing fetus. Pregnant rats have on several occasions been used to test the hypothesis that animals can adapt their eating habits to accomodate changing nutritional needs (Leshner, Siegel, & Collier, 1972; Richter & Barelare, 1938). In those studies the dietary self-selection patterns of the pregnant rat were found to follow the varying nutritional requirements of the organism. In both these studies, protein intake was observed to increase during pregnancy while carbohydrate intake either declined moderately (Richter & Barelare, 1938) or remained constant (Leshner et al., 1972).

The present experiment was designed to determine whether the reduction in consumption observed in Experiment 1 was specific to ethanol, or whether pregnant rats would also reduce their intake of other calorically-rich

nutrients. A sucrose solution with a caloric density equal to that of the 10% ethanol solution used in Experiment 1 was chosen to test this hypothesis. In addition, the effect of pregnancy on the intake of a noncalorically sweetened solution was determined. It was considered interesting to monitor the intake of a saccharin solution because there have been reports that pregnancy in the rat is associated with changes in taste reactivity (Wade & Zucker, 1969b, 1970b). If the intake of the saccharin solution were to change during gestation, there would be reason to suspect that the reduction in ethanol consumption observed in Experiment 1 was due to changes in responsivity to the gustatory property of an ethanol solution.

Method

In this study sixteen female rats weighing 175-200 g at the outset served as subjects. The housing of the animals, the presentation of the drinking tubes and the calculation of fluid intake were carried out as in Experiment 1.

Eight animals were presented with a choice between a 14% sucrose solution (calculated to be isocaloric to the 10% ethanol solution) and water. The other eight animals were given a choice between a .75% sodium saccharin solution (Wade & Zucker, 1969b) and water.

At the end of four weeks, half of the animals in each

condition were housed with stud males of the same strain, the other half were housed with gonadectomized males. The day of conception was determined by the sighting of sperm in the vaginal smear. As in the previous experiment both water and one of the sweet solutions were available during this period, but measurements were not taken. After sperm was sighted animals were rehoused singly and fluid consumption was measured daily.

Results

Separate analyses of variance were carried out on the data from animals in the sucrose and saccharin conditions. A repeated measures analysis revealed a significant interaction between Groups (Pregnant vs Nonpregnant) by blocks of Days in the sucrose condition ($F(9,54) = 3.36$, $p<.01$) and no significant interaction in the saccharin condition ($F(9,54) = 0.77$, $p>.6$). It can be seen from Figure 3a that in the case of the sucrose condition the significant interaction arises from the fact that consumption of sucrose decreased dramatically during the last week of pregnancy, paralleling the decrease in ethanol intake seen in Experiment 1.

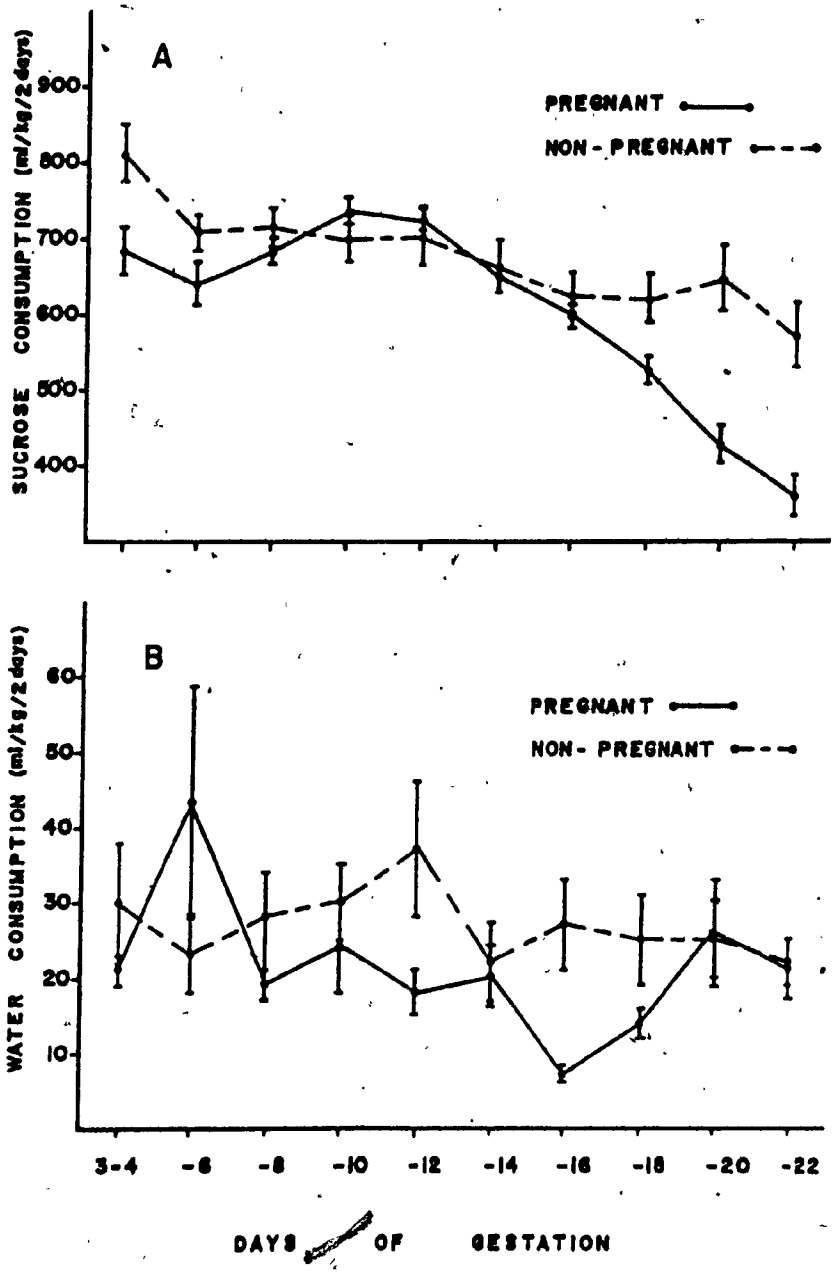

Figure 3. a) Mean (±SEM) sucrose consumption (14%, w/v) by pregnant and non-pregnant rats over the course of gestation. b) Mean (±SEM) water consumption by pregnant and non-pregnant rats offered choice of sucrose and water. Each point represents the total consumption for two days.

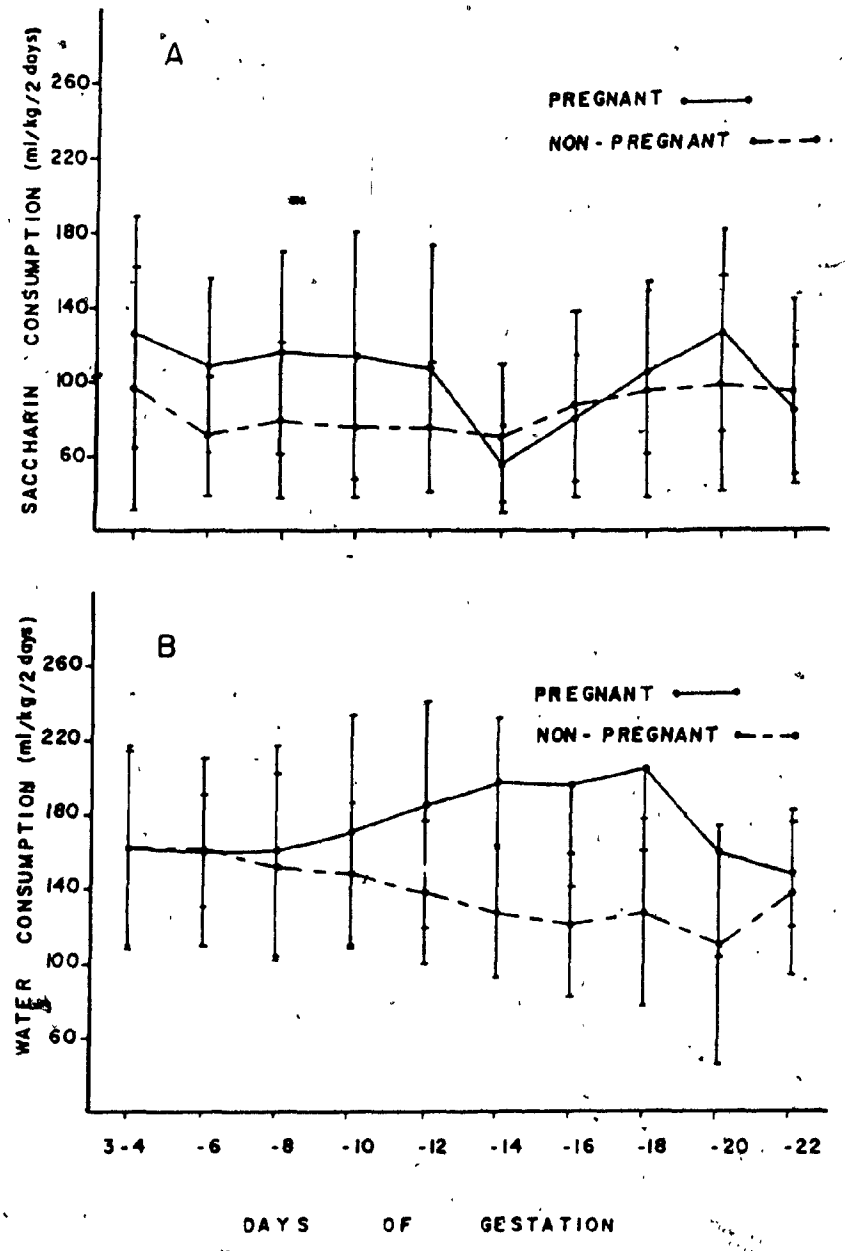

Figure 4. a) Mean (±SEM) saccharin consumption (0.75%,w/v) by pregnant and non-pregnant rats over the course of gestation. b) Mean (±SEM) water consumption by pregnant and non-pregnant rats offered choice of saccharin and water. Each point represents the total consumption for two days.

Post hoc analyses of individual blocks of days showed that pregnant rats drank significantly less sucrose on Days 19 to 22 than did nonpregnant rats ($p<.05$). Figure 4a shows, on the other hand, that saccharin intake remained relatively stable throughout pregnancy showing no tendency to decrease systematically over the last trimester although there was considerable variability.

Water intake, recorded for the same period, is shown in the lower half of each of Figures 3 and 4. No statistically significant effects were found for water intake in either the sucrose or saccharin conditions. It should be noted that the very large increases in water consumption found during pregnancy in Experiment 1 did not occur in the sucrose condition. This may be due to the fact that the volume of fluid intake was extremely high, approximately three times that observed in the ethanol study. On the other hand, in the saccharin condition, where the volume of saccharin consumed was closer to the volume of ethanol drunk in Experiment 1, there was a tendency for water intake to increase during pregnancy.

Discussion

In Experiment 2, it was seen that pregnant rats reduced their intake of a solution of high caloric value (sucrose)

in the same way they had reduced their intake of ethanol in Experiment 1. One explanation of this result might be found in the changes in carbohydrate metabolism that occur in both rats and humans. During the last trimester there is decreased carbohydrate tolerance despite increased concentrations of circulating insulin (Freinkel, 1980; Friesen, 1973). In Experiments 1 and 2, consumption of both ethanol and sucrose decreased during the same period, days 19-22. It is possible, therefore, that the observed decrease in ethanol intake reflects a change in carbohydrate metabolism. Such an explanation would also account for the fact that the intake of the noncaloric substance, saccharin, did not decrease systematically over days.

The findings of Experiment 2 suggest that common factors control the intake of ethanol and carbohydrates in the pregnant rat. The fact that the levels of estrogens rise sharply during the last trimester of pregnancy may be relevant (Shaikh, 1971; Soloff et al., 1979). As previously mentioned, the administration of contraceptive hormones (K. Eriksson, 1969), diethylstilbestrol or estradiol benzoate (Aschkenasy-Lelu, 1960a,b) to rats has been shown to inhibit ethanol consumption by rats. Furthermore, Wurtman and Baum (1980) have recently demonstrated that estradiol benzoate selectively reduces carbohydrate intake in animals given a choice of diets varying in concentrations of carbohydrates and proteins.

It would appear that diet selection in pregnancy,

including the choice of a substance such as ethanol, is dictated by the changing nutritional requirements and metabolism of the pregnant animals. If such an hypothesis is correct, then the decrease in ethanol intake seen in Experiment 1 can be viewed as a fortuitous consequence of the changes in nutritional needs rather than the result of a specific feto-protective mechanism. Furthermore, the finding that pregnancy does not affect saccharin intake suggests that the reductions observed in both ethanol and sucrose consumption are not the result of altered taste reactivity.

In the following study, the role that estrogens play in the modulation of ethanol consumption was assessed.

Experiment 3

Studies of the effects of ovarian hormones on ethanol consumption have found in general that increased estrogen levels are associated with decreased ethanol intake (Aschkenasy-Lelu, 1960a,b; K. Eriksson, 1969; Jones & Jones, 1976). Since the concentration of estradiol in the blood of pregnant rats rises sharply only during the last trimester of pregnancy (Shaikh, 1971; Soloff et al., 1979), the finding in Experiment 1, that ethanol consumption decreased only at that time is consistent with the hypothesis that estrogens maintain an inhibitory influence on ethanol consumption.

On the basis of the finding in Experiment 2, that pregnant rats suppress their intake of a sucrose solution just as they had ethanol, it was decided to study the effects of estradiol benzoate (EB) on ethanol consumption in an experiment that was designed to parallel studies on the effects of estradiol on food intake (Mook, Kenney, Roberts, Nussbaum, & Rodier, 1972; Nance & Gorski, 1975; Wade, 1975).

Daily injections of EB to ovariectomized rats lead to suppression of food intake and to weight loss (Wade & Gray, 1979). Food intake is suppressed for a period of approximately two weeks, but with continued daily EB administration total intake recovers to levels comparable to oil-treated control animals (Tarttelin & Gorski, 1973; Wade, 1975). It was predicted that if the rat was responsive to

the caloric property of ethanol then the administration of EB would be followed by decreases in ethanol consumption similar to those reported for food intake (Wade, 1975).

Method

In this and all following experiments, female Wistar rats were ovariectomized soon after arrival in the animal colony and at least one month prior to their participation in an experiment. Twenty-seven ovariectomized rats weighing 300-360 g at the outset of the study served as subjects. The animals had continuous access to both an ethanol solution and tap water. Ethanol consumption was computed in terms of daily absolute amount of ethanol ingested (mean g per kg body weight). Water intake was calculated in terms of body weight. The daily water and ethanol consumption data were summed over periods of two days and all statistical analyses were performed on these two-day totals. Body weights were averaged over two-day periods to make these data directly comparable to the drinking data. Furthermore, body weights are reported and analyzed in terms of change in body weight prior to hormone administration. The mean body weight of an animal over six days prior to treatment was taken as its baseline.

Upon initial exposure to ethanol the concentration of the solution was increased by steps of 2% (v/v; 95% ethanol) every two days commencing with a 2% and terminating with a 10% solution. The concentration of the ethanol solution remained fixed at 10% for the remainder of the study. After an additional three weeks of exposure to this concentration, the animals were divided into three groups matched for ethanol and water consumption and for body weight. Subcutaneous injections of either EB (5 µg in 0.1 ml peanut oil) or the oil vehicle alone (0.1 ml) were begun at this time. This dose of EB was chosen because it produces a plasma level of estradiol that is roughly equal to that seen during the proestrous peak in intact and regularly cycling rats (Henderson, Baker, & Fink, 1977). One group (DAILY EB) received daily injections of EB for a period of 22 days. A second group (SINGLE EB) was administered EB for one day only. Subsequently, this group continued to receive injections of the oil vehicle alone for an additional 21 days. The third group (OIL) received injections of the oil vehicle for each of the 22 days of the treatment period. All injections were administered at the time when solutions were changed and body weights recorded. At the end of the treatment period all injections were discontinued and the intake of the animals was measured for an additional 12 days.

Results

Ethanol consumption in response to EB administration is illustrated in Figure 5a. The repeated measures analysis of variance revealed a significant Days effect, $F(16, 384) = 5.34$, $p<.001$ and Group x Days interaction, $F(32, 384) = 4.71$, $p<.001$. No differences were evident prior to hormone administration. The group receiving the SINGLE EB injection consumed less ethanol during the first four days following the injection. The trend was not statistically significant, however. On the other hand, DAILY EB injections led to a much greater decrease in consumption that was statistically different from that of the oil-treated control group animals even during the first two days of treatment (Tukey, $p<.01$). In spite of the continuation of hormone administration, ethanol consumption recovered to levels of control group animals by Days 13-14 (Block 7) of daily EB administration. Withdrawal of the hormone did not lead to any further changes in consumption.

The effects of SINGLE and DAILY EB administration on body weight are illustrated in Figure 6. The mean body weights for the rats were 325 ± 5 g, 331 ± 6 g and 321 ± 6 g, respectively, and were not statistically different from one another.

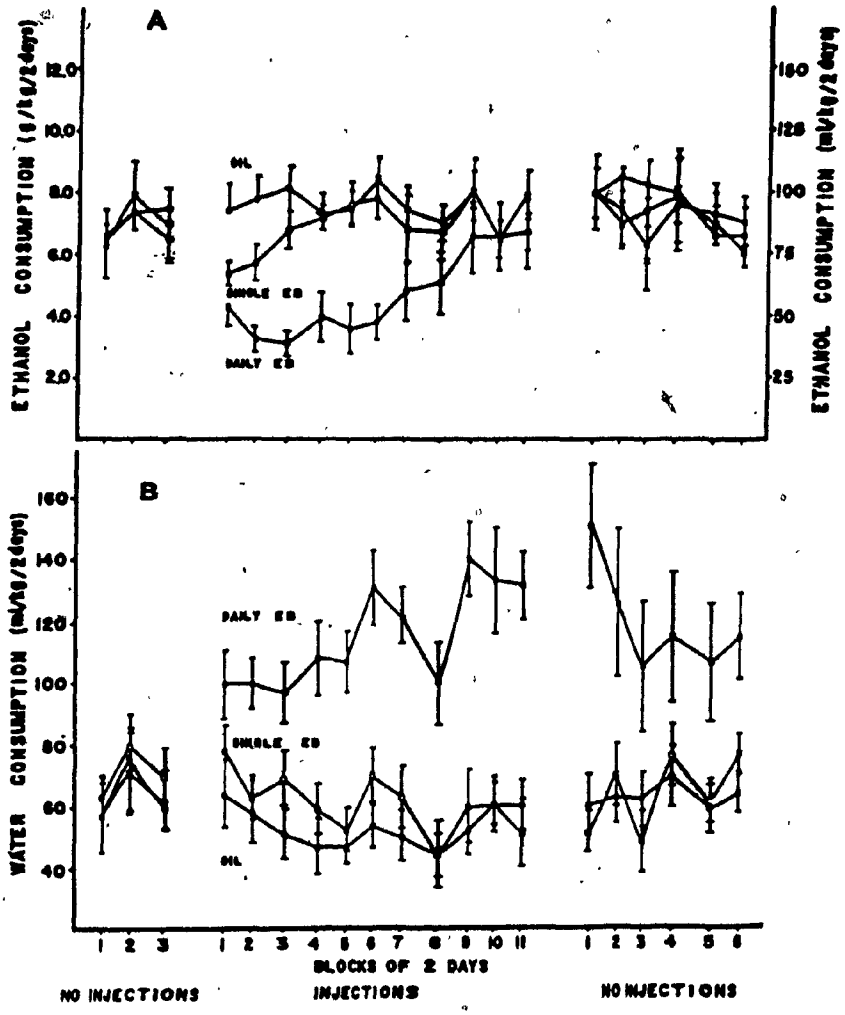

Figure 5. Mean (±SEM) ethanol (Panel A) and water (Panel B) consumption of ovariectomized rats in Experiment 3 administered either a single injection of estradiol benzoate (5 µg; SINGLE EB) on Day 1 of the injection period, daily injections of estradiol benzoate (5 µg/day; DAILY EB) on Days 1 through 22 of the injection period, or peanut oil (OIL) throughout the injection period.

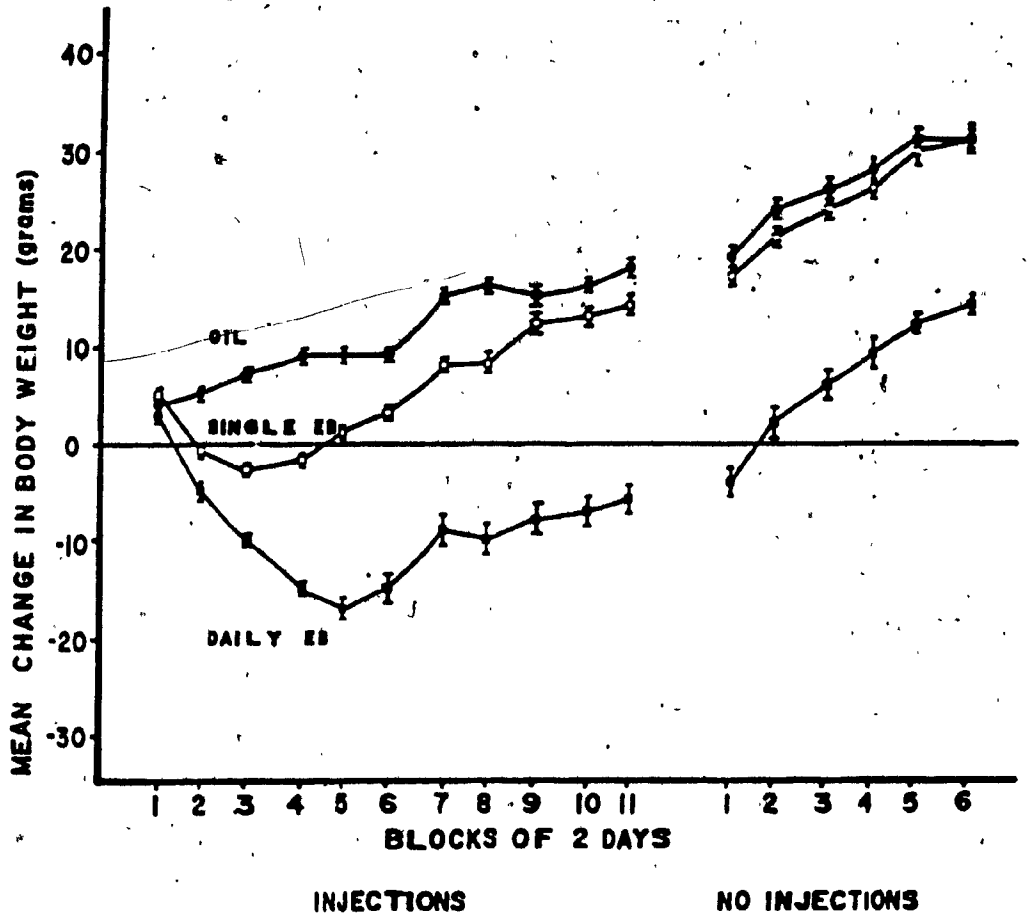

Figure 6. Effects of either a single (5 µg estradiol benzoate on Day 1) or daily injections of estradiol benzoate (5 µg/day) on mean (±SEM) change in body weight in ovariectomized rats in Experiment 3. Injections took place during the first 22 days. This was followed by an additional 12 days during which no injections were administered.

The analysis of variance conducted on the change scores following hormone administration revealed significant Group, $F(2, 24) = 39.29$, $p<.001$ and Days, $F(16, 384) = 185.67$, $p<.001$ effects and a Group x Days, $F(32, 384) = 10.38$, $p<.001$ interaction. It can be seen that DAILY EB administration produced the largest decreases in body weight. The SINGLE EB injection produced a mild and transient decrease in body weight that first became statistically significant on Days 3-4, (Tukey, $p<.05$). The body weight of this group was not different from that of the OIL group by Days 17-18 (Block 9) of the study. In contrast, the DAILY EB group showed a much more pronounced weight loss during the injection period, first expressing itself on Days 3-4 (Block 2), (Tukey, $p<.01$). These animals continued to lose weight for an additional six days. From that point onwards they gained weight at a rate similar to the group receiving injections of the oil vehicle alone, but their body weight remained approximately 8% below that of the OIL control group. Upon termination of the injections there appeared to be an accelerated rate of weight gain.

An additional finding of interest was that DAILY EB injections led to large increases in water intake (Figure 5b). While consumption by all groups was virtually identical during the baseline period, a repeated measures analysis of variance revealed significant Group, $F(2, 24) = 16.84$, $p<.001$ and Days, $F(16, 384) = 2.22$, $p<.005$ effects. It can be seen that the DAILY EB group showed large

increases in water consumption relative to the other two groups. The difference between consumption by the DAILY EB group and the OIL control group became statistically significant by Days 7-8 (Block 4) of treatment (Tukey, $p<.01$). In addition there were further increases in consumption during the second half of the injection period. After discontinuation of EB injections water consumption by the DAILY EB group returned to levels comparable to the OIL group (by Block 3 of the 'no injection' period). Messiha and Webb (1981) have reported a similar finding from a study in which they determined the effects of estrogen treatment on both water and ethanol consumption. Other studies measuring changes in water consumption subsequent to variations in estrogen levels have produced mixed results. Tarttelin & Gorski (1973) assessed the effects of EB on both food and water intake in ovariectomized rats. Compared to an oil-treated control group the water intake of animals receiving estradiol treatment increased. These enhanced levels of consumption were not different, however, from those of intact females. There also exists evidence against a direct dipsogenic action of estradiol. First, water intake declines on the day of vaginal estrus in a paradigm in which the rat has free access to food and water (Tarttelin & Gorski, 1971). In addition, increases in estradiol levels at the time of estrus (Danielson & Buggy, 1980) or exogenous administration of EB (Fregly, 1980) both result in an attenuated dipsogenic response to angiotensin

in rats. The possibility thus exists that the increaes in water consumption in this present study are the result of an effect peculiar to the present paradigm. It is as yet unclear which features of the situation or possibly characteristics of the subjects can account for these discrepant findings.

An alternative interpretation for the increased water consumption might be that the rats in the group receiving DAILY EB injections compensate for decreased ethanol consumption by increasing water intake. This explanation does not seem likely however. First, increases in water intake, in particular during the second half of the treatment period, are more dramatic than decreases in ethanol. Also, the time course of the two effects is very different. While it is true that the water intake begins to rise as ethanol intake decreases, water consumption continues to increase and peaks at a time when ethanol consumption has returned to control levels (Figure 5a and 5b).

Discussion

The results of Experiment 3 indicate that estradiol inhibits consumption of a 10% ethanol solution in paradigm in which the rat has continuous access to both ethanol and water. The time-course of change in ethanol consumption

resulting from DAILY EB injections is strikingly similar to that seen when changes in food intake are measured subsequent to EB administration. Wade (1975) reported that daily administration of 2 μg EB to ovariectmized rats led to decreases in food intake. With continued administration of EB, food intake returned to control levels by Day 18. In the present study, DAILY EB, while initially suppressing ethanol consumption, failed to maintain this effect; ethanol consumption returned to the levels of the oil-treated animals by Day 14. It is on the basis of this similarity together with the results of Experiment 2, that it is suggested that the mechanism leading to inhibitory effects of estradiol on food intake is the same that leads to decreases in ethanol consumption.

Experiment 4

As a further test of the idea that the mechanisms mediating the effects of EB on ethanol intake are the same as those involved in the effects of EB on food intake, it was decided to assess the effects of MER-25, an antiestrogen on ethanol consumption. MER-25, while interfering with many of the actions of estradiol, mimics in part the action of estradiol on food intake and body weight (Roy, Maass, & Wade, 1977; Roy & Wade, 1976; Wade & Blaustein, 1978). The mechanism mediating this effect is as yet undetermined (Roy, Schmit, McEwen, & Wade, 1979). It was reasoned, however, that if the rat when exposed to the situation described in Experiment 1 is drinking ethanol, in part, for its caloric content, then MER-25 injections would be expected to suppress ethanol consumption just as it does food consumption (Roy & Wade, 1976).

Method

Eighteen ovariectomized rats weighing 295-350 g at the outset of the study served as subjects. The housing of the animals, the presentation of the drinking tubes, and the calculation of fluid intake were carried out as in Experiment 3. The only change from the previous experiment was that animals received a 6% (v/v; 95% ethanol) ethanol

solution on the first exposure. The concentration of the solution remain fixed at this level for three days and was finally set at 10% for the remainder of the study. Just as in Experiment 3, the rats had three weeks experience with a choice between a 10% ethanol solution and water, prior to hormone administration. At the end of this period the animals were divided into two equal groups, matched on all measures. The injection period began the following day and lasted for 36 days. One group received daily subcutaneous injections of 10 mg/animal MER-25 dissolved in 0.1 ml peanut oil. This dose has previously been shown to suppress food intake (Roy & Wade, 1976). A second group (OIL) received daily injections of the oil vehicle alone for the same period. At the end of this phase of the study, injections were discontinued and the fluid intake of the animals was measured for an additional 14 days.

Results

Ethanol consumption in response to MER-25 administration decreased rapidly and dramatically (Figure 7a).

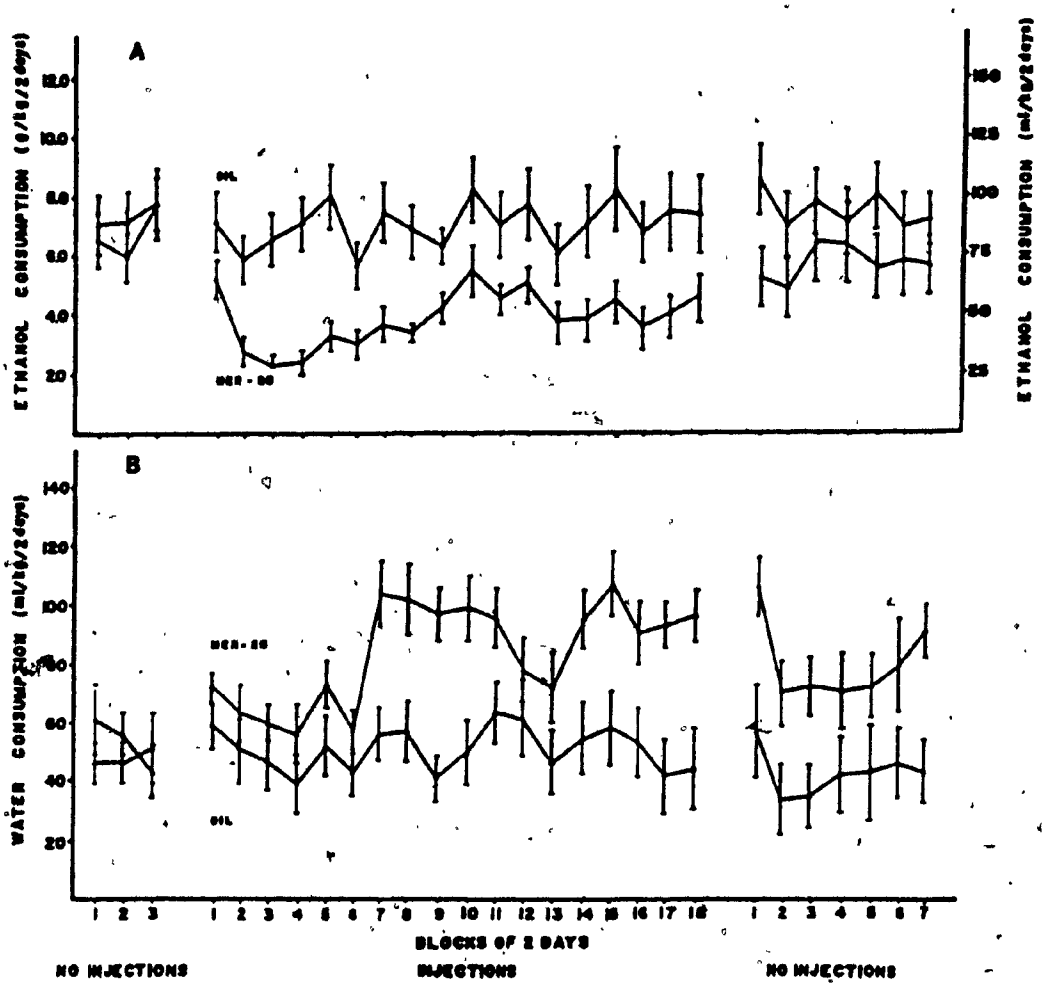

Figure 7. Mean (±SEM) ethanol (Panel A) and water (Panel B) consumption of ovariectomized rats in Experiment 4 administered either MER-25 (10mg/day) or peanut oil (OIL) on Days 1 through 36. Each point represents the total consumption for two days.

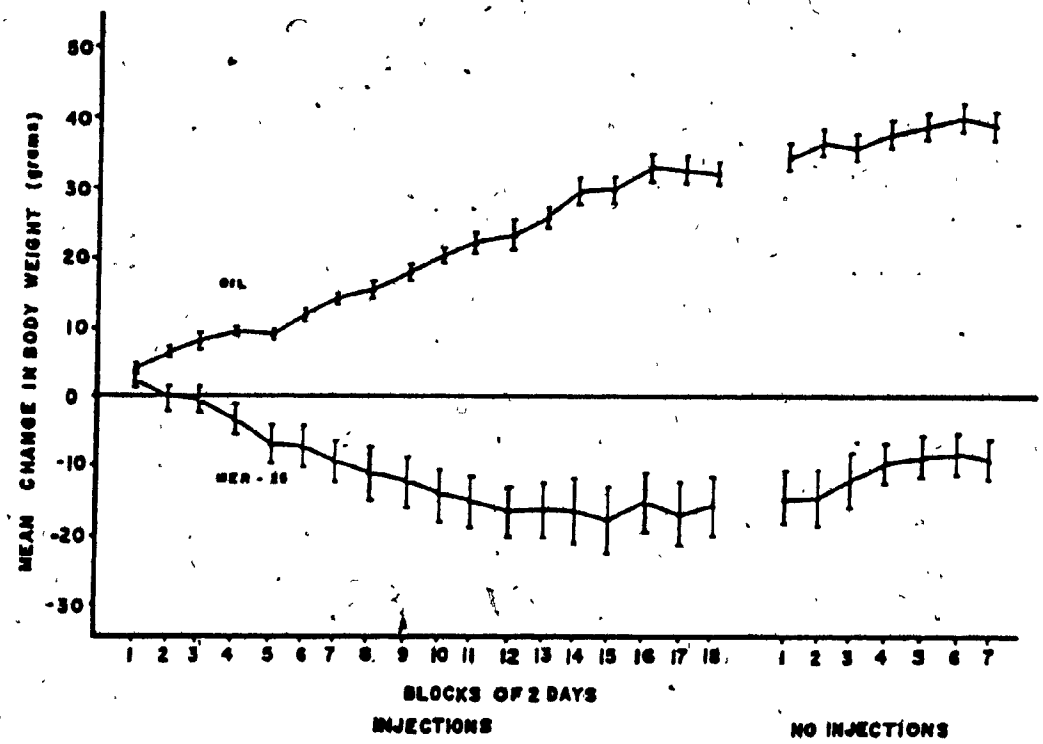

Figure 8. Effects of daily MER-25 injections (10mg/day) on mean (±SEM) change in body weight in ovariectomized rats in Experiment 4.

The repeated measures analysis of variance revealed both Group, $F(1,16) = 5.89$, $p<.05$ and Days, $F(24, 384) = 4.36$, $p<.001$ effects in addition to a Group x Days interaction, $F(24, 384) = 2.23$, $p<.001$. Ethanol intake in the MER-25 group was depressed by Days 3-4 of treatment (F-test for simple main effects, $p<.05$). With continued injections, however, consumption by these animals returned to levels comparable to those of the OIL group (by Days 17-18 of the treatment period, F-test for simple main effects, $p>.10$). Subsequent sporadic decreases in ethanol consumption were seen at several points during the continuation of treatment. Intake returned and stayed the same as that of the OIL group once hormone administration had ceased.

Water intake, illustrated in Figure 7b, increased in the group receiving daily MER-25 injections. The analysis of variance revealed statistically reliable Group, $F(1, 16) = 9.13$, $p<.01$ and Days, $F(24, 384) = 3.72$, $p<.001$ effects, in addition to a significant Group x Days interaction, $F(24, 384) = 1.74$, $p<.05$. The intake of the MER-25 group first became significantly elevated on Days 13-14 of the injection period (F-test for simple main effects, $p<.01$), but subsequently decreased on Days 23-26 to levels not significantly different from that of the OIL control group. Intake spontaneously rose once again and remained significantly elevated until 12 days after the termination

of treatment with the hormone.

The effect of MER-25 injections on body weight is illustrated in Figure 8. A repeated measures analysis of these data revealed highly significant Group, $F(1, 16) = 106.61$, $p<.001$ and Days, $F(24, 384) = 22.05$, $p<.001$, in addition to a significant Group x Days interaction, $F(24, 384) = 63.73$, $p<.001$. The MER-25 group was first observed to lose weight by Days 7-8 of the treatment period (F-test for simple main effects, $p<.01$), and the animals in this group continued to lose weight for an additional 24 days. The percent weight loss at this time, as calculated by considering the weight of the OIL control group as the predicted weight of the MER-25 group had they not received the antiestrogen, was approximately 12%. This weight loss is somewhat higher than the 8% decrease in body weight produced by chronic estradiol administration (Experiment 3). It can be seen that with termination of the MER-25 injections these animals gained weight at an accelerated rate.

Discussion

The results of Experiment 4 demonstrating that the paradoxical effects on food intake of the antiestrogen MER-25 are generalizable to ethanol consumption, lend support to the notion that the rat given continuous access

to ethanol is responsive to the caloric content of the solution. Not only did daily injections of MER-25 suppress ethanol consumption, but the time course of the effect paralleled to a remarkable degree the effect of MER-25 on food intake reported by Roy and Wade (1976).

Although the significance of the effect remains unclear, the water intake of the group receiving MER-25 injections increased just as it had subsequent to EB administration (Experiment 3). In both cases the intake was seen to return to levels observed in oil-treated animals once the agent had been withdrawn.

In summary, the results of the last two experiments (Experiments 3 and 4) demonstrate that both EB and MER-25 bring about decreases in ethanol consumption. Moreover, the time course of change seen in response to these hormonal manipulations is very similar to that observed when food intake is considered (Roy & Wade, 1976). These similarities suggest that the mechanism leading to hormonally-induced inhibition of food intake is the same that contributes to decrements in ethanol consumption.

Alternative interpretations for these findings do exist, however. Decrements in ethanol consumption subsequent to a pharmacological manipulation could be the result of either a direct toxic effect exerted by the injected substance or as a result of a malaise developing through an interaction with ethanol. These possibilties were tested in the next two experiments. In Experiment 5

the effects of EB and MER-25 on ethanol and acetaldehyde elimination from the blood were determined.

Experiment 5

Only 2%-10% of the ethanol absorbed by the gut is eliminated through the kidneys and lungs, the rest must be oxidized principally in the liver, which contains the bulk of the enzymes capable of ethanol oxidation (Wallgren & Barry, 1970). Ethanol is metabolized by alcohol dehydrogenase (ADH) into acetaldehyde. Acetaldehyde is, in turn, rapidly oxidized to acetate by the mitochondrial enzyme aldehyde dehydrogenase (ALDH). ALDH-inhibitors (e.g. disulfiam) can result in the accumulation of toxic levels of acetaldehyde subsequent to ethanol administration (Kitson, 1977). Excessively high blood acetaldehyde levels could thus result in the reduction of voluntary ethanol consumption, or any other consummatory response for that matter. It was considered important, therefore, to evaluate the possibility that the suppression of ethanol intake following EB administration resulted from altered ethanol metabolism. The need to do this was further reinforced by the fact that several studies have indicated a role for ovarian hormones in the regulation of ethanol metabolism.

Jones and Jones (1976) have reported that upon ingestion of a fixed dose of ethanol, blood ethanol levels in women were higher during the premenstrual than during other phases of the menstrual cycle. In other studies conducted by these same investigators (Jones & Jones, 1977),

it was reported that women using oral contraceptive pills consumed less ethanol than women not using them. Together, these findings suggest a reduction in the rate of ethanol metabolism might be associated with high estrogen levels.

Several studies, conducted in rats, have specifically looked at the effects of estrogens on the activities of both ADH (Mezey, Potter, & Tsitouras, 1981) and ALDH (Messiha, Lox, & Heine, 1980; Messiha, Lox, Heine, & Webb, 1981; Messiha & Tyner, 1980). Reports of an inhibitory action on ALDH activity are of particular interest because such an action could result in the accumulation of acetaldehyde during the course of voluntary ethanol consumption. Because the accumulation of acetaldehyde, has been linked to decreases in the voluntary consumption of ethanol in rats (C. Eriksson, 1980), it seemed important to study the effects of the hormone treatment used in our experiments. The elimination of ethanol and acetaldehyde from the blood of rats intubated with a moderate dose of ethanol was studied. In addition, it became possible, by using hormonal treatment regimens that replicated those of Experiments 3 and 4, to evaluate whether any observed changes in metabolism could account for the behavioral data.

Method

Thirty-three female rats ovariectomized three months

prior to the outset of the study and weighing 260-375 g with a mean weight of 333±29 g served as subjects. The animals had continuous access to both a 10% ethanol solution and tap water dispensed from Richter tubes for 22 days prior to the intubation of ethanol. Tubes were refilled with fresh solutions daily. Intake of fluids was not recorded but body weight was measured on alternate days. The animals were randomly assigned to one of four groups, eight animals in each of three groups and nine animals in the fourth. All injections were administered subcutaneously in a volume of 0.1 ml between 1400-1600 h. The DAILY EB group received daily injections of EB (5 µg/animal) for a period of 22 days, a duration identical to the treatment received by the DAILY EB group in Experiment 3. A second group (MER-25) received daily injections of MER-25 (10 mg/animal) over the same period as the DAILY EB group. The third group (SINGLE EB) received the oil vehicle alone for the first 21 days but were administered EB (5 µg/animal) at 2000 h on Day 22 of the study. The fourth group (OIL) received the oil vehicle alone (0.1 ml) and served as a control group.

On the morning (0900 h) of Day 23 of the study all animals were intubated with a 2.0 g/kg body weight dose of ethanol (30% v/v; 95% ethanol). Blood samples (0.2 ml) were taken from the from the tip of the tail at 0.5, 1, 2, 3, and 5 h after intubation. The samples were analyzed for ethanol and acetaldehyde according to the procedure described by Stowell (1979).

Results

A repeated measures analysis of variance of the data presented in Figure 9a revealed that the concentration of ethanol in the blood decreased over time, $F(4,112) = 142.90$, $p<.001$. Neither the Group nor Group x Time interaction achieved statistical significance. Thus it can be concluded that under the conditions of the present experiment neither administration of EB nor MER-25 significantly affected the rate at which ethanol was eliminated from the blood.

Acetaldehyde levels are illustrated in Figure 9b. These data reveal that one group was distinctively different from the others. Higher blood acetaldehyde levels were exhibited by the group that had received chronic EB treatment prior to intubation (DAILY EB). The overall analysis of variance revealed significant Group, $F(3,29) = 9.01$, $p<.001$, and Time, $F(4,116) = 18.14$, $p<.001$ effects. In addition, the increased peak of the DAILY EB group with a gradual decay to control levels led to a significant Group x Time interaction, $F(12,116) = 3.29$, $p<.001$. The blood acetaldehyde levels of the DAILY EB group returned to control levels by the third hour after intubation (Tukey, $p>.05$).

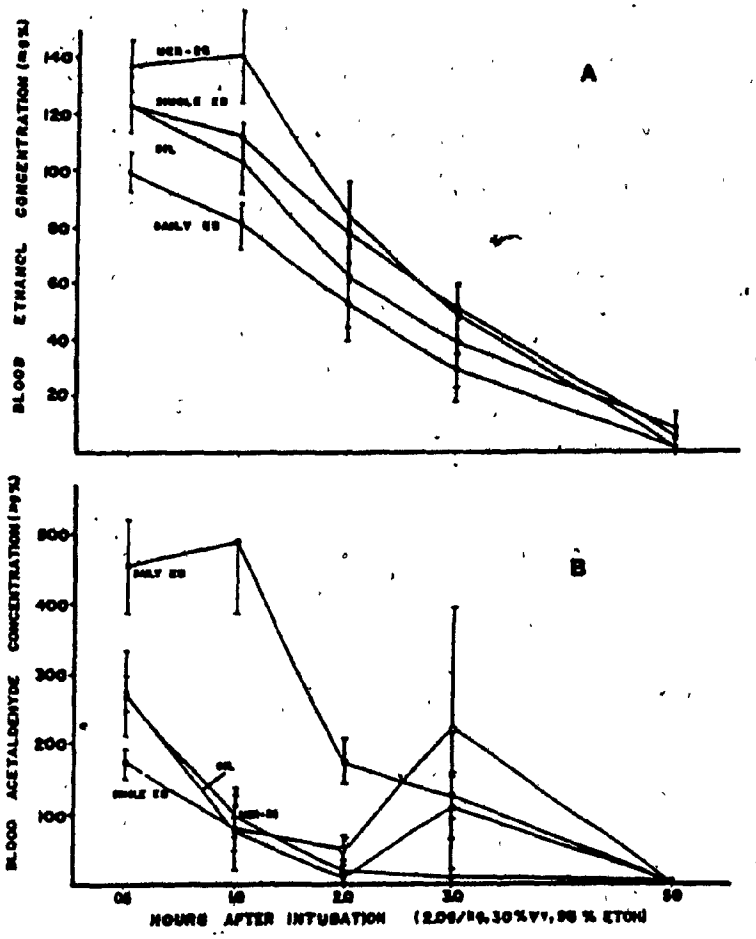

Figure 9. Mean (±SEM) blood ethanol (Panel A) and acetaldehyde (Panel B) levels of ovariectomized rats in Experiment 5 injected for 22 days with either estradiol benzoate (5µg/day; DAILY EB), MER-25 (10mg/day), or peanut oil (OIL). A fourth group received a single injection of estradiol benzoate (5 µg; SINGLE EB), 13 hr prior to intubation of ethanol.

With the exception of the SINGLE EB group, none of the other treatment conditions led to significant differences from the oil-treated animals either in the presence or rate of elimination of acetaldehyde, as determined by a post-hoc analysis (F-test for simple main effects) of the data. The blood acetaldehyde level of the SINGLE EB group was lower than that of the oil-treated group at 0.5 h (Tukey, $p<.05$).

Discussion

The present finding that DAILY EB can enhance levels of acetaldehyde subsequent to ethanol administration is interesting but not altogether surprising. There is a recent report that estradiol can interact with ethanol metabolism by inhibiting liver mitochondrial aldehyde dehydrogenase (Messiha & Tyner, 1980). This, in principle, could lead to increases in blood acetaldehyde subsequent to ethanol exposure. In the present experiment the only group demonstrating increased levels of acetaldehyde was the group that had received 22 daily injections of EB at the time of ethanol intubation. It should be recalled that while EB produced a transient inhibition of voluntary ethanol consumption (Experiment 3), intake had returned to the level of the control animals within 14 days and remained comparable to the oil-treated group in spite of continued EB

administration; ethanol consumption by the DAILY EB group was essentially identical to that of the control group after 22 days of daily EB treatment. Furthermore, the group that had received daily injections of MER-25 did not reveal an accumulation of acetaldehyde relative to the oil-treated control group. This treatment was extremely effective, however, in suppressing ethanol consumption in Experiment 4. In conclusion, while chronic estradiol administration can be demonstrated to interfere with the elimination of acetaldehyde, this fact would not appear to explain the decreased ethanol consumption in the previous two experiments.

Experiment 6

In this experiment the possibility that the EB-produced decreases in ethanol consumption were attributable to a malaise resulting from an interaction between ethanol and estradiol was examined. To that end a conditioned taste aversion (CTA) study was devised in which ovariectomized animals, injected with either EB or the oil vehicle, were exposed to a novel sodium saccharin solution and subsequently injected with ethanol. Several studies have reported that administration of ethanol by various routes, paired with the oral intake of a saccharin solution, induces in rats a CTA to the flavored solution (Berman & Cannon, 1974; Cannon, Berman, Baker, & Atkinson, 1975; Cappell & LeBlanc, 1973; Lester, Nachman, & LeMagnen, 1970). The rationale behind this study was that if, in Experiment 3, EB were interacting with ethanol to produce a toxic reaction, and thereby resulting in decreased ethanol consumption, then EB combined with ethanol as the unconditioned stimulus in the CTA paradigm should lead to an enhanced aversion to the novel saccharin solution.

Method

The 31 ovariectomized subjects used in this study had previously been used in Experiment 5. A period of 30 days had elapsed since the time of the last hormone injection of Experiment 5 and the beginning of the present study. The animals were assigned to one of four groups in such a way that animals coming from different groups in Experiment 5 were equally represented in each group. Food was available ad libitum but water was presented only 10 min/24 h. The presentation occurred daily at noon. An ethanol solution was not present. Water was dispensed from plastic tubes with double ball-bearing spouts. Fluid intake and body weight were measured at the end of this 10-min period. This phase of the experiment continued until fluid consumption during the 10-min period had stabilized.

Pre-exposure stage. During this phase of the study all animals received two pre-exposures to the unconditioned stimulus (1.5 g/kg ethanol; 20% w/v in saline) injected intraperitoneally 7 and 4 days prior to the presentation of the conditioned stimulus (0.1% sodium saccharin). The purpose of these pre-exposures was to attempt to partially recreate the experience with ethanol that the rats in Experiments 3 and 4 had had prior to hormone administration.

EB administration. Two days after the last pre-exposure to ethanol, two of the groups (EB + EtOH and EB

+ SAL) began to receive daily subcutaneous injections of 5 µg EB in 0.1 ml oil at approximately 1000 h. Injections continued until the end of the study. The remaining two groups (OIL + EtOH and OIL + SAL) received daily injections of the vehicle alone (0.1 ml oil) during the same period.

Pairing days. Two days following the beginning of hormone administration, the animals in all groups were given a 0.1% sodium saccharin solution for 10 min in place of water. Immediately following the 10-min drinking period two of the groups (EB + EtOH and OIL + EtOH) were given an i.p. injection of ethanol (1.5 g/kg ethanol; 20% w/v in saline). The remaining groups (EB + SAL and OIL + SAL) received injections of saline, alone. Two additional pairing days identical to that described above were given. Each pairing followed at 2-day intervals. On intervening days water was presented instead of saccharin. Subsequent to the third pairing day, animals in all groups received an additional three exposures to saccharin, but these trials were not paired with injections.

Results

A repeated measures analysis of variance of the data from the six days on which the saccharin solution was available revealed highly significant Group, $F(3, 27) = 46.39$, $p<.001$ and Days, $F(5, 135) = 14.84$, $p<.001$ effects, in addition to a Group x Days interaction, $F(15, 135) = 15.79$, $p<.001$ (Figure 10). There were no statistically significant differences between the groups either in consumption of water on the day prior to or on the first day of saccharin exposure. Furthermore, it can be seen that the levels of saccharin intake in all the groups were comparable to those seen on the previous day when water was available. Differences between the groups appeared first on the second day of conditioning (F-test for simple main effects, $p<.01$). This in fact would be the earliest point at which the degree of aversion to the saccharin solution induced through its association with ethanol could be assesed. Ethanol administration served as an extremely effective unconditioned stimulus for the development of a conditioned avoidance of the sodium saccharin solution. Moreover, it can be seen that the presence of EB did not in any way enhance the degree or rate of extinction of the aversion to the saccharin solution.

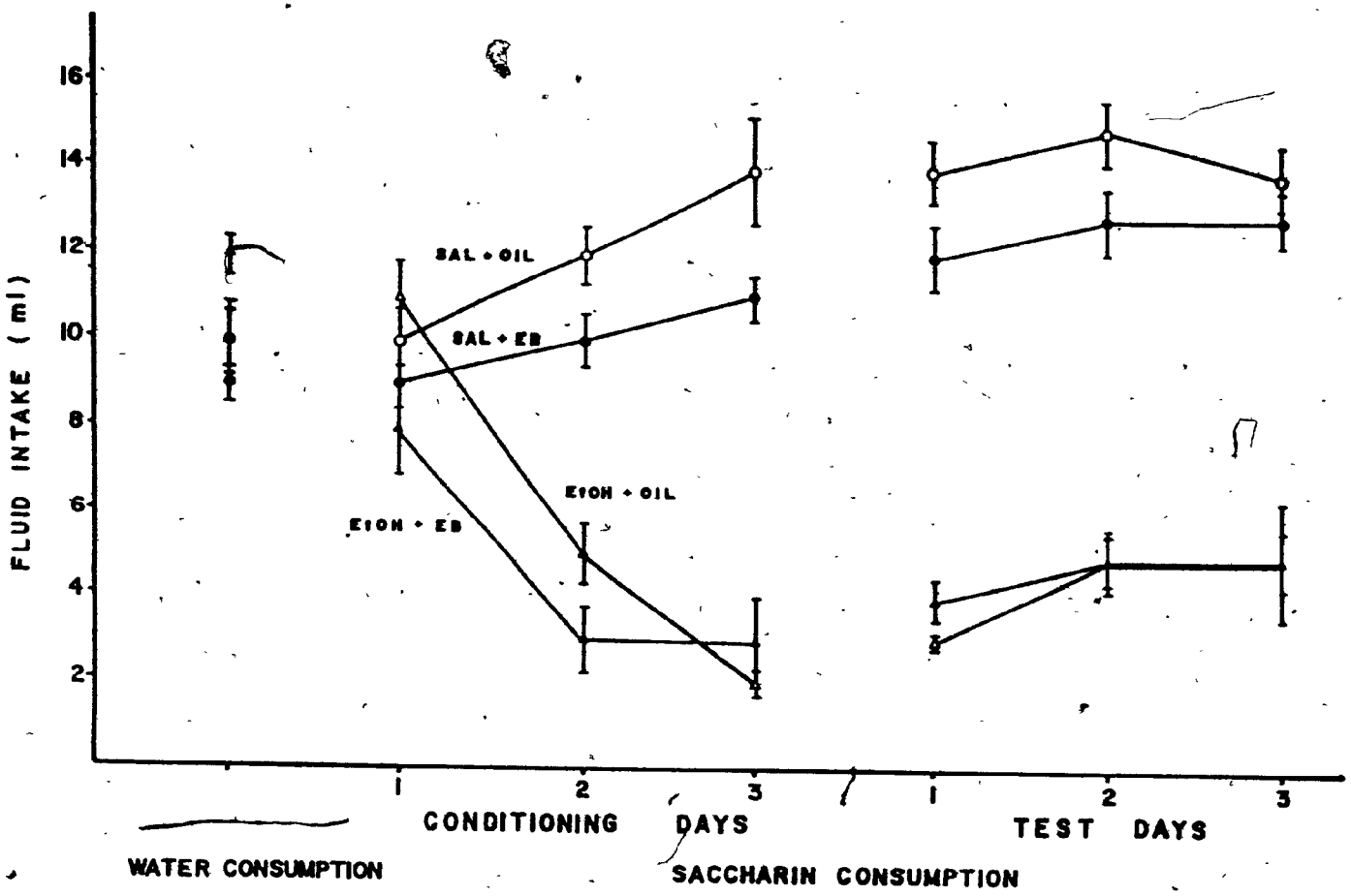

Figure 10. Mean (±SEM) intake of 0.1% sodium saccharin solution following pairings with either 1.5 g/kg, i.p. ethanol injections or with saline in ovariectomized animals pretreated with estradiol benzoate or oil in Experiment 6.

Discussion

The results of Experiment 6 confirm and extend those of Experiment 5 by demonstrating behaviorally that EB does not enhance the toxicity of ethanol. Together, the results of Experiments 5 and 6, by eliminating the toxic-reaction explanation of the effects of EB on ethanol intake, lend support to the hypothesis that estradiol inhibits ethanol consumption by virtue of its anorectic properties.

Experiment 7

The present experiment extends the comparison of the effects of EB on food intake and ethanol intake to a study of meal patterns. Lasting changes in meal patterns of rats occur in response to EB administration. Blaustein and Wade (1976) have shown that EB administered to ovariectomized rats leads to an immediate and permanent shortening of the duration of each meal consumed, accompanied by a gradual increase in the frequency of meals initiated. Initially, this change in frequency only partially compensates for the decreases in duration. The trend towards increased frequency of meals continues, however, and the total food intake returns to levels indistinguishable from those of an oil-treated control group within three weeks of the onset of hormone treatment (Blaustein & Wade, 1976). By demonstrating an effect of EB on the duration of food intake that persists beyond the period of temporary hypophagia, these data reinforce the idea that estradiol can directly influence consummatory behavior. In the present experiment ovariectomized rats were given free access to food, a 10% ethanol solution and water. The effects of daily injection of EB on the pattern of feeding and drinking were evaluated.

Method

Five female Wistar rats, purchased from Canadian Breeding Farms Laboratories and weighing 175–200 g at the beginning of the study were ovariectomized and one month later individually housed with water and a 10 % ethanol (v/v; 95% ethanol) solution and standard laboratory chow available at all times. Prior to the present study the animals had been maintained in this fashion for approximately four months during which four animals served as oil-treated control subjects in Experiment 4. The fifth animal had been given a single subcutaneous injection of 5 µg EB three months prior to participation in the present study.

In this, as in the previous studies, animals were given prolonged experience with ethanol in order to stabilize consumption prior to EB treatment. This permitted a comparison, without any confounding influences of the novelty of the ethanol solution, of the effects of EB on ethanol drinking with its effects on food intake.

For purposes of the present study the animals were transferred to and housed at all times in transparent plastic boxes with metal grid floors. Water and the 10% ethanol solution were dispensed from plastic centrifuge tubes with rubber stoppers affixed with double ball-bearing metal spouts to reduce leakage. In addition, the spouts were recessed behind plastic covers in order to further minimize the possibility of leakage resulting from

inadvertent shaking of the tube. In order to permit continuous recording over the 24 h period, the tubes were connected to drinkometer circuits and an Esterline-Angus multi-pen recorder. Contact with the water or ethanol spouts caused the pens to deflect but unless the animal broke contact with the spout the record would be continuous. In addition, even if the animal paused within bout, it would be impossible to record individual licks unless the pause lasted several seconds. The position of the tubes was alternated daily. Fluid consumption was measured daily (to the nearest ml) between 1400-1700 h; fresh solutions were provided at that time. Body weights of the animals were also recorded daily. Each cage was also equipped with a lever which when pressed led to the release of a 45 mg food pellet (P.J. Noyes Company, Lancaster, N.H.) and was automatically noted on the same record as the drinking data. The cumulative number of bar-presses (pellets consumed) for the previous 24 hr period was recorded on a counter.

The criteria used for the calculation of both meals and drinking bouts closely approximates those of Levitsky (1970). In order to be scored as a meal or drinking bout contact with the lever or drinking tube had to have lasted a minimum of 35 sec. Any activity of a shorter duration was not scored. This minimum duration was long enough to ensure that inadvertent contact with a drinking spout or the bar would not be counted as drinking or eating. Analysis of the data revealed that such short duration responding occurred

very infrequently. Further, when calculating the duration of an individual eating or drinking bouts the pauses in behavior within a bout were subtracted from the total duration of the meal or drinking bout. Additional licks or bar-presses within 10 min of the last event were scored as part of the same meal or drinking bout.

The hormone treatment period lasted 20 days during which each rat received daily subcutaneous injections of 5 µg EB dissolved in 0.1 ml oil. The timing of the injections coincided with all other daily measurements. This treatment period was preceded and followed by eight daily injections of the oil vehicle alone (0.1 ml).

Results

Data are presented as daily mean values for blocks of four days. In each case the effects of treatment were evaluated by t-test for dependent samples in which a comparison was made between the last four-day block prior to EB treatment, the first (Days 1-4) and last (Days 17-20) blocks of EB treatment. The effects of EB on body weight are illustrated in Figure 11. Body weight was reduced significantly ($p<.02$) within the first four days of treatment. With continued hormone administration body weight decreased further, resting finally 29 g or approximately 8% below baseline ($p<.01$).

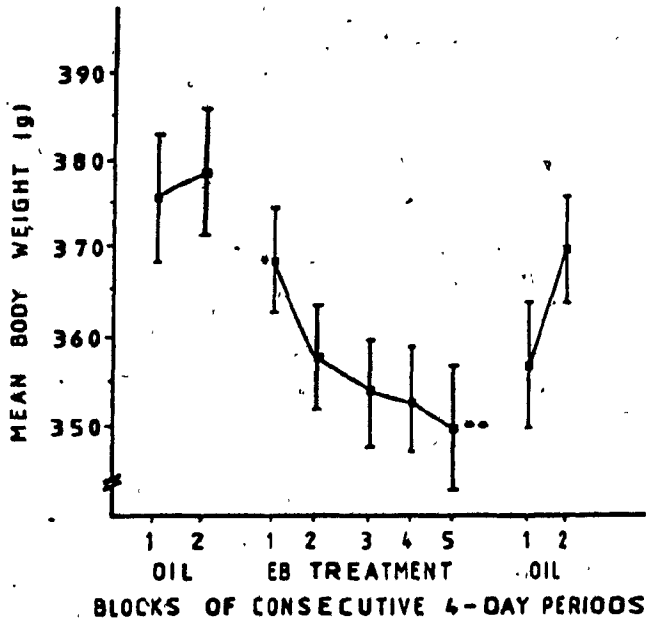

Figure 11. Mean (±SEM) daily body weight of ovariectomized rats prior to, during, and following daily EB treatment. Each point represents a block of four days. In this and following figures, the first and last blocks of treatment were compared to the last baseline block prior to EB treatment using two-tailed paired t-tests (*$p < .05$; **$p < .01$).

Increases in body weight coincided with hormone withdrawal.

The effects of EB injections on food, ethanol and water consumption are illustrated in Figures 12 through 14. Changes from baseline consummatory behavior were assessed along a number of dimensions. The effects of EB on the daily total consumption of food, ethanol, and water are illustrated in the top panel of each of these three figures. Meal and drinking bout durations are summarized in the middle panels, and frequency of bouts in the bottom panels. The data for the day and night of the light/dark cycle are illustrated separately.

It can be seen that daily hormone treatment led to an approximately 40% suppression of food intake within the first four days of treatment ($p < .001$, Figure 12a). This suppression was transient, however, and absolute intake approached baseline levels by Days 13-16 of EB treatment (Block 4). Following the termination of EB injections, large increases in food intake were observed; by Days 5-8 after removal of EB, food intake was approximately 160% of baseline.

These changes in overall food intake resulting from DAILY EB treatment were associated with changes in meal patterns. The duration of individual meals decreased significantly during both the day and night periods within the first four days of EB treatment ($p < .05$) and stabilized at approximately 50% of baseline (Figure 12b).

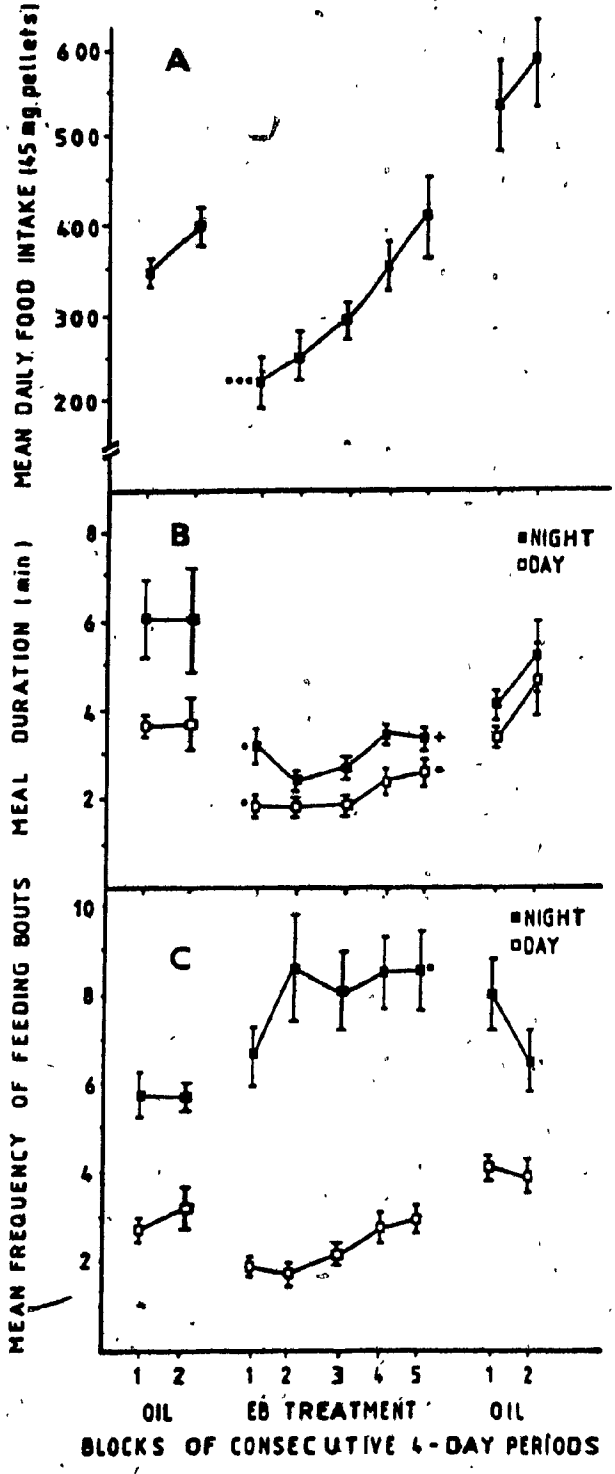

Figure 12. Mean (±SEM) daily food intake (Panel A), meal duration (Panel B) and meal frequency (Panle C) of ovariectomized rats prior to, during, and following daily EB treatment. Each point represents a block of four days. Meal duration and frequency during the day and night are graphed separately (+p< .01; *p< .05; ***p, .001).

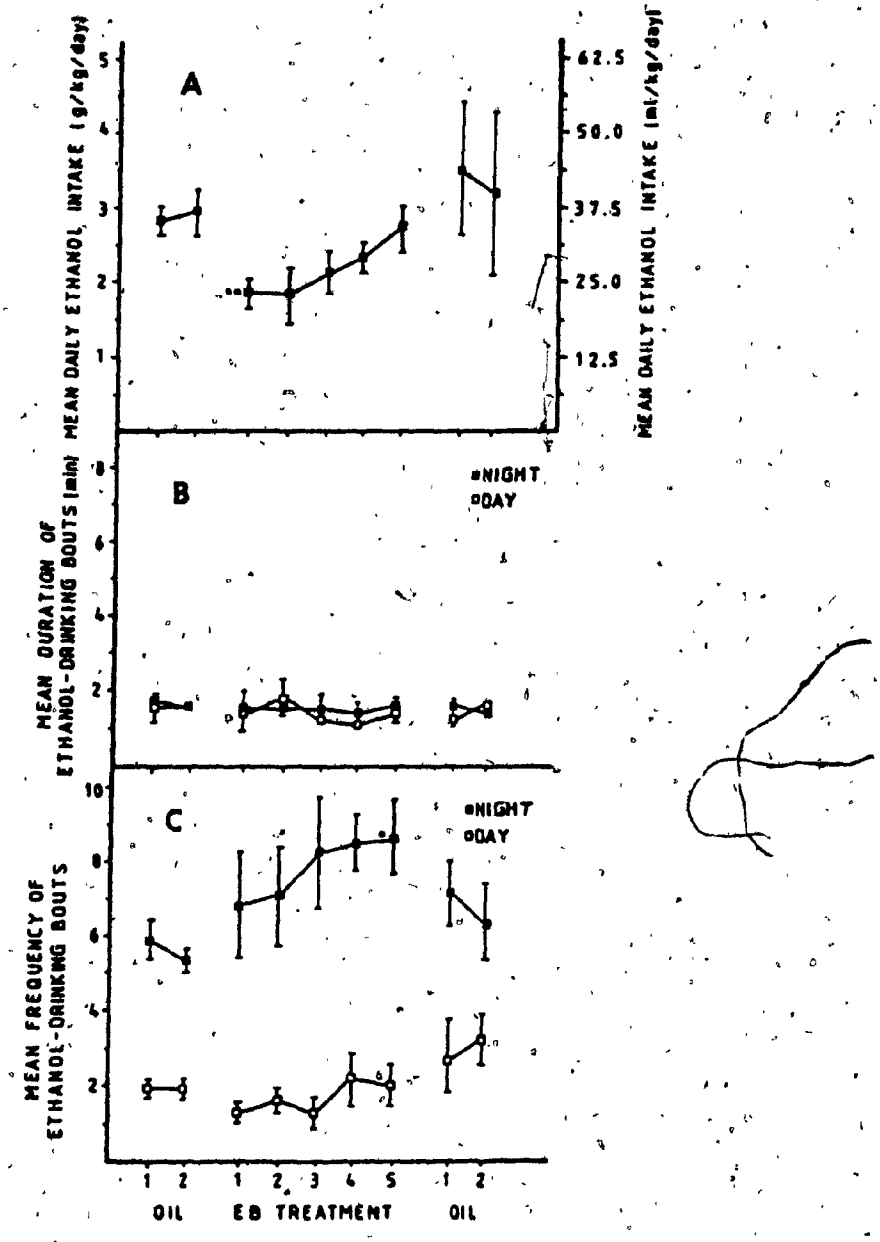

Figure 13. Mean (±SEM) daily ethanol intake (Panel A), ethanol-drinking bouts duration (Panel B) and ethanol-drinking bout frequency (Panel C) of ovariectomized rats prior to, during, and following daily EB treatment. Each point represents a block of four days. Ethanol-drinking bout duration and frequency during the day and night are graphed separately (*$p < .05$; **$p < .01$).

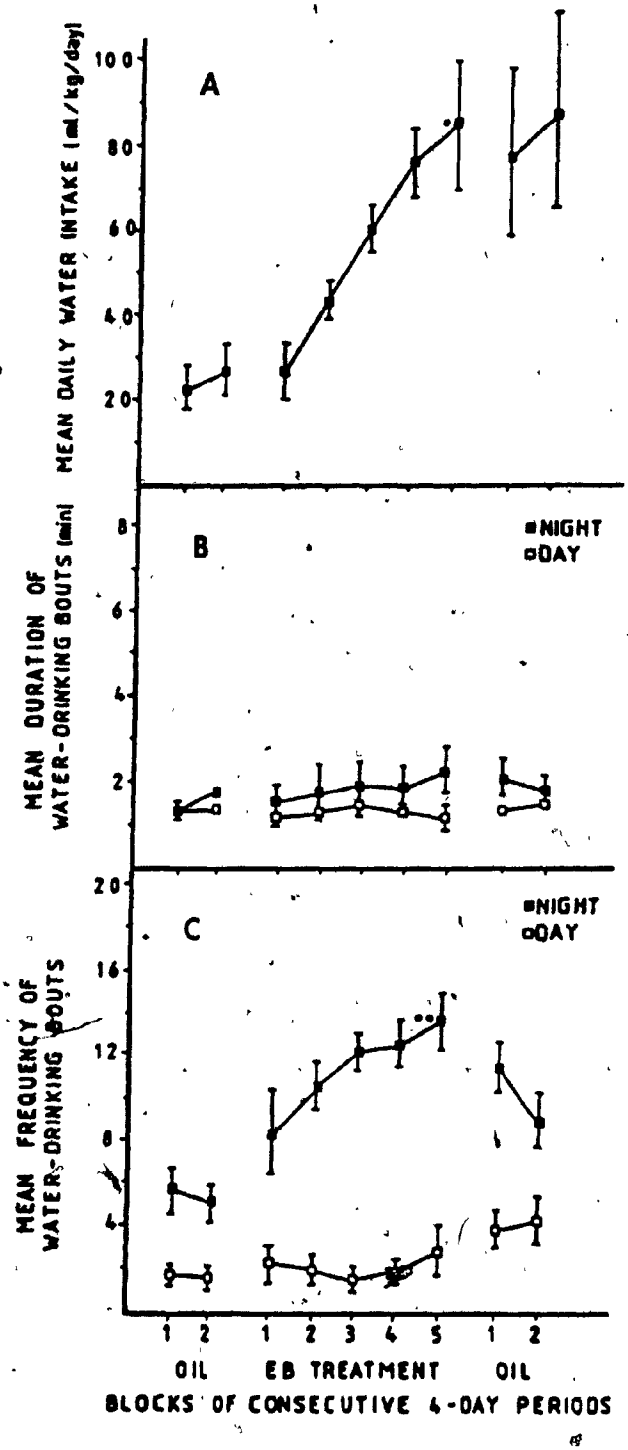

Figure 14. Mean (±SEM) daily water intake (Panel A), water-drinking bout duration (Panel B) and water-drinking bout frequency (Panel C) of ovariectomized rats prior to, during, and following EB treatment. Each point represents a block of four days. Water-drinking bout duration and frequency during the day and night are graphed separately (*p< .05; **p< .01).

It can be seen from Figure 12c that the frequency of meals increased during the night ($p<.02$), but was not significantly affected during the day. The frequency of night meals increased by Days 5-8 of EB treatment to approximately 150% of baseline, and remained stable thereafter. In summary, the anorexia produced by EB results from a change in meal duration. Its transience is due to an increase in frequency that occurs gradually and consistently, bringing the total food intake to baseline levels.

Changes in the pattern of ethanol consumption in response to EB are illustrated in Figure 13. Ethanol consumption declined during EB treatment (Figure 13a). The suppression was most marked early in treatment, when animals consumed approximately 60% of their baseline ethanol intake ($p<.01$). Just as was the case with food intake, however, total ethanol intake increased gradually over days, despite continued hormone administration and returned to baseline by Days 17-20 of EB treatment ($p>.05$). It can be seen from Figure 13b that there was little effect of EB on the duration of ethanol-drinking bouts. EB did, however, produce an increase in the frequency of bouts (Figure 13c) similar to that seen in the frequency of meals (Figure 12c).

The effects of EB on water intake are illustrated in Figure 14. Total water intake increased three-fold over the

course of EB treatment ($p<.05$) with mean mean intake rising from 27±6 ml/kg/day prior to hormone administration to 86±17 ml/kg/day during Days 17-20 of EB treatment (Figure 14a). This increased overall intake was accompanied by increased frequency of drinking bouts (Figure 14c). There was no effect of EB however, on the duration of water-drinking bouts (Figure 14b). Upon withdrawal of EB treatment, mean water intake remained elevated but did not differ significantly from baseline. This lack of significance is attributable to the fact that one animal showed a large reduction immediately following hormone withdrawal.

Discussion

In the present study, EB administration induced the pattern of changes in food intake reported by others in the ovariectomized rat (Blaustein & Wade, 1976; Mook et al., 1972; Tarttelin & Gorski, 1973; Wade, 1975). Hypophagia was transient, lasting approximately two weeks, but was nonetheless accompanied by weight loss which persisted throughout the injection period. Furthermore, as reported previously, meal duration decreased and frequency of meals gradually increased (Blaustein & Wade, 1976). We can now compare directly the effects of EB on ethanol consumption to these highly replicable findings of EB on food intake. In the present study the rats exhibited shifts in their pattern

of ethanol consumption in response to treatment with EB that paralleled both in time-course and magnitude the effects observed in food intake. This finding of decreased ethanol consumption in ovariectomized rats in response to treatment with EB confirms the finding in Experiment 3 and extends it by demonstrating that in the same animal the magnitude and time course of the effects on both food and ethanol intake are virtually identical.

At first glance it is puzzling that while a decrease in meal duration was clearly observed, no systematic change in the duration of ethanol drinking bouts was recorded. Considering the similarity in effects along the other two dimensions described (i.e., decreased total intake and increased frequency of meals and drinking bouts), the absence of a decrease in the duration of ethanol drinking bouts is difficult to understand. A decrease in total ethanol intake that is associated with an increase in the overall frequency of drinking bouts, must necessarily be accompanied by a decrease in the volume consumed during an individual bout. It therefore must be concluded that the measure of ethanol bout duration used in the present experiment was not an adequate index of the volume of ethanol consumed. While efforts were made to eliminate pauses in responding from the calculations of the duration of about, this effort was not sufficient to detect changes in the density or rate of licking (see Method). Thus, it must be inferred from the results illustrated in Figure 13

that the rate of ethanol drinking must have decreased with EB treatment despite the absence of change in the duration of the bout. This decrease in the rate of licking would thus contribute to the decrease observed in total ethanol intake.

In sharp contrast to those similarities described above in the effects produced by EB on the patterns of both food and ethanol intake, water consumption underwent very different types of changes. Total water intake increased dramatically early during EB treatment, resting finally 300% above baseline levels. This dipsogenic effect was achieved by increases in the frequency of drinking bouts during the night period. Durations during the light period remained uninfluenced by the presence of EB. Messiha and Webb (1981) have reported similar increases in total water intake in response to EB administration in a situation in which the rat had free access to both an ethanol solution and water. That the increases in water intake are not simply a compensatory response to EB-induced decreases in ethanol consumption becomes apparent when both the magnitude and the time course of the two events are compared. First, water increases are greater than decreases in ethanol. Also, water intake continues to rise and peaks at a time when ethanol consumption has returned to control levels (Figures 13a and 14a). As already mentioned in Experiment 3, these large increases in water intake that occur when animals consuming ethanol are administered EB are puzzling in view

of several reports that increased levels of estradiol inhibit angiotensin-induced drinking (Danielson & Buggy, 1980; Fregley, 1980). In view of the fact that the same effect was observed in Experiments 3 and 4 and once again in the present study, it would appear that this phenomenon is reliable.

By demonstrating that daily EB administration to ovariectomized rats leads to similar changes in both overall intake and the patterns of eating and ethanol consumption, the results of the present study further support the notion that that the rat, given free access to ethanol, responds to the caloric property of the solution.

General Discussion

The consumption of ethanol by animals, in particular rodents, has been demonstrated to be under the influence of several factors such as the taste and odor of the ethanol solution, the source of calories that it provides, and the ability of the species studied to metabolize ethanol (Wallgren & Barry, 1970). Furthermore, estrogens have been reported to alter sensitivity to gustatory and olfactory stimuli, to produce changes in energy balance, and, as discussed in Experiment 5, to alter ethanol metabolism directly. It was concluded in that discussion that changed ethanol metabolism, brought about by estrogens, did not provide a satisfactory explanation of the data. In the following section, the known effects of estrogens on taste and smell and on energy balance will be reviewed. The possibility that the present findings could be accounted for by one or more of the proposed mechanisms of action of estrogens will be explored.

Ovarian hormone effects on taste and olfactory stimuli

One way in which estrogens might act to suppress ethanol intake would be by intensifying the aversive taste and smell properties of ethanol solutions. Ethanol consumption has been demonstrated to be suppressed in experimental animals

by both the taste and smell of higher concentration solutions. K. Eriksson (1969; cited by Wallgren and Barry, 1970) reported that in rats, increases in intake of a 10% ethanol solution are observed when a very low concentration of nonnutritive sweetener (0.0034% saccharin) is added to the solution. Under these circumstances, a heavy-drinking strain of rats consumed quantities of ethanol that apparently exceeded their metabolic capacity.. Similarly, Rogers and McClearn (1964) demonstrated in mice, that the addition of a range of quantities of sucrose to a 10% ethanol solution, resulted in concentration-dependent (sucrose) increases in the volume of the ethanol solution consumed. Dicker (1958; cited by Wallgren and Barry, 1970) reported that the administration of a drug which interferes with taste, temporarily enhanced consumption of a 20% ethanol solution in a majority of rats that had previously rejected it. These studies all indicate that ethanol consumption is partially influenced by the aversive gustatory properties of higher concentration solutions.

Evidence indicating that the odor of high concentration ethanol limits intake, comes from a study by Kahn and Stellar (1960) in which olfactory bulbectomized rats demonstrated a marked shift in preference for ethanol solutions of higher concentrations. In contrast, the rat's preference for lower concentrations of ethanol was disrupted by the same manipulation.

Ovarian hormones have been reported to influence

responsivity to both gustatory and olfactory stimuli. Rats exhibit a sexually dimorphic response to palatable solutions, such that nondeprived female rats consume significantly greater quantities of both nutritively- and nonnutritively sweetened solutions than do males (Gabric & Soljacic, 1975; Valenstein, Kokolewski, & Cox, 1967). Ovariectomy has been reported to attenuate the preference of female rats for a 0.75% saccharin solution in a situation in which the rat is given unlimited access to the test solution, and to water and laboratory chow (Nance, 1976; Zucker, 1969). Prior experience with the saccharin solution, however, blocks this inhibitory effect of ovariectomy, indicating an important role for learning in the expression of taste preferences (Wade & Zucker, 1969a).

Injections of low doses of both estradiol and progesterone to ovariectomized rats has been shown to elevate the saccharin preference of these animals to the level of intact animals (Zucker, 1969). Note, however, that neither estradiol nor progesterone administration alone was effective in altering the saccharin preference. The importance of this point will become apparent later in this discussion.

Ovarian hormones have been found not only to increase responsivity towards sweetened solutions but also to heighten the aversiveness of a bitter taste stimulus, quinine-adulterated water. Ovariectomized rats have been reported to drink significantly more of a very bitter .0075%

solution of quinine sulphate than intact females (Wade & Zucker, 1970b). Treatment of ovariectomized rats with the estradiol and progesterone combination, effective in enhancing the preference for saccharin, also enhanced the aversion to the quinine solution (Wade & Zucker, 1970b). Neither estradiol nor progesterone alone, however, was effective in restoring responsiveness of ovariectomized rats to the quinine solution. Thus, once again it appears that these two hormones act synergistically in a fashion that cannot be reproduced through the administration of either hormone alone.

It should be mentioned that an adaptation of the procedure used in the Wade & Zucker (1970b) study, using a lower concentration of quinine, failed to demonstrate an effect of ovariectomy on quinine aversion (Hirsch & Bronstein, 1976). It is possible that this discrepancy is attributable to the fact that lower concentrations of the quinine solution were chosen in this later study. This idea is suggested because it has previously been observed that the effect of ovarian hormones on saccharin taste preference depends upon the specific concentration of the test stimulus employed (Nance, 1976). Should this factor account for the discrepancies between these two studies, then the finding that ovarian hormones enhance the reactivity to an aversive taste stimulus would provide support for the idea that EB suppresses ethanol intake by heightening the aversive gustatory properties of an ethanol solution.

To summarize, the findings presented above are consistent with the conclusion that ovarian hormones can alter the responsivity of female rats to tastes and these changes, in turn, result in altered intake. Could such effects on taste responsivity account for the suppression of ethanol intake observed in ovariectomized rats in Experiment 3 subsequent to EB administration? Such a possibility appears unlikely. First, taste preferences and aversions in ovariectomized rats have been demonstrated to change only when both estrogen and progesterone were administered; either of the hormones in isolation was ineffective (Wade & Zucker, 1970b; Zucker, 1969). In Experiment 3, daily injections of EB alone suppressed ethanol consumption. Second, it has been shown that prior experience with the test solution interfered with the effects produced by these combined hormonal manipulations (Wade & Zucker, 1969a). In Experiment 3, EB inhibited ethanol consumption in rats that had received four weeks experience with the ethanol solution prior to hormone administration. Finally, the effects of EB administration on ethanol consumption in Experiment 3 were transient. In contrast, combined estrogen/progesterone injections resulted in sustained alterations in taste preference that persisted beyond the termination of hormone treatment (Zucker, 1969).

Finally, it is interesting to note that the saccharin consumption of rats in Experiment 2 did not change during pregnancy. This finding provides further support for the

idea that reduced consumption of ethanol can not be readily accounted for in terms of altered taste reactivity.

It thus appears that the effects of estradiol on ethanol consumption are probably not mediated by changes in gustation. But what of the possibility of estradiol-induced changes in olfaction resulting in suppressed ethanol intake? In a series of elegantly conducted experiments, Pietras and Moulton (1974) delineated the role that ovarian hormones play in olfactory detection. The performance of intact cycling females on an odor detection task was shown to fluctuate according to the phase of the estrous cycle. The induction of pseudopregnancy both eliminated these fluctuations and depressed detection performance. In contrast, ovariectomy elevated but still eliminated the cyclicity in performance. It was concluded by these investigators that low levels of estrogens in combination with high levels of progesterone increase discrimination thresholds, and that high levels of estrogens in combination with low levels of progesterone decrease thresholds. Further support for the view that increased levels of circulating estrogen are accompanied by lowered detection thresholds comes from an experiment in which it was found that detection performance improved following elevations in the level of endogenous estrogens (Pietras & Moulton, 1974).

As already mentioned, the odor of ethanol can either facilitate or suppress consumption in rats depending upon the concentration of the ethanol solution (Kahn & Stellar, 1960). A 10% ethanol solution (the concentration that had been chosen for all the behavioral experiments in this thesis) has been shown to be well beyond those concentrations that support optimal intake. Anosmia has been demonstrated to result in a shift in the preference-aversion function of rats towards higher concentrations (Kahn & Stellar, 1960). Since it has been proposed that estrogen reduces olfactory detection thresholds of rats (Pietras & Moulton, 1974), it is possible that suppression of ethanol intake by estradiol is the result of the enhancement of the aversive olfactroy property of the ethanol solution. While such a possibility is certainly feasible, a number of important questions relevant to verification of this hypothesis remain to be answered. First, it is unclear whether a reduction in the olfactory threshold produced by estrogen necessarily implies an enhancement of the aversive odor of a solution that lies above the detection threshold. Also, to the best of my knowledge, there are no data indicating that the effects of repeated estrogen administration on olfactory sensitivity change over time. If estradiol's suppression of ethanol consumption results from changes in olfactory acuity, then it would be necessary to demonstrate that chronic estrogen administration produces only a temporary enhancement of

sensitivity. It is not unlikely, however, that this would occur: habituation to the olfactory stimulus could serve as the basis for such changes to occur over time.

Estrogenic effects on energy balance

It has repeatedly been suggested that the food-like quality of ethanol is a primary factor involved in the manintenace of ethanol consumption by experimental animals (Aschkenasy-Lelu, 1962; Freed, 1972; Freed & Lester, 1970; Lester & Freed; 1973; Marfaing-Jallat, Larue, & LeMagnen, 1970; Palfai & Reckhow, 1977; Purdy & Lee, 1962; Richter, 1953; Westerfeld & Lawrow, 1953). Since a gram of ethanol contains almost twice as many calories as a gram of sucrose, ethanol can comprise a substantial portion of the caloric intake of an animal when it is consumed in significant volumes. The idea that animals consume ethanol as a source of calories has received support from studies that have utilized widely differing experimental paradigms.

Richter (1953) demonstrated that rats that were either forced to drink ethanol or that were given a choice between ethanol and water, reduced their food intake directly in proportion to the calories obtained from the ethanol solution. The fact that weight gain under such circumstances was normal is consistent with the idea that rats maintain their caloric intake regardless of whether the

calories came from stock food or ethanol. In a more recently conducted study, golden hamsters were observed to reduce their intake of a stock food source when their intake of ethanol increased. The compensatory response resulted in the caloric total being unaffected by ethanol consumption. This relationship endured even when ethanol-derived calories comprised over half of the caloric intake (McCoy, Haisley, Powchik, & Tambone, 1981). Rodents will not only decrease their food intake to compensate for the calories ingested in the form of ethanol, but they will also increase their consumption of ethanol when the supply of standard food is limited (Aschkenasy-Lelu, 1962; Palfai & Reckhow, 1977; Waller, McBride, Lumeng, & Li, 1982; Westerfeld & Lawrow, 1953).

Procedures that result in overeating in animals also bring about increases in ethanol intake. Lesions in the area of the ventromedial nucleus of the hypothalamus (VMH) result in hyperinsulinemia, hyperphagia and obesity (Powley, 1977). Rats sustaining such lesions have been reported to increase their intake of an 8% (w/v) ethanol solution (Marfaing-Jallat et al., 1970). The effect was so marked in the case of some VMH-lesioned animals that the "hyperphagia" was expressed entirely in terms of an enhanced intake of the ethanol solution. This finding lends additional support to the contention that, under numerous circumstances, rats treat an ethanol solution as an alternative source of calories.

A different procedure that can also result in the elicitation of hyperphagia and the eventual development of obesity is treatment with insulin (Larue-Achagiotis & LeMagnen, 1979; DeCastro, Paullin, & DeLugas, 1978). If an animals' response to an ethanol solution is dictated by the same factors that determine food intake in general, then one might expect that injections of insulin that lead to increased feeding, would also lead to increased ethanol consumption. Forsander, Kohonen and Suomalainen (1958) demonstrated that rats that were treated daily with either insulin (1 I.U. zinc protamine insulin; administered subcutaneously) or with another insulin-like agent, substantially increased their intake of a 15% (v/v) ethanol solution. In addition, they showed that diabetes produced by treatment with the β-cell toxin, alloxan, resulted in the development of an apparent aversion towards ethanol.

In summary, ethanol consumption in rodents appears to covary with changes in feeding. This statement is based upon several convergent findings: caloric intake from a stock food source declines when ethanol is consumed; restrictions on feeding are followed by compensatory increases in ethanol intake; and, manipulations that result in the development of hyperinsulinemia and hyperphagia also result in the enhancement of ethanol consumption. It thus appears that rodents respond "adaptively" to the calorically-related consequences of ethanol ingestion, and, under certain circumstances, select ethanol specifically for

such post-ingestional effects. This conclusion is most recently reinforced by the results of the present experiments. In Experiments 3 and 4, EB and MER-25 were shown to suppress ethanol consumption as they do food intake. The results of Experiment 7 replicated and extended these findings by demonstrating in the same animal that the suppression of ethanol intake by EB administered to ovariectomized rats parallels both in time course, magnitude and pattern of effects observed in food intake.

At this point it becomes necessary to consider in detail the possible ways in which estrogens bring about changes in feeding and to determine whether these actions can account for the pattern of effects produced by EB administration in the present set of experiments.

Initially, investigators concluded that estrogens were exercising a direct control over consummatory behavior. Speculation along such lines was encouraged by the observation that implantation of crystalline estradiol directly into the region of the VMH led to decreases in food intake (Beatty, O'Briant, & Vilberg, 1975; Jankowiak & Stern, 1974; Roy, Maass, & Wade, 1977, Wade & Zucker, 1970a). Strong suppression of food intake was achieved when estradiol implants were restricted to the area of the VMH (Wade & Zucker 1970a). At the time that the earliest of these studies was conducted, the VMH was considered by many to be acting as a "satiety" center, producing its effects by inhibiting neural activity in the lateral hypothalamus, the

"hunger" center. Activation of the VMH, either by electrical or chemical stimulation, or by changes in the constituents of the blood, was thought to lead to changes in the neuronal activity in the lateral hypothalamus, that would, in turn, modulate consummatory behavior (see review by Rabin, 1972). According to such a view, estradiol implants into the VMH were thought to stimulate directly the satiety center (Wade & Zucker, 1970a). Accompanying changes in body weight were considered to result from estradiol's effects on behavior. A finding that undermines this hypothesized link between behavior and body weight changes, however, is that changes in food intake are neither necessary nor sufficient to cause the body weight changes induced by ovarian hormones (Roy & Wade, 1977). This and related findings (Cox & King, 1980; Gentry, Wade, & Roy, 1976) encouraged investigators to investigate peripheral metabolic factors associated with the actions of estradiol.

Wade & Gray (1979), in their review of gonadal hormone effects on energy balance, focused on adipose tissue as the site where both estrogens and progestins act to elicit changes in fat metabolism. They suggested that these changes in metabolism are responsible for estrogen-induced anorexia. Intra- and extracellular lipases are the rate-limiting factors in the clearance of triglycerides from the blood. These enzymes control the storage of triglycerides into adipose tissue in times of caloric

surplus and their breakdown under circumstances of caloric deficiency. Gonadal hormones can alter the synthesis of these enzymes and could in turn result in shifts in the storage and breakdown of fats. Such an action could potentially result in alterations in the amounts of nutrients that freely circulate in plasma and that would be available for use as metabolic fuels. According to this formulation, estrogens reduce the activity of lipoprotein lipase, thereby reducing the uptake of lipids into adipose tissue. The resulting elevation in the level of lipids in the circulation could in turn serve as an additional source of oxidizable substrate (Wade & Gray, 1979). It has previously been suggested that such changes in the availability of metabolic fuels can serve as signals for the central nervous system to either initiate or terminate feeding (Friedman & Stricker, 1976).

The "metabolic hypothesis", proposed by Wade and Gray (1979) has helped to clarify how gonadal hormones could produce changes in both body weight and composition. There is at this time, however, reason to suspect that the proposed relation between estrogen-induced anorexia and alterations in fat metabolism is incorrect. Logically, if changes in fat metabolism are considered to be the immediate cause of the anorexia that follows estrogen treatment, then it would have to be demonstrated that the changes in lipids in the circulation precede the suppression of feeding. Ramirez (1980) attempted to verify this prediction in a

series of studies that were designed to examine the relation between estradiol-induced changes in food intake and changes in plasma triglycerides. It was found that although increases in the level of triglycerides followed EB administration this occurred only after the maximal suppression of food intake was observed. This particular change in fat metabolism would not appear to be responsible for the suppression of food intake. This conclusion is supported by the observation that nafoxidine, an antiestrogen that mimics estrogenic effects on food intake, suppressed food intake without causing an elevation in the level of plasma triglycerides. In addition, combined administration of EB and nafoxidine led to the suppression of food intake even though the elevation in plasma triglycerides that had previously been observed when EB was administered alone was completely abolished (Ramirez, 1980). The related finding, that EB implants into the region of the VMH suppress food intake without affecting LPL activity, is further suggestive of the possibility that estrogens have a direct action on consummatory behavior (Nunez, Gray, & Wade, 1980).

An alternative mechanism that might account for the effects of EB on sucrose and ethanol consumption involves the actions of estrogens on carbohydrate metabolism. The possibility that alterations in carbohydrate metabolism may be responsible for the reduction of ethanol and sucrose intake observed during the last trimester of pregnancy and

subsequent to treatment with EB will now be described. In Experiments 1 and 2, intake of either an ethanol or sucrose solution declined at a point during pregnancy that has previously been associated with the development of insulin resistancy and increases in the level of circulating insulin (Freinkel, 1980; Friesen, 1973). The observation that elevations in placental estrogens develop concomitantly with these changes in carbohydrate metabolism in both humans (DeHertogh, Thomas, Bietlot, Vanderheyden, & Ferin, 1975) and rats (Shaikh, 1971) is suggestive of the possibility that estrogens mediate these effects. There are reports, however, that estradiol can produce hyperinsulinemia that is accompanied by improved glucose tolerance (Bailey & Matty, 1972; Costrini & Kalkhoff, 1971). It thus appears that estrogens may contribute to the development of hyperinsulinemia during pregnancy without being a major factor in the etiology of insulin resistance. Could an estrogen-mediated increase in insulin secretion itself be responsible for the suppression of ethanol and sucrose intake? The finding that administration of insulin to C57Bl mice dramatically reduces ethanol consumption in this strain is consistent with such an idea (Goas, Pelham, & Lippa, 1979).

The development of the insulin-resistant state in the near-term rat could quite independently contribute to the reduction of both sucrose and ethanol intake. Continued consumption of large volumes of a sucrose solution under

such circumstances would be maladaptive since the uptake of glucose into tissue would be reduced due to insulin insensitivity. Such an action could result in the development of hyperglycemia. At the same time, sustained intake of the ethanol solution could potentiate an hyperglycemic response by suppressing the conversion of glucose to glycogen (Dornhorst & Ouyang, 1971). It is generally acknowledged that rats possess the capacity to modify their diet under those circumstances in which continued intake would result in the development of a metabolic imbalance. Little is known, however, of the mechanism by which animals make such adjustments in diet selection. It has been proposed that conditioned taste aversions develop towards those foods that are associated with such imbalances (Rozin, 1976).

But what of the suppression of ethanol consumption by EB administered to ovariectomized rats? It is unlikely that this effect is mediated by insulin resistancy inasmuch as estradiol increases plasma insulin levels, enhances glucose tolerance (Bailey & Matty, 1972; Costrini & Kalkhoff, 1971), and increases glycogen deposition in rats (Ahmed-Sorour & Bailey, 1981). As already mentioned, insulin administration suppresses ethanol consumption in mice (Goas et al., 1979). Even more interestingly, Vanderweele and his colleagues (Vanderweele, Pi-Sunyer, Novin, & Bush, 1980) have reported that slow infusion of modest doses of insulin using the 'Alzet' osmotic minipump results in suppressed food intake

and declining body weight. When insulin is administered in this fashion, total food intake is reduced through a reduction in the size of individual meals. These effects produced by low dose infusions of insulin are of course similar to those produced by injections of EB. Further support for the idea that EB-induced increases in insulin may be responsible for the decrements in ethanol consumption observed in Experiments 3 and 7 comes from the supplementary finding of these studies that the EB injections led to significant increases in water intake. Other investigators have reported that administration of insulin also leads to increased water intake (Forsander et al., 1958; Goas et al., 1979; Vanderweele et al., 1980).

In summary, the reduced ethanol intake by pregnant rats may be the consequence of either increased secretion of insulin or the result of the development of insulin resistancy in the near-term rat. It is still unclear what factors are responsible for the development of insulin resistancy in the pregnant rat. The increased insulin levels, on the other hand, could be due to elevations in estradiol levels that are associated with this period of gestation. In the case of ovariectomized rats, augmentation of insulin secretion by administration of EB could be the immediate cause of suppressed ethanol intake and increased water consumption.

A very different account of the effects of EB on food and ethanol intake comes from recent studies on the effects

of estrogens on the serotoninergic system of the brain. Cone, Davis and Goy (1981) demonstrated that acute administration of EB to ovariectomized rats results in elevated levels of basal serotonin (5-HT) in the raphé nuclei. In a different study designed to assess the effects of estrogens on 5-HT receptors, it was found that EB administration resulted in increased 5-HT receptor levels. This effect was observed in the hypothalamus, the preoptic area, and amygdala, all of which normally accumulate estrogens (Biegon & McEwen, 1982).

There are several reports indicating that enhancement of serotoninergic activity through the use of 5-HT agonists results in the development of anorexia (Blundell & Latham, 1978; Blundell, Tombros, Rogers, & Latham, 1980; Pollock & Rowland, 1981). Similar pharmacological manipulations also result in reduced ethanol intake (Geller & Hartman, 1981; Rockman, Gustafson, & Amit, 1982; Rockman, Amit, Carr, & Ogren, 1979). It thus is plausible that the effects of estrogen on food and ethanol intake could be mediated via changes in serotoninergic activity.

Injections of either EB or agents that enhance serotoninergic neurotransmission result in decrements in total food intake. In both cases, the anorexia has been shown to result from a selective avoidance of carbohydrates. Protein and fat intake remain relatively unaffected by such treatments (Wurtman & Baum, 1980; Wurtman & Wurtman, 1977, 1979). In their discussion of these findings, Wurtman and

Baum (1980) suggested that EB may produce its effects on diet selection via its effects on 5-HT activity.

The transience of the anorexia produced by EB and by 5-HT agonists is suggestive of the possibility that EB may be acting through 5-HT to produce its effects on consummatory behaviors. Daily administration of the 5-HT agonist fenfluramine results in only a temporary suppression of food intake (Levitsky, Strupp, & Lupoli, 1981) an effect similar to that following daily administration of EB (Experiments 3 and 7; Wade, 1975). Recently, it has been shown that daily injections of either zimelidine or fluoxetine, both of which act as 5-HT reuptake blockers, suppress ethanol intake in rats but only transiently (Rockman et al., 1982).

A final similarity between the effects of EB and 5-HT agonists on feeding has been found in studies of patterns of feeding in rats. Both EB (Blaustein & Wade, 1976; Experiment 7) and drugs that enhance serotoninergic activity either by promoting synthesis and release, or by blocking 5-HT reuptake (Blundell et al., 1980), reduce total food intake by decreasing the size of meals eaten. Furthermore, it should be noted that drugs that produce anorexia through the enhancement of catecholaminergic activity (e.g. amphetamine) have markedly different effects on meal patterns from those produced by EB or the 5-HT agonists (Blundell et al., 1980).

It is hoped that the ideas presented in this section

will help to generate experiments designed to further elucidate the roles that carbohydrate metabolism and that brain 5-HT activity play in the modulation of ethanol consumption in rodents. It would be an added benefit if the present results and discussion also serve to stimulate new thinking about the ways in which estrogens alter consummatory behaviors. The idea that estrogen-mediated changes in carbohydrate metabolism might be responsible for some of the effects on feeding has been all but put aside (Dudley, Gentry, Silverman, & Wade, 1979; Wade & Gray, 1979). Perhaps the reasoning that leads one to conclude that EB may be suppressing ethanol consumption by altering carbohydrate metabolism might also serve to revive interest in the possible relation between estrogenic effects on carbohydrate metabolism and feeding. In addition, the possibility that estrogens may be acting through 5-HT to produce some of its effects of feeding is particularly exciting. Little attention to date has been given to the idea that estrogens may interact with monoamines to elicit change in consummatory behavior. This is peculiar in view of the substantial amount of evidence that is supportive of the hypothesis that these systems contribute to the regulation of feeding. The techniques and strategies necessary for experiments designed to study such a relationship are readily available.

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