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Maternal Social Stress Disrupts Reproduction of Hamsters Drinking High-Calorie Fluids

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WISE, D. A. AND N. L. ELDRED. *Maternal social stress disrupts reproduction of hamsters drinking high-calorie fluids.* PHARMACOL BIOCHEM BEHAV 25(2) 449–456, 1986.—Adding maternal social stress, induced by housing in pairs, to maternal consumption of high-calorie fluids adversely affected hamster reproduction. Pregnancy outcomes of 67 dams housed either alone or in pairs, and given either water (WAT), or isocaloric solutions of either 30% ethanol (ETH), sucrose (SUC) or propylene glycol (PG), were compared. Pups of unpaired dams drinking any high-calorie fluid weighed less than those drinking water, but no other deficits were found. Among paired dams, those designated submissive on the basis of agonistic behavior and drinking either SUC, ETH, or PG, had fewer viable pups and more fetal resorptions and stillborn pups than WAT dams. Also, dominant PG dams delivered few pups and both dominant and submissive PG dams delivered malformed pups or embryos. Whether paired or unpaired, litters of SUC and ETH dams were similar. Drinking any high-calorie fluid reduced maternal food and fluid intake and weight gains below that of water, but pairing had no additional effect on dams.

Maternal aggression Crowding Fetal development FAS Teratology Sucrose Ethanol Propylene glycol Nutrition

INFANTS born to mothers who consume alcohol during pregnancy often exhibit severe developmental deficits at birth. Possible contributing factors to the deficits include the drug and food properties of alcohol and the condition of the mothers themselves. Because alcohol interferes with nutrient utilization [36] and is a low-nutritive, high-calorie fluid, mothers of severely affected infants often are undernourished [4]. Besides being undernourished, women who deliver affected infants probably are under social stress during pregnancy. Reports of their personal histories include indications of high social stress and women frequently cite social stress as a precipitating factor [42] in their use of alcohol.

Using animal models, researchers have attempted to unravel the possible factors involved in the developmental dysfunctions associated with maternal drinking, but, except for maternal nutrition [49], maternal status factors such as social stress have received little attention. Because stress acts to increase glucocorticoids [48], maternal social stress could act to exacerbate the nutritional effects of consuming any high-calorie substance such as alcohol as well as, or instead of, the drug effects of alcohol. Hence, its potential role in disrupting fetal development in drinking women warrants investigation.

Even in the absence of known drinking or undernutrition, maternal social stress has been associated with infant developmental complications such as low birth weight, impaired fetal growth, and fetal malformations [46,47] that are similar to those reported for infants of drinking women. Among rodents, maternal social stress has been used to explain the developmental disorders seen in pups born to females living in high population densities [17]. Crowding rodents can result in intrauterine mortality, fetal resorptions, and failed pregnancies with a consequent reduction in the number of pups born [16, 18, 20, 27, 28, 35]. In addition to prenatal pup loss, other signs of maternal social stress among crowded rodents include impaired fetal growth and fetal malformations [27].

Although results have been variable, studies of maternal ethanol consumption in rodents also have found litter deficits at birth. Some studies have found decreased pup weights [1, 2, 6, 7, 37, 43] and reduced pup number [1,7]. However, other studies have found no decrease in pup weights [13, 24, 50] and no reduction in pup number [6, 13, 22, 24, 33, 37, 40, 43]. Except in mice [5], congenital malformations, which are seen among children of drinking mothers, have not been found in animal studies.

Because drug and nutritional effects of alcohol are difficult to disentangle experimentally, inconsistencies in results across studies probably reflect variations in experimental control procedures. In the study reported here, procedures did not permit clear distinctions between the effects of alcohol as a drug and as a high-calorie, low-nutritive substance. We considered that information about potential con-

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tributory effects of maternal social stress to developmental dysfunction would be useful regardless of whether the effects exacerbate the drug effects of alcohol or the maternal nutritional state or both.

In the present study, therefore, the combined effects of maternal social stress and maternal consumption of ethanol and non-drug, high-calorie fluids other than ethanol were investigated. The non-drug fluids were isocaloric solutions of sucrose and propylene glycol which have been used previously as nutritional controls in alcohol studies [8, 41, 45]. Using two caloric fluids also controlled for the possibility that fluid preferences might result in differential calorie consumption.

Hamsters were chosen as subjects because they exhibit signs of social stress when crowded in very small populations [21,26] which can be as small as two [29,52]. Using pairs of pregnant hamsters, relative social rank can be determined, and it has been shown that lower social rank results in poorer reproduction [53].

Another desirable characteristic of hamsters is that, unlike most strains of rats and mice, hamsters voluntarily consume high concentrations of ethanol [32]. Given a choice between 10% ethanol and water, male hamsters choose ethanol for approximately 90% of their fluid intake, but they consume the maximum amount of ethanol when 30% ethanol is given as a choice [39]. At concentrations between 25% and 35%, male hamsters prefer ethanol to dextrose [23]. Females prefer ethanol less than males, but, in a free choice situation, grouped female hamsters drink 15 percent alcohol in preference to water and will drink some alcohol when concentrations are as high as 60 percent [9]. In our laboratory, pilot studies have shown that pregnant hamsters consuming 30% ethanol have blood alcohol levels of 0.10 mg/dl and exhibit behavioral signs of intoxication [10].

We report here that the combined effects of maternal social stress and chronic consumption of 30% ethanol or another high-calorie fluid can be detrimental to fetal development. Maternal social stress was induced by pairing hamsters during pregnancy. Comparison groups were dams living alone and drinking water.

METHOD

Subjects

Subjects were 76 virginal, female ENG/SYR golden hamsters. Upon arrival, females, weighing 90–100 g, were housed individually in 45 L × 24 W × 16.5 H cm polyethylene cages in windowless rooms under a reversed light cycle (14 L:10 D) with Purina laboratory chow and water available ad lib. Thirty-six males, obtained at the same time, were housed in pairs and used for matings.

Procedure

Each female delivered two litters. Initial pregnancies were untreated and served as controls for individual differences in litter sizes. Following two weeks of laboratory acclimation and monitoring of estrous cycles, first pregnancies were obtained by placing estrous females with males. After 30 min of copulation with a male, females were weighed, and returned to their individual cages. The day of copulation was considered to be the first day of pregnancy (P1). On P7, females were weighed and placed individually in 93 L × 45 W × 37 D cm observation chambers. Chambers were fitted with water bottles at each end and were illuminated with red lights mounted inside. Except for weighing on P10, dams were left undisturbed until the last day of pregnancy (P16) when they were weighed, removed from observation chambers, and returned to polyethylene cages. On the following day, females were observed during parturition. After two hours without a pup being born, parturition was considered complete. Pups then were counted, weighed, examined under a magnification glass and returned to their dams for four days.

Two weeks after removal of first litters, females were mated as before for their second pregnancy. Except for assignment to treatment groups and the various treatments, procedures for second pregnancies were the same as first pregnancies.

Dams were assigned randomly to be housed in observation chambers either alone or paired with another dam. For each pair, one dam was chosen randomly and marked for identification by clipping fur from its back near the tail. After the initial pairing and placement of dams in chambers, all dams remained there until P16, the day preceding parturition.

Both paired and unpaired dams were given either water (WAT), 30% (v/v) ethanol (ETH), or solutions of either sucrose (SUC) or propylene glycol (PG), isocaloric (1.67 Kcal/ml) to the 30% ethanol. Making solutions isocaloric resulted in concentrations of 0.0316 ml/ml of ethanol, 0.0286 ml/ml of propylene glycol, and 0.0418 g/ml of sucrose. This procedure gave four different fluid groups: WAT, ETH, SUC, and PG. Dams in a group received only the solution specified as their sole drinking fluid during the P7–P16 period.

Based on a pilot study using spectrophotometric analysis (Cal-Bering Biochem Ethyl Alcohol Stat Pack) of blood samples taken by postmortem cardiac puncture on the fifteenth day of pregnancy, it was found that mean blood alcohol levels of 0.03, 0.10, and 0.11 mg/dl could be expected for dams chronically drinking, respectively, 20%, 30%, or 40% ethanol (v/v). As measured by open field activity, chronic drinking of 30% and 40%, but not 20%, solutions of ethanol were behaviorally intoxicating. Additionally, 40% solutions, but not 30%, were found to depress significantly maternal fluid intake, maternal weight gain, and fetal weight.

Fluid intake was monitored daily by weighing drinking bottles. To determine if evaporation or spillage differed between fluids, control bottles of each fluid, mounted on boards, were weighed daily with the observation bottles. Food intake was determined by weighing remainders on P16 of food quantities given in equal amounts to all females on P7.

Observations of paired dams for agonistic behavior began one minute following their placement in observation chambers on P7, two hours after the dark cycle began. Trained observers recorded aggressive and defensive response of pairs for ten minutes daily. The responses and other conditions of observation have been described previously [52,53].

After completion of observations on P16, pairs were separated and placed individually in polyethylene cages. Unpaired dams also were placed in polyethylene cages on P16, and all dams were given water and food ad lib.

On the following day, parturition was observed as in the first pregnancy. Two hours after parturition, viable, stillborn, and cannibalized pups were counted and examined for malformations. Viable pups were weighed and returned to their dams. If dams failed to deliver within 12 hours following the onset of the dark cycle, they were sacrificed by cervical dislocation and their uteri were removed and examined for the presence of fetuses and resorption sites.

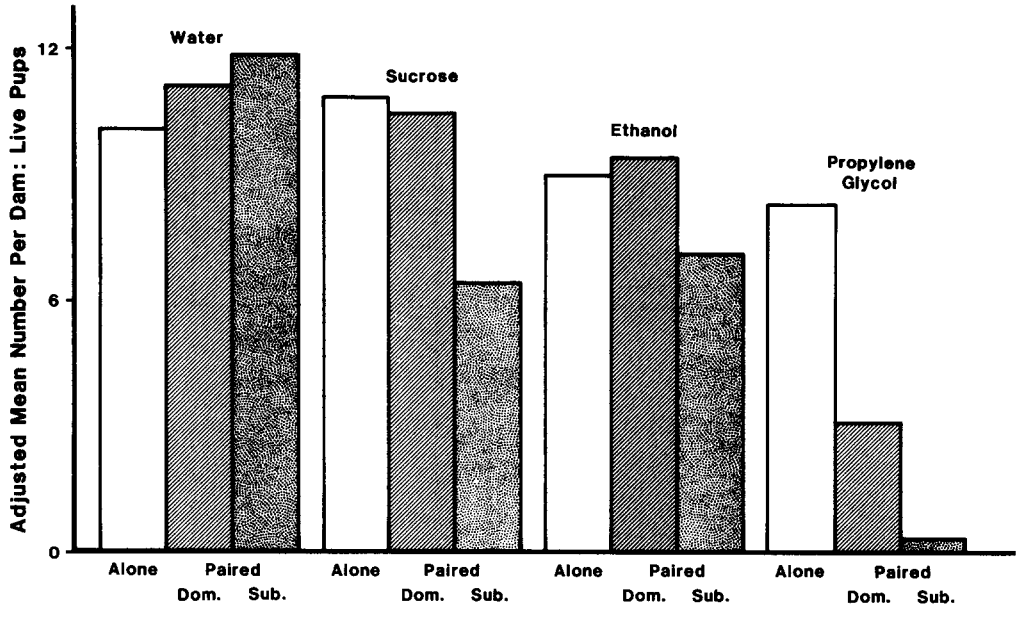

FIG. 1. Adjusted mean number of viable pups born per dam for dams housed alone or paired and given either water or 30% solutions of ethanol, sucrose, or propylene glycol. Based on covariate data derived from untreated pregnancies, means were statistically adjusted to correct for individual differences. Standard errors inappropriate for adjusted means.

Statistical Analyses

For each pair, the total number of aggressive and defensive responses across all days of observation was determined. The pair member with the higher number of aggressive responses was designated as "dominant" and the other as "submissive." With the social status of paired dams assigned as dominant or submissive, and with unpaired dams, there were three social conditions of dams which had been given one of four fluids, water (WAT), ethanol (ETH), sucrose (SUC), or propylene glycol (PG).

Using first pregnancy outcomes as a covariate, analysis of covariance [31] was used to compare litter production following social and fluid treatments of the second pregnancies. To detect other potential confounding factors, analysis of variance was used for differences in aggressive behavior, food consumption, and fluid consumption. When significant F-ratios were obtained, Duncan New Multiple Range Tests [31] with a significance criterion of 0.05 were used to determine differences between groups.

RESULTS

Of 76 dams, 67 completed the study. Five dams were eliminated when they did not return timely to estrous cycling following delivery of the first litter. Because submissive members died during treatment, a WAT and ETH pair were eliminated from the study.

Differences Among Litters

Number of pups. Figure 1 presents adjusted means for number of viable pups per dam for each treatment group. The adjusted means, derived from the analysis of covariance, reflect a statistical correction for individual differences found in litter production following the first, untreated pregnancies. As determined by analysis of covariance, both social condition, $F(2,54)=4.71$, $p=0.01$, and fluid type, $F(3,54)=11.73$, $p=0.00001$, had significant effects on the number of viable pups delivered per dam for their second litters. A significant interaction was not found, $F(6,54)=2.03$, $p=0.08$.

As seen in Fig. 1, unpaired dams delivered about the same number of pups regardless of the type of fluid consumed. Among paired dams, on the other hand, dominant dams drinking PG and submissive dams drinking any of the high-calorie fluids delivered fewer viable pups than dams drinking water. Of seven submissive PG dams, only one delivered viable pups, all of which died within 24 hours. Of the remaining submissive PG dams, one delivered a litter of stillborn pups, three were sacrificed due to failures to deliver, and two died on L1 preceding delivery. Post-hoc tests showed that differences between the number of pups born for paired and unpaired dams were significant.

Although instances of cannibalism were observed, we do not believe that pup numbers were affected systematically by undetected cannibalism. Parturition was observed hourly and, further, it has been our experience that dams rarely cannibalize whole pups, thus, we would have seen body parts. The highest frequency of observed cannibalism (3 of 6) occurred among litters of unpaired ETH dams and none occurred among litters of WAT dams, but Chi square tests indicated no significant differences.

Resorptions and stillbirths. The reduced number of live pups resulted from both resorptions and stillbirths. As shown in Fig. 2b, unpaired dams showed no resorptions whereas submissive members of paired dams drinking any of the high-calorie fluids, SUC, ETH, or PG, frequently showed resorptions. Resorptions also occurred among paired, dominant dams drinking PG. An analysis of variance of the frequency of resorptions showed that the effects of

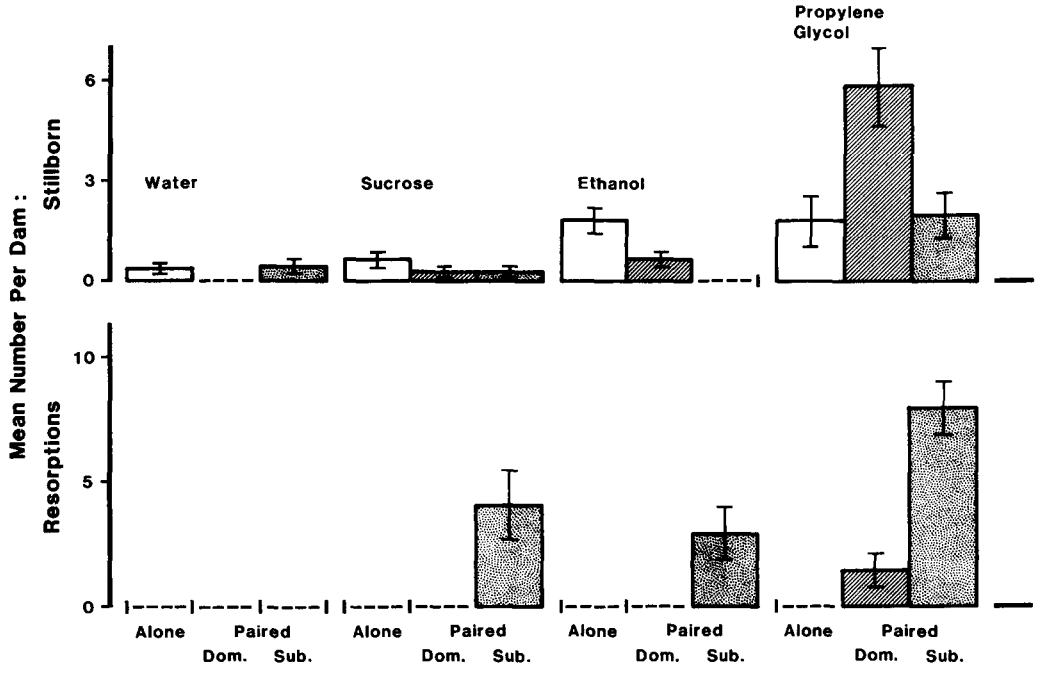

FIG. 2. (a) Mean number of stillborn pups per dam for dams housed alone or paired and given either water or 30% solutions of ethanol, sucrose, or propylene glycol. (b) Mean number of resorption sites per dam for dams housed alone or paired and given either water or 30% solutions of ethanol, sucrose, or propylene glycol.

TABLE 1

MEAN VIABLE PUP BIRTH WEIGHT (g) PER DAM FOR DAMS HOUSED ALONE OR IN PAIRS AND GIVEN WATER OR 30% ISOCALORIC FLUIDS OF EITHER ETHANOL, SUCROSE OR PROPYLENE GLYCOL

	Type of Fluid Consumed			
Social Condition	Water M (n)*	Ethanol M (n)	Sucrose M (n)	Propylene Glycol m (n)
Alone	2.74 (6)	2.53 (6)	2.15 (5)	1.86 (5)
Paired				
Dom	2.46 (5)	2.25 (5)	2.18 (5)	2.20 (3)
Sub	2.62 (5)	2.01 (3)	2.18 (3)	2.17 (1)

*(n) refers to number of dams for which mean pup weights were based.

both social condition, $F(2,55)=10.39$, $p=0.0001$, and fluid type, $F(3,55)=3.37$, $p=0.3$, were significant and the interaction term, $F(6,55)=2.01$, $p=0.08$, approached significance. Subsequent post-hoc tests indicated that the frequency of resorptions for submissive dams drinking any high-calorie fluid was significantly different from that for all unpaired dams. Also, resorptions among submissive PG dams were significantly more frequent than the resorptions for dominant PG dams, submissive ETH dams, and submissive SUC dams. Differences in resorptions between SUC and ETH dams were not significant.

As shown in Fig. 2a, the frequency of stillbirths was very high among dominant dams drinking PG. An analysis of variance for stillbirth frequency indicated that only fluid type had a significant effect, $F(3,55)=4.82$, $p=0.005$, on stillbirths. Post-hoc tests for fluid effects showed that dams drinking PG had significantly more stillbirths than all other groups, but other groups were comparable.

Fertility. By summing frequencies of viable pups, resorptions, and stillbirths, it was determined that all dams initially had shed and implanted about the same number of ova. The mean frequency of total "pups," live, dead, and resorbed, for all groups was 10.65. Submissive WAT dams had the highest mean total, 12.24; submissive ETH dams had the lowest, 9.10. Statistically, though, none of the differences in summed frequencies were significant.

Morphological deformities. In addition to the high incidence of resorptions and stillbirths among litters of paired dams drinking PG, there also were many pup deformities. Four of six dominant PG dams had litters in which virtually all pups were stillborn, and at least 60% of these pups had deformities that included cleft palates, twisted rear legs, micrognathy, and edema. Retarded development and skeletal abnormalities also were apparent among the fetuses and the few pups born to submissive PG dams.

Pup weights. As seen in Table 1, mean weights of pups born to WAT dams were greater than those of any group drinking high-calorie fluids regardless of social condition. An analysis of covariance for mean pup weight per litter for all groups of dams yielded significant differences for fluid type, $F(3,39)=9.51$, $p=0.0001$, but not social condition. The interaction term approached significance, $F(6,39)=2.19$;

$p=0.06$. Post-hoc comparisons among fluid type groups showed that dams drinking water had significantly heavier pups than dams drinking any high-calorie fluid, but there were no significant differences among high-calorie groups.

Because PG had some unique effects on pups, for example, developmental abnormalities and frequent stillbirths, and, because submissive PG dams delivered virtually no viable pups, additional statistical comparisons were made omitting litters of PG dams. However, the analysis omitting pups of PG dams gave essentially the same results. No differences were found between weights of pups delivered by SUC and ETH dams.

In summary, litters of unpaired dams were very similar. With the exception of depressed pup weights for litters of dams drinking high-calorie fluids, no significant differences among pups of unpaired dams were found. For paired dams, on the other hand, submissive dams drinking any of the high-calorie fluids and dominant dams drinking PG had litters that were smaller in number than any unpaired dams and there was a corresponding higher number of resorptions. Also, pups and fetuses of paired PG dams showed morphological abnormalities.

Differences Among Dams

Agonistic behavior. Total frequency of aggression during the observation period was highest between pairs of WAT dams (M=130) and lowest between SUC dams (M=49). However, variability was high and the type of fluid consumed had no significant effect on agonistic behavior. Because of the variability, a log transformation of frequencies of aggression was performed and submitted to analysis of variance with repeated measures over three day blocks. Again, no significant differences were found among fluid groups for frequencies of either aggressive or defensive responses, but the factor of "days" was significant, $F(2,35)=3.48$, $p<0.05$. The effect of days was seen as a decline in aggression during the last three days of pregnancy.

Maternal weight. Maternal weight gains were determined for the treatment period between P7, when dams were given different fluids, and P16, when all dams were returned to water. Groups were compared for differences in relative weight gains expressed in terms of a percentage by the formula: P16−P7/P16 × 100 where P16 and P7 refer to maternal body weight on the appropriate day of pregnancy. Because dams that lost litters through resorption would not gain very much weight, these dams were omitted from the analysis of maternal weight gain.

Table 2 presents a comparison of mean relative weight gains for dams drinking water and dams drinking any of the high-calorie fluids. Analysis of variance yielded significant differences in relative weight gain for fluid type, $F(3,43)=26.22$, $p=0.00001$. Post-hoc tests indicated that groups drinking water differed from all others, but among groups drinking high-calorie fluids, there were no significant differences. Because there were no significant differences between the SUC, ETH, or PG groups for maternal weight gain, Table 2 presents a comparison of these groups combined as a single high-calorie group with the WAT groups. No significant differences in relative weight gain were found for social condition or the interaction.

Food and fluid consumption. Table 2 also presents the mean total food and fluid consumption between P7 and P16 for WAT dams and the high-calorie groups. Unlike maternal weight gain, individual intake of food and water for paired dams could not be determined. Using mean amounts per animal per pair, analyses of variance showed that type of fluid had significant effects on the amounts of food, $F(3,37)=19.61$, $p=0.00001$, and fluid consumed, $F(3,36)=12.10$, $p=0.00001$. As found for maternal weight, post-hoc tests showed that there were significant differences in food and fluid consumption between groups drinking water and any group drinking a high-calorie fluid, but there were no significant differences among ETH, SUC, or PG groups. Therefore ETH, SUC, and PG groups were combined again as a single high-calorie fluid group.

TABLE 2

MEAN RELATIVE MATERIAL WEIGHT GAIN (RWG)* AND MEAN TOTAL FOOD AND FLUID CONSUMPTION FROM P7 TO P16 FOR DAMS HOUSED ALONE OR IN PAIRS AND GIVEN EITHER WATER OR A HIGH-CALORIE FLUID

Drinking Fluid		Social Condition: Alone	Dom	Sub
Water				
n		6	5	5
RWG	M	18.7	20.2	25.4
	SE	(4.5)	(4.4)	(6.0)
Food (g)	M	64.8	71.8	†
	SE	(5.3)	(2.3)	
Fluid (g)	M	168.5	147.8	†
	SE	(29.0)	(11.5)	
High Calorie Fluid				
n		17	16‡	8‡
RWG	M	1.9	1.7	0.1
	SE	(3.2)	(2.2)	(3.1)
Food (g)	M	35.7	37.6	†
	SE	(3.1)	(3.1)	
Fluid (g)	M	96.6	83.2	†
	SE	(7.8)	(6.0)	

*See text for calculation.

†Individual intake could not be determined within pairs; amounts given for food and fluid are mean/dam/pair.

‡Omits dams with resorbed litters.

Table 2 shows, then, that dams drinking water gained more weight, ate more food, and consumed more fluid than did dams drinking high-calorie fluids. Neither social condition effects nor any interaction effects were significant.

Based on fluid consumption, dosages for animals drinking high-calorie fluids were 29.7 g/kg/da for ETH dams, 16.4 g/kg/da for PG dams, and 31.6 g/kg/da for SUC dams. As noted above, there were no significant differences in fluid consumption and, hence, dosages, that could be attributed to social condition. However, as noted in Table 2, fluid consumption of dams housed alone tended to be higher than that of paired dams.

DISCUSSION

The results show clearly that the combination of maternal social stress, induced by pairing, and maternal consumption of any of the high-calorie fluids, ethanol, sucrose, or propylene glycol, decreases the number of viable pups born to hamsters. Without social stress, maternal consumption of

high-calorie fluids had little effect on the number of viable pups born.

Maternal social stress had the greatest effect on litters of dams consuming PG. While the litters of alone PG dams were similar to those on other high-calorie fluids, litters of dominant PG dams showed a high incidence of stillbirths and submissive PG dams had so many resorptions that virtually no viable pups were born. In addition, pups and fetuses of paired PG dams exhibited many skeletal malformations and signs of retarded growth.

Paired ETH and SUC dams also delivered small litters of viable pups. Although some stillborn pups were delivered, the reduction of viable pups occurred primarily through intrauterine resorptions among the submissive dams. Unlike litters of paired PG dams, though, malformations were not seen among offspring of dams drinking either ETH or SUC.

For all treatment groups, the sum of viable pup number, resorptions, and stillbirths was comparable to the number of viable pups typically delivered by unpaired WAT dams. This, and because experimental treatment was begun after implantation occurred, indicated that the joint effects of social stress and high-calorie fluids interfere with intrauterine embryonic and fetal development.

Some of the deficits seen in litters of paired dams could have resulted from differential consumption of food and fluids, rather than social stress. However, both dominant and submissive PG dams had litters that were strikingly abnormal when compared with unpaired PG dams, and neither dominant nor submissive WAT dams had litters with any observable deficiencies.

Furthermore, differences in consumption did not result in differences among litters. There were large differences in both maternal consumption and maternal weight gain between dams drinking water and dams drinking high-calorie fluids for both unpaired and paired dams. However, even though WAT dams consumed much more food and water and gained more weight than ETH and SUC dams, their litters were not significantly different from the litters of either unpaired or dominant ETH and SUC dams.

Also, there were no indications of differential maternal weight gains or consumption between dominant and submissive members of pairs. Maternal weight gain, which was obtained independently for each dam, was comparable for all unpaired, dominant, and submissive dams drinking the same fluid. Further, on a per dam basis, the amounts of food and fluid consumed by pairs were comparable to that of unpaired dams drinking the same fluid.

If there were any differences in fluid and food intake between dominant and submissive dams, then the effects should have occurred for pairs drinking high-calorie fluids and pairs drinking water. For WAT dams there were no differences between litters from dominant and submissive dams. Hence, it seems unlikely that the differences in pup number that were found between litters of dominant and submissive dams drinking high-calorie solutions should be attributed to differential food and fluid consumption.

Because dams drinking ethanol showed signs of intoxication, we expected that their agonistic behavior would be affected. However, ETH dams were more similar to WAT dams than other groups and no significant differences among groups were found. The decrease in aggression found during late pregnancy for all dams confirms previous findings [35].

The lack of differences between litters of dominant and submissive dams drinking water was unexpected. Previously, Huck *et al.* [29] and Wise *et al.* [53] found that paired submissive dams drinking water bore litters that were smaller in number than unpaired dams. In both studies, however, dams were paired earlier in pregnancy than in the present study. A later day of pairing was chosen here to reduce implantation failures and early abortions, thereby increasing completed pregnancies.

Not surprising was the finding that pup weights of litters born to females drinking any of the high-calories were low. Because dams drinking high-calorie fluids ate and drank much less and gained much less weight than those drinking water, low pup weights and pup loss would not have been unexpected. However, only when dams were paired did drinking high-calorie fluids have effects on fetal development other than birth weight. Submissive dams drinking any of the high-calorie fluids did have a higher incidence of resorptions and fewer viable pups than did unpaired dams.

Our findings show clearly that, when combined with social stress, PG can have devastating effects on fetal development. PG is described as harmless in the Merck Index [51], and it has been used as a nutrient in animal diets [44]. The Federal Register [19] lists PG as a generally recognized safe substance (GRAS) which can be used in various preparations in concentrations ranging from 2.0% for food to 97% for seasonings and including 5% for alcoholic beverages. We chose PG as an isocaloric control for ETH because it had been used previously in alcohol studies [12,41] without untoward effects.

Clinical reports of adverse reactions to inadvertent use of PG, as, for example, in intravenous injections of vitamins in a PG solution, have included lactic acidosis [14] and hyperosmolality [11,25]. Hyperosmolality *per se*, however, is not considered harmful [38]. Further, ethanol ingestion also is accompanied by hyperosmolality [15,38]; but, because ethanol moves freely across membranes, as does PG, fluid shifts do not occur and elevated osmolality is not considered to be one of the harmful effects of ethanol ingestion [38]. Because sucrose does not move freely across membranes, elevated osmolality, and resultant fluid shifts could have occurred in the dams consuming sucrose. However no signs of diarrhea or diuresis were apparent.

Our data did not show any differential effects between drinking ethanol and sucrose. Although the fluid intake of dams drinking ethanol was low, as it was for dams drinking either sucrose or propylene glycol, the amount of ethanol consumed was high. As estimated from fluid consumption, the ethanol dosage was 22.5 g/kg/da which compares closely to that of 23.2 g/kg reported by McMillan *et al.* [40] for male hamsters drinking the same concentration of ethanol.

Our findings suggest that nutritional deficits and social stress could play a major role in the developmental disorders associated with maternal drinking. Effects of social stress presumably are mediated through hormonal response of the hypothalamic-pituitary-adrenal axis [17]. Because the adrenal hormones released in response to stress, corticosterone and cortisol, are involved in metabolism [48], the effects of any nutritional deficits among drinking women may be exacerbated with social stress.

The most serious disorder associated with maternal drinking is the fetal alcohol syndrome (FAS), characterized by low birth weight, developmental lags, and craniofacial abnormalities [30]. Although all mothers of FAS infants drink ethanol, they also may live in stressful social situations and eat inadequate diets. In a retrospective study of FAS mothers, Abel [5] has described them as young, having several children (thus, closely spaced), and frequently refer to

their husbands as "alcoholic." Abel also reports that the mothers were underweight during and prior to pregnancy.

Our findings suggest the possibility that the social situation and nutritional state of mothers of FAS deserves as much attention as does their drinking. The data also suggest that, like propylene glycol, other presumably safe substances may have damaging effects when combined with social stress. We do not know the mechanism by which PG could exert its deleterious effects on fetal development. The important finding here is that those effects become manifest only when combined with maternal social stress.

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