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Reduced Preference for Alcohol During Pregnancy and Following Lactation in Rats

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MEANS, L. W. AND H. B. GOY. *Reduced preference for alcohol during pregnancy and following lactation in rats.* PHARMAC. BIOCHEM. BEHAV. 17(6) 1097–1101, 1982.—The present study was conducted to examine alcohol preference in rats during cohabitation, pregnancy, lactation, and post-lactation. Nine females who became pregnant and nine who served as controls were compared on their daily consumption of 0.01 M saccharine solution and a 0.01 M saccharine solution containing 5% ethanol (v/v) throughout the study. Alcohol preference ratios (alcohol solution/total fluid consumed) were significantly lower in the pregnant animals during the pregnancy ($p<0.01$) and the 32-day post-lactation period ($p<0.01$).

Alcohol preference Pregnancy Cohabitation Lactation Post-lactation Long-Evans rats

RECENTLY, it has been reported that mice show a decreased preference for alcohol during pregnancy, lactation, and the first week post-lactation relative to a nonpregnant control group [9] and that rats show decreased ethanol preference during the last days of gestation [10]. An earlier report presented evidence that pregnant nonalcoholic women ingest fewer alcoholic drinks during pregnancy, with many of the women reporting the reason for the reduction being distaste for alcohol [6,7]. Finally, there are brief reports indicating that hamsters [2] and macaques [3] reduce their ethanol consumption during pregnancy.

The observations that many women reduce their ethanol intake during pregnancy and report a distaste for alcohol in conjunction with the findings that three other species all reduce their alcohol intake suggest that there is some protective mechanism for the developing fetus [4,5]. The present study was conducted to determine if rats, the most frequently used species for research on the fetal alcohol syndrome [1], show a reduction in self-selection of an ethanol-containing solution during pregnancy. Also, self-selection of ethanol was examined during lactation and for thirty-two days following the weaning of the pups.

METHOD

Animals

Eighteen female Long-Evans hooded rats, 75–95 days of age at the beginning of the experiment, were used as subjects. Eighteen males of the same age and strain were used as studs. Throughout the study each female was housed in a wire mesh cage measuring 34×18×18 cm that was modified so that two 100 ml Richter drinking tubes could be mounted on the front. Lab chow (Wayne Lab-Blox) was available continuously and the drinking tubes were present from 8:30 a.m. to 6:30 p.m. daily. A 16-hr/8-hr light–dark cycle, with the lights being turned on at 7:00 a.m., was maintained throughout the experiment.

Procedure

During each daily 10-hr drinking session one Richter tube contained a 0.01 M saccharine solution and one tube held the same saccharine solution mixed with ethanol. The position of the saccharine- and ethanol-containing tubes was randomly changed each day. The ethanol tube contained 2%, 3%, 4%, 5% (v/v) ethanol on the initial four days and 5% ethanol on all subsequent days. Following the initial four days, baseline fluid consumption was measured for 10 days.

At the end of the baseline measurements, a male was placed in cohabitation with each female. Males were present only during the 14 hours that fluids were not available. Each morning, the paper under each cage was examined for sperm plugs. Upon finding a sperm plug, the male was permanently withdrawn from the cage of that female and from the cage of one other randomly selected nonpregnant female. This procedure resulted in nine animals becoming pregnant, the experimental group, and nine animals remaining nonpregnant, the control group. On approximately the seventeenth day of pregnancy of the experimental animals a fine screen floor was attached to the bottom of the cages of all animals in both groups to prevent the expected pups from falling through the bottom of the cage. Also, strips of paper towelling were placed in the cages to be used for nesting materials. At birth, the pups were counted, weighed, examined and returned to their natural mothers.

Fluid consumption of subjects in both groups was measured daily throughout cohabitation (1–3 days) and during the time that the experimental animals were pregnant (20–21 days) lactating (20 days) and following weaning of the pups (10 days). All subjects were then given three days continuous access to tap water. Finally, after examination of the initial 10 days of postweaning fluid consumption, it was decided to measure fluid consumption for another 22 days. Following the last day of fluid measurements, the nine control animals were placed in cohabitation with the same nine males that

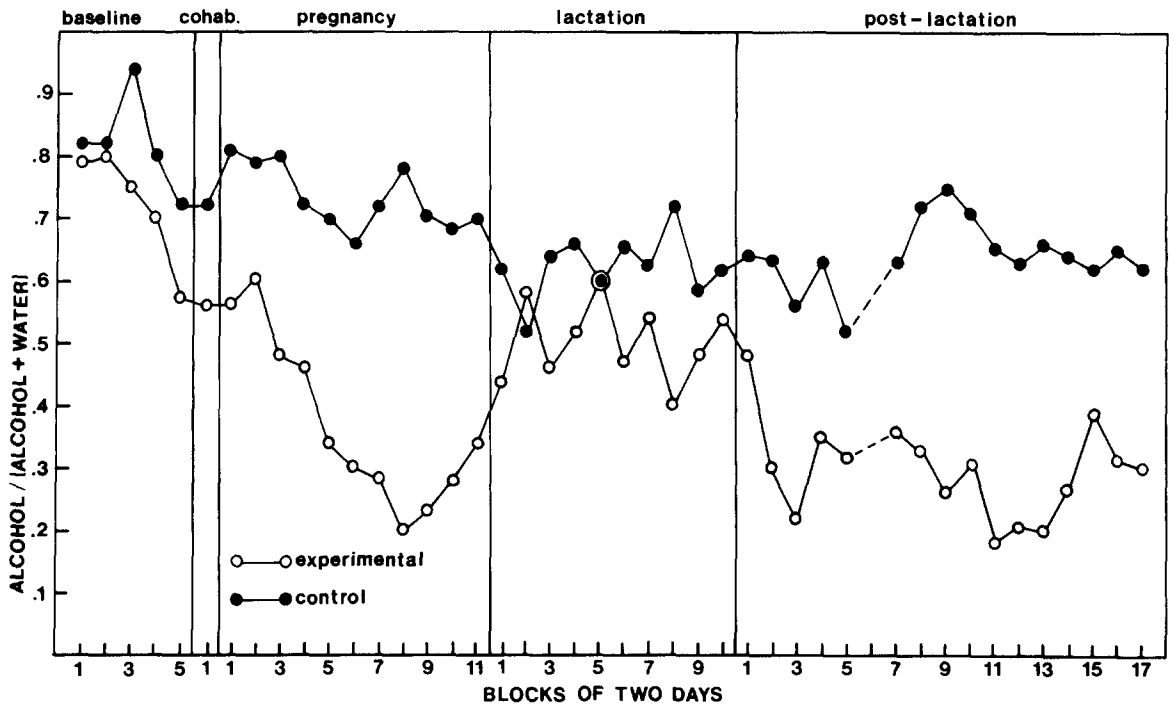

FIG. 1. Mean proportion alcohol/total fluid consumed during 2-day blocks by the experimental and control animals throughout the experiment. Both the "alcohol" and "water" solutions contained saccharine (0.01 M).

they were in cohabitation with earlier. Impregnation of the control animals was done to determine that they were capable of reproduction. Thus, any observed differences between the groups could not be attributed to differences in reproductive potential. The subjects were weighed weekly throughout the experiment.

RESULTS

Pregnancy and Litters

Four experimental animals became pregnant on the first day of cohabitation, 4 on the second day, and 1 on the third. The experimental animals had 12.0±0.65 (mean±SEM) pups per litter, with the pups weighing 5.8±0.23 g. The experimental animals gained 57.44±4.81 g during their pregnancy, while the control animals gained 9.56±4.35 g during the same three weeks, a difference which is highly significant, $t(16)=7.14$, $p<0.01$. These values are similar to the values we get for normal Long-Evans litters bred in our laboratory. For example, in one recent unpublished study normal dams had 11.3±0.3 pups weighing 6.1±0.73 g each. Following completion of the fluid consumption measurements all 9 control animals became pregnant within 3 days.

Liquid Consumption

Figure 1 shows the alcohol preference ratio, alcohol solution/total fluid consumed, in 2-day blocks for both groups throughout the entire experiment. Mean preference ratios were determined for all subjects during the baseline, cohabitation, pregnancy, lactation, post-lactation 1 (first 10 days following lactation), and post-lactation 2 (22 days following the three days during which data were not collected) periods. These means were then subjected to a mixed-factors analysis of variance, experimental condition × period. The analysis resulted in a significant experimental condition effect, $F(1,16)=6.72$, $p<0.025$, period effect, $F(5,85)=12.72$, $p<0.001$, and interaction, $F(5,85)=4.45$, $p<0.005$. Newman-Keuls tests performed on the cell means revealed that the experimental group had a lower mean preference ratio than did the control group during pregnancy and both post-lactation periods ($p<0.01$ in all cases). The two groups did not differ during the other periods. Further, the preference ratios of the experimental group during pregnancy, post-lactation 1, and post-lactation 2 did not differ from one another, but were all lower than experimental group preference ratios during the baseline, cohabitation, and lactation periods ($p<0.01$ in all cases). Clearly, the experimental group had depressed preference ratios during pregnancy and following lactation. Also, it is interesting to note that the control group showed a slight, but relatively consistent decrease in alcohol preference during the course of the experiment. In fact, the preference ratio of the control group during post-lactation 1 was significantly lower than it was during the baseline period ($p<0.05$).

To further examine the alcohol preference ratios of the experimental group, weekly mean preference ratios were determined for the three weeks of pregnancy. The ratios for the first, second and third weeks were 0.54, 0.33, and 0.27, respectively. A one-way analysis of variance for repeated measures resulted in a significant weeks effect, $F(2,16)=25.12$, $p<0.001$. Newman-Keuls follow-up tests revealed the preference ratios were lower during the second and third weeks of pregnancy than during the first week ($p<0.01$ in both cases), but that the second and third week ratios did not differ from one another.

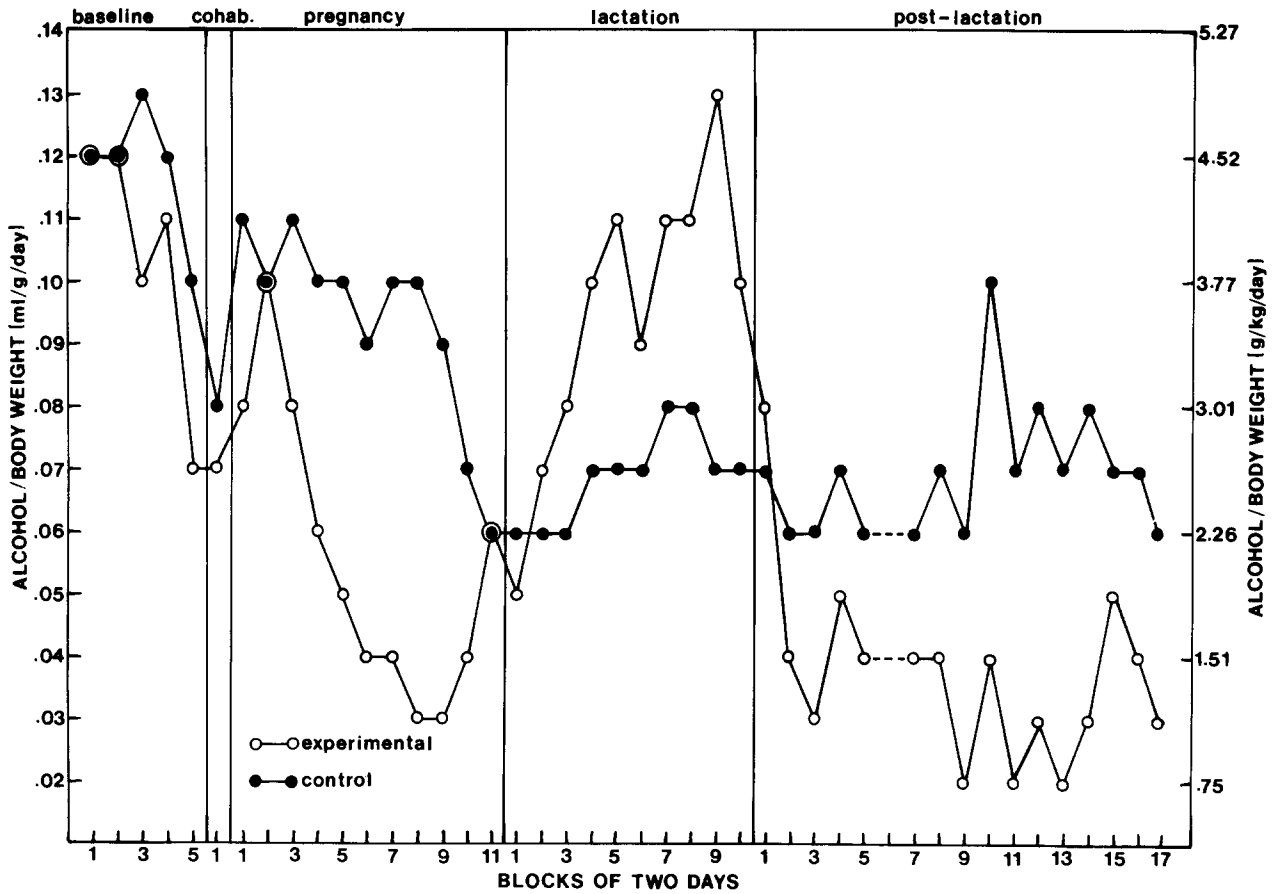

FIG. 2. Mean alcohol solution/body weight consumed during 2-day blocks throughout the experiment. The alcohol solution contained saccharine (0.01 M).

Because the pregnant animals gained approximately 6 times as much weight during the pregnancy period as the control animals, a separate mixed factors analysis of variance was done on the absolute amount of alcohol solution consumed. The analysis resulted in a significant interaction, $F(5,85)=11.25$, $p<0.01$. Subsequent Newman-Keuls tests revealed that the experimental animals consumed less alcohol solution during their pregnancy and both lactation periods than they did during the baseline period and lactation ($p<0.01$ in all cases). The experimental animals also consumed less alcohol than the control animals during pregnancy and both post-lactation periods ($p<0.01$ in all cases). Thus, the experimental animals showed a decrease in alcohol consumption during pregnancy and following lactation, that was not an artifact of their rapid weight gain during pregnancy.

To determine if the change in alcohol preference ratios was due to a change in alcohol solution consumption, a change in water solution consumption, or both, alcohol solution/body weight and water solution/body weight were also graphed in two-day blocks for the entire experiment (Figs. 2 and 3). Separate mixed factors analyses of variance were conducted on the alcohol solution and water solution consumption data. The analysis on alcohol solution/body weight data resulted in a significant periods effect, $F(5,85)=15.42$, $p<0.001$, and a significant interaction, $F(5,85)=5.28$, $p<0.001$. Newman-Keuls comparisons revealed that the experimental group consumed less alcohol than did the control group during the pregnancy and post-lactation 2 periods ($p<0.01$ in both cases). The two groups did not differ during the other periods. The significant period effect and observation of Fig. 2 make it clear that both groups were decreasing their alcohol consumption relative to their body weight during the course of the experiment, the only exception being the increased alcohol consumption of the experimental group during lactation. The analysis of the water consumption data resulted in a significant experimental condition effect, $F(1,16)=9.05$, $p<0.01$, period effect, $F(5,85)=5.12$, $p<0.001$, and interaction, $F(5,85)=3.14$, $p<0.025$. Subsequent Newman-Keuls comparisons revealed that the experimental group consumed more water solution than the control group during the pregnancy, lactation, and post-lactation 1 periods ($p<0.01$ in all cases). Thus, from the analyses and examination of Figs. 2 and 3, it is evident that the difference in alcohol preference ratios between the two groups during pregnancy is due to the experimental group consuming both less alcohol solution and more water solution than did the control group. The difference in preference ratios during the post-lactation 1 period is, in large part, due to the increased consumption of the water solution by the experimental animals, while during the post-lactation 2 period the difference is due primarily to the decreased con-

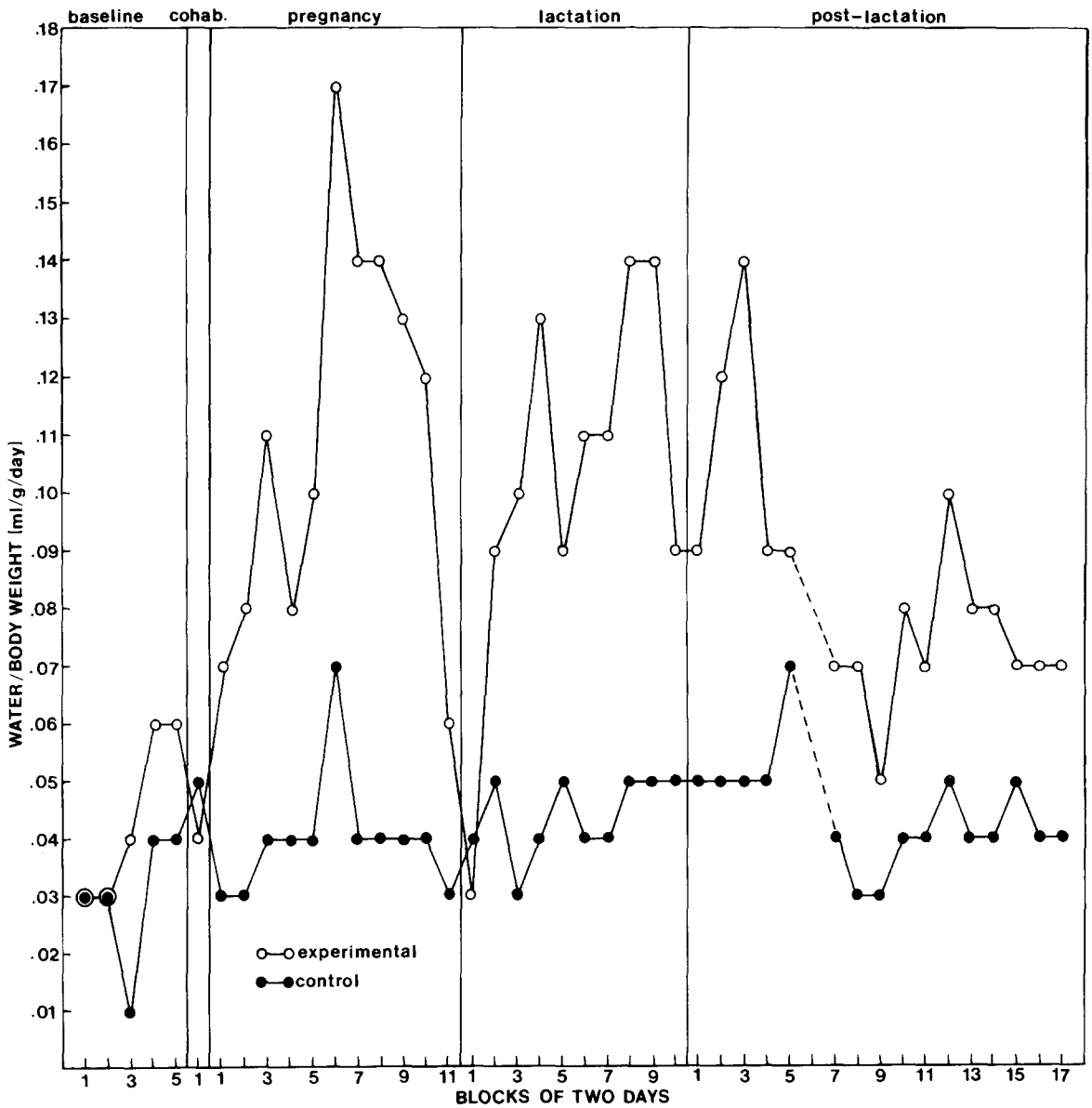

FIG. 3. Mean water solution/body weight consumed during 2-day blocks throughout the experiment. The water contained saccharine (0.01 M).

sumption of the alcohol solution by the experimental animals. During lactation the experimental animals consumed more of both the alcohol and the water solutions than did the control animals, resulting in a preference ratio that did not differ from the control animals.

DISCUSSION

The results clearly indicate that the preference for alcohol decreases during pregnancy in rats, reaching its lowest levels during the second and third week. During lactation consumption of all liquid increases, with no evidence of preference between the 5% ethanol and water solutions as the animals consumed equal amounts of each. Following lactation, rats show a strong preference for the water solution over the alcohol solution, and the preference persists for at least a month.

The decrease in self-selection of alcohol during pregnancy appears to be a general phenomenon among mammals, having previously been reported in rats [10], in hamsters [2], mice [9], monkeys [3], and humans [6,7]. The fact that several species show decreased ethanol consumption during pregnancy strongly suggests the presence of an inborn fetal protective mechanism [4,5], one probably mediated by reproductive hormones. Also, the fact that the ethanol solution accounted for 73% of the total fluid consumed before pregnancy by the experimental animals, suggests that the protective mechanism is not dependent on the ingestion of a normally distasteful substance or novel substances. The ethanol solution may become distasteful during pregnancy, as re-

ported by 53% of the women in one study [6], or result in illness as reported by 65% of the women in the same study.

The failure of rats to demonstrate a preference between the ethanol and water solutions during lactation differs from what has been observed in mice [9], who show an even more pronounced decrease in ethanol preference during lactation than during pregnancy. There are several possible explanations for the difference between our observations on lactating rats and the observations made on mice [9], besides the obvious species difference. In our study a 5% (v/v) ethanol solution was used while a 10% (w/v) solution was used in the study on mice. In our study, so that preference measurements could be taken during cohabitation, the liquid solutions were only available 10 hours each day, while the solutions were continuously available in the other study. Several studies have shown that periods of alcohol deprivation increase alcohol preference [8, 11, 12]. Finally, in our study saccharine was added to both solutions to partially mask the flavor of the ethanol. One interpretation of the indiscriminant selection of the fluids by the rats is that during a period of increased thirst, a broader range of fluids may be nearly equally acceptable. The studies on humans [6,7] and monkeys [3] have not examined the effects of lactation on ethanol self-selection.

It has been shown that during pregnancy and lactation, rats show a decreased preference for saccharine solutions compared to water [13,14]. While it is possible that the present results are due to changes in the relative preference for saccharine and saccharine-alcohol solutions, this is probably not the case. First, both solutions contained the same amount of saccharine. Second, the results of our study parallel other studies which have not used saccharine [3, 5, 9]. Third, saccharine preference decreases during both pregnancy and lactation [14], while decreased ethanol preference did not occur during lactation in the present study. Fourth, in a recent study [10] it was found that while alcohol preference dropped during pregnancy, saccharine preference remain unchanged.

Several observations of the present study are consistent with the hypothesis that the pregnant rats learned to decrease their self-selection of ethanol. First, self-selection of ethanol decreased during pregnancy, being significantly lower during the second and third weeks than during the first week. Second, even during lactation, a period of increased fluid need and consumption, the lactating rats selected a lower proportion of alcohol than they did during baseline measurements taken before cohabitation. Third, for at least a month following weaning of the pups, the experimental animals showed significantly reduced ethanol selection. It may be that pregnancy provided an opportunity for the acquisition of a conditioned taste-aversion for the ethanol solution. If ethanol consumption resulted in illness or discomfort during pregnancy, then each daily 10-hour drinking session would essentially constitute a taste-aversion conditioning trial.

The mean litter size and pup weights of the experimental animals in the present study were comparable to those of the nonexperimental litters of Long-Evans pups that have been raised in our laboratory. The alcohol consumed before and during pregnancy (approximately 2 g/kg/day during pregnancy) did not have a noticeable effect on number of pups in a litter or pup birth weight.

Examination of Fig. 1 reveals that control animals show a slight decrease in ethanol preference when tested over a three month period of time. Such a decrease indicates that proper assessment of the effects of time related variables, such as pregnancy and lactation, on alcohol preference requires the inclusion of a control group experiencing the same tests at the same temporal intervals.

Finally, the decreased alcohol preference shown by the experimental animals cannot be attributed to the control and experimental animals being different in their reproductive capabilities. The control animals immediately (1–3 days) became pregnant when placed with males.

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