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Blood Alcohol Level and Caloric Intake in the Gravid Rat as a Function of Diurnal Period, Trimester, and Vehicle[1]

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MARTIN, J. C., D. C. MARTIN, B. RADOW AND G. SIGMAN. *Blood alcohol level and caloric intake in the gravid rat as a function of diurnal period, trimester and vehicle.* PHARMAC. BIOCHEM. BEHAV. 8(4) 421–427, 1978. – Blood ethanol levels, caloric intake and weight gain were monitored over the 21-day gestational period in the gravid Sprague-Dawley rat as a function of the administration of ethanol in either a liquid diet (Ensure) or an aqueous saccharin solution. The mean daily percentage of ethanol consumed (38% vs 31%), and g/Kg of ethanol consumed (11.9 vs 9.7 g/Kg) were higher for the liquid diet group than the aqueous solution group. Ethanol consumption varied by the trimester in the Ensure but not in the saccharin solution rats. Proportional maternal weight gain, live litter size, and live litter weight did not vary as a function of the method of ethanol administration. Both groups exhibited significant diurnal periodicity in ethanol consumption, and the greatest caloric intake during the second trimester. The implications of these results for ethanol administration in gravid rats is discussed.

Blood alcohol level Caloric intake Ethanol Maternal weight gain Gravid rats Fetal development

BOTH animal and human studies have clearly demonstrated that alcohol crosses the placenta to adversely affect the fetus [3, 4, 9, 20, 21]. A study of ethanol and acetaldehyde content in peripherai blood of gravid and nongravid rats following one IP injection of 1.2 g/kg of ethanol found slower elimination of acetaldehyde but not of ethanol in the pregnant rats. Since the aldehydes exert sympathomimetic effects including blood pressure and heart rate changes, it is possible the fetus may suffer side effects [11].

Although other investigators have utilized a variety of administration routes including inhalation, intubation, and injection, the oral route via drinking has been found by us to provoke the least maternal trauma with a predictably high ethanol caloric intake (36–41%) while still allowing for weight gain during pregnancy. It certainly is the closest approximation to the human model. Our method of choice has been ethanol in a 0.1% sodium saccharin solution as the sole fluid source with Purina rat chow ad lib. See Studies 3 and 4 in Table 1.

Another oral method which has been utilized by several investigators is the administration of ethanol in a complete liquid diet [8, 15, 17]. One such study [10] administered 25% of the total daily calories in ethanol to gravid inbred mice, with controls receiving an isocaloric sucrose diet. Increased resorption and skeletal and cardiac anomalies were found in the ethanol fetuses on Day 19 of gestation.

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TABLE 1
ALCOHOL ADMINISTRATION TO GRAVID RATS

Method of Ethanol Administration	Percent Ethanol Solution (v/v)	Daily Amount Ethanol Ingested	Total Calories Consumed	Calories of Ethanol Consumed	% Calories in Ethanol	Weight Gain Over Pregnancy	Number Born Alive	Individual Newborn Weight
I. Past Studies								
1. Injection SC twice daily	20	4.0 g/kg	98.8 k/cal	9.2 k/cal	10	143.4 g	10.6	6.9 g
2. Intubation twice daily	20	8.5 g/kg	84.6 k/cal	20.1 k/cal	24	142 g	12.7	7.4 g
3. Oral saccharin	20	11.9 g/kg	77.6 k/cal	28.3 k/cal	36	87.5 g	10.5	6.3 g
4. Oral saccharin solution + SC injection twice daily	20	10.9 g/kg	80.2 k/cal	32.9 k/cal	41	71 g	11.9	6.1 g
5. Purina chow control (ad lib)	0	0	93.1 k/cal	0	0	143.1 g	12.2	6.9 g
6. Purina chow control (pairfed)	0	0	75.9 k/cal	0	0	79.5 g	10.3	6.5 g
II. Present Study								
1. Oral saccharin solution + blood withdrawal	20	9.7 g/kg	65.8 k/cal	20.3 k/cal	31	33.3	10.7	5.7 g
2. Oral Ensure + blood withdrawal	10	11.9 g/kg	65.9 k/cal	24.9 k/cal	38	41.4 g	11.0	5.8 g
3. Oral Ensure	10	14.6 g/kg	73.0 k/cal	27.6 k/cal	38	71.7 g	11.0	5.9 g
4. Oral Ensure	0	0	98.7 k/cal	0	0	150 g	12.5	7.1 g

One investigator [6] examined drinking patterns when ethanol was administered in either water or Metrecal in the nongravid mouse. The liquid diet resulted in a more even circadian consumption pattern than found with the aqueous solution which was consumed primarily at night. The mice also failed to take more than 23% of their calories in ethanol with several concentrations of the aqueous solution. He concluded that the aqueous method was not useful as a model for chronic alcoholism.

In order to test the liquid diet method in the gravid rat pilot studies were performed in this laboratory with Ensure (Ross Laboratories). This was avidly consumed in the plain state by the Sprague-Dawley strain, (93 ml/day) and did not result in diarrhea or gastric upset, either of which could be disastrous in the gravid animal.

It was anticipated that the rate of absorption would probably be more uneven with the aqueous solution since food delays gastric emptying and thus slows ethanol absorption [10].

Very little is known of the way in which blood ethanol levels and caloric intake shift as a function of the trimester of pregnancy, diurnal period, and length of time the animal has been drinking ethanol. The following study was an attempt to delineate these factors in the rat utilizing the aqueous saccharin/ethanol solution with the Ensure/ethanol as a comparison method.

METHOD

Apparatus

The Varian 2100 gas chromatograph with a flame ionization detector and a 1 mV recorder was used with a six foot Poropak Q column. The carrier gas was N_2 which had a flow rate of 30 ml/min. The hydrogen flow rate was 30 ml/min and the air flow was 200 ml/min. The column temperature was maintained at 190° after initial conditioning by heating overnight at 200°C with the carrier gas flow.

Reagents

The internal standard was 1-propanol. The standards were prepared by volumetric dilution in distilled water to produce ethanol concentrations in the range of 50–200 mg/100 ml with 1-propanol in the ratio of 1–1000. The concentration was chosen to yield a propanol peak approximately equal to that of the middle ethanol standard. Twenty μl of blood were diluted with 100 μl of the aqueous propanol solution and one μl samples of the dilutant were injected into the gas chromatograph with a Hamilton μl syringe. Standards and blood dilutions were mixed at the start of each assay solution. The standards were first run through the gas chromatograph and any necessary recalibrations performed at this time.

Blood extraction involved placing the rats in Lucite restraining containers with the tail extruded. A small bit of the end of the tail was removed with sterile surgical scissors and 20 μl of blood squeezed from the tail into a heparinized capillary tube. These were sealed and stored at 0°C until the assay was run. Assays were performed weekly.

Procedures

Experimental design. Table 1 presents the protocol. This was an unbalanced repeated measures design with each rat

TABLE 2

Treatment	Diurnal Period	Trimester 1	2	3	Total
Ethanol in saccharin solution n = 9	7 A.M.	n = 3	3	3	n = 9
	1 P.M.	3	4	2	
	7 P.M.	2	4	3	
	1 A.M.	3	4	2	
Ethanol in Ensure n = 9	7 A.M.	3	3	3	n = 9
	1 P.M.	3	4	2	
	7 P.M.	2	4	3	
	1 A.M.	3	4	2	

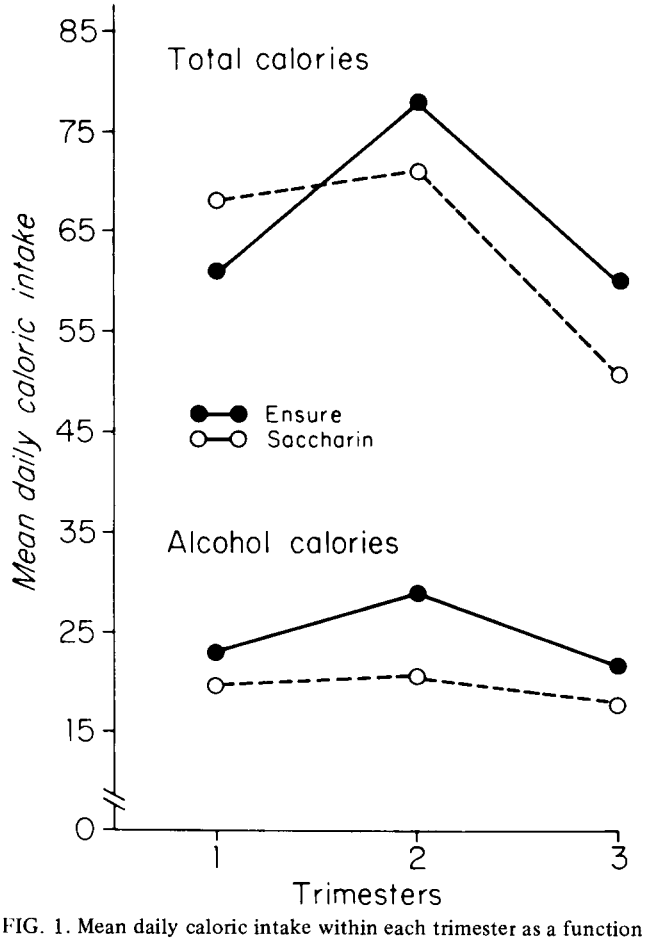

FIG. 1. Mean daily caloric intake within each trimester as a function of treatment. n = 9 in each group.

having blood withdrawn four times over the three trimester period, once at each time of day. Each gravid rat in the ethanol/saccharin group was matched with one in the ethanol/Ensure group and blood was withdrawn on the same day of pregnancy and at the same time of day from each throughout the gestational period. Initially n = 10 in each treatment but one rat in the Ensure treatment conditioned failed to deliver, so the scores of the corresponding rat in the saccharin group were excluded from the analyses.

Animals

Twenty-five Sprague-Dawley derived, barrier-sustained rats were received over a two-day period on the morning following evening impregnation as determined by the presence of sperm in the vaginal lavage. (The caesarian-derived, barrier-sustained timed pregnant, Sprague-Dawley rats were supplied by Tyler Laboratories, Bellevue, WA.) Day 1 weights ranged from 240–270 g. The 10 rats which arrived on the first day were assigned to the Ensure group, and the 10 which arrived the following day to the saccharin solution group. Treatment was started at 9 a.m. on the day of arrival and continued for 21 days. Group 1 received a 20% ethanol in a 0.1% sodium saccharin solution. The concentration of ethanol was chosen to be applicable to previous studies in our laboratory [12]. Purina rat chow was available ad lib. The bottles were removed at 9 a.m. on Day 22 and water was substituted.

Group 2 received 10% ethanol in a vanilla-flavored liquid diet (Ensure, Ross Laboratories) with the addition of 0.5 g/100 ml of U.S.P. Salt Mixture XIV, and 0.3 g/100 ml of Vitamin Diet Fortification Mixture (ICN Pharmaceuticals). No additional food was available. The percentage of ethanol added to the Ensure was chosen empirically from pilot studies cited previously as the most concentrated solution the rats would drink and still maintain their weight. Plain Ensure replaced the alcohol solution beginning on Day 22.

Each animal had 20 μl of blood withdrawn from the tail vein four times during the 21 day gravidity period: once each at 7 a.m., 1 p.m., 7 p.m. and 1 p.m. Each rat had blood withdrawn in each of the three trimesters with one additional drawing. The four blood withdrawals were spaced as far apart as possible, given the constraints of one withdrawal for each diurnal period and once in each trimester.

Five additional gravid animals were monitored for Ensure consumption without ethanol. Two of the rats received Ensure alone and three received Ensure with 10% ethanol but no blood withdrawal. It was felt that the blood withdrawal process itself might be stressful for the gravid animals. All rats were weighed daily at 9–10 a.m. throughout gestation. All were housed individually in Lucite cages containing Sani-cell, an absorptive material made of ground corn cobs. Temperature was maintained at 22–25°C with humidity at 50% on a 14/10 hr light/dark cycle.

Maternal measures. The ethanol intake, caloric intake other than ethanol, and maternal weight gains were measured daily for 21 days. The blood ethanol levels were computed four times during pregnancy. Beginning at 10 a.m. on Day 22, one hr following ethanol removal, the rats were observed for 8 hr for any behavioral signs of withdrawal. The behavior of each rat was observed for a two-min period in each hour, and rated on the scale in Table 3.

Offspring measures. Gestation length, live litter size, birth weights, sex ratio, and litter weights at birth were tallied for all treatments.

RESULTS

Since maximizing intake resulted in different ethanol concentrations between the two methods, intragroup analyses were performed except for comparisons of litter size, weight and maternal weight gain.

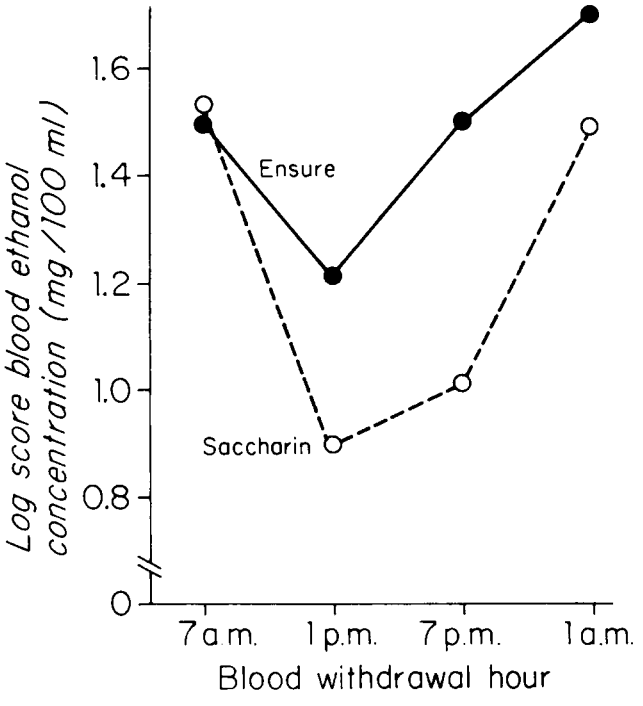

FIG. 2. Mean BAL as a function of diurnal period.

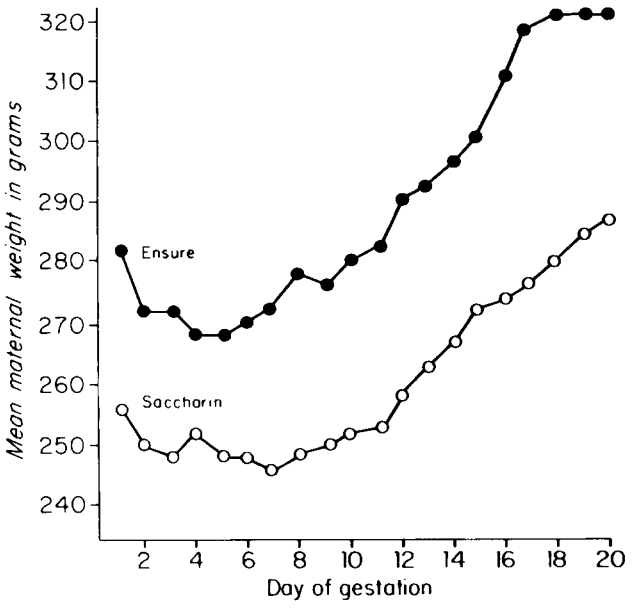

FIG. 3. Mean maternal weight gain over the gestational period.

Total Caloric Consumption

Friedman Rank tests [1] were performed on the total calories consumed for each trimester to determine if total caloric intake differed over trimesters for each treatment condition: S = 14.0, p=0.009 for the Ensure rats, and S = 8.7, p = 0.013 for the saccharin solution group. Figure 1 illustrates the total vs ethanol caloric consumption for both treatment groups as a function of trimester pregnancy.

A Hotelling's T-Squared Two Group Profile Analysis [19] was used to test for parallelism between the two treatment conditions over trimesters. An F(2,15) = 4.86, p = 0.02 was obtained.

Ethanol Caloric Consumption

In order to test for differences over trimesters Friedman Rank tests were performed on the K/cal of ethanol consumed. The results were: S = 13.7, p = 0.001 for the Ensure rats and S = 4.67, p = 0.096 for the saccharin solution rats.

A Hotelling's T^2 test for parallelism just failed to reject the null hypothesis with an F = 3.42, p 0.059.

Non-Ethanol Caloric Consumption (Food)

Similarly, a Friedman Rank test resulted in an S 14.0, p = 0.0009 for the Ensure group and an S = 8.67, p = 0.013 for the saccharin solution group.

A Hotelling's T^2 test for parallelism resulted in an F(2,15) = 4.88, p = 0.023. Unsurprisingly, the pattern was identical to the total calorie pattern.

Blood Ethanol Levels

Time of day. A log transformation was performed on the blood ethanol scores and two repeated measures analyses of variance [22] were performed at 4 x 2 (diurnal period x treatment) ANOVA yielded an F(3,48) = 2.79, p = 0.05 for the time of day component. The treatment term did not approach significance, nor did the treatment by hour interaction term (see Fig. 2).

Order of blood withdrawal. A 4 x 2 (order x treatment) repeated measures analysis of variance resulted in no treatment differences and a non-significant interaction term. The order of blood withdrawal resulted in an F(3,48) = 2.64, p = 0.06.

Maternal Litter Measures

Weight gain. Weight gain over pregnancy is plotted in Fig. 3. A growth curve analysis [19] was utilized which fitted a cubic polynomial approximation to the weight gain curves. The null hypothesis of no difference between weight gain for the Ensure and saccharin solution groups was barely rejected with a p = 0.049. A Hotelling's T^2 test of parallel growth curves failed to reject the null hypothesis with a p = 0.29, i.e., the curves were indeed parallel. However, a univariate t-test for mean weight differences resulted in a rejection of the null with a p = 0.01. The Ensure group was significantly heavier on the average as is apparent in Fig. 3. The last test for the proportion of weight gain over the gestational period, was a Hotelling's T^2 test of parallelism on the logs of the weights. The null was not rejected with a p = 0.34, i.e., the proportional weight gain was the same for both groups over the gestational period.

Other comparisons. There were no significant differences in length of gestation, live litter size or litter weights between the liquid diet and saccharin solution conditions. No signs of withdrawal in either group were observed over the eight hour observational period.

Ensure/ethanol without blood withdrawal and Ensure control comparisons. The weight gain during pregnancy of the three Ensure/ethanol rats without blood withdrawal was considerably higher than that of the BAL group, which is some indication that successive blood withdrawals may

TABLE 3

Activity	Gait	Tremor	Convulsion
0. somnulent	1. normal	0. None	0. none
1. normal	2. ataxic	1. intermittent	1. circling
2. hyperactive	3. falls, loses balance	2. sustained	2. mild rhythmic tremor
			3. contractions
		1. face, limbs	4. rolling and thrashing
		2. whole body	5. alternate spasms and contractions (tonic/clonic seizure)
		1. gentle tremor	6. tail curled over back, body rigid (opisthotonus)
		2. moderate tremor	
		3. severe tremor	7. apnea and coma (usually preceded by gasp)

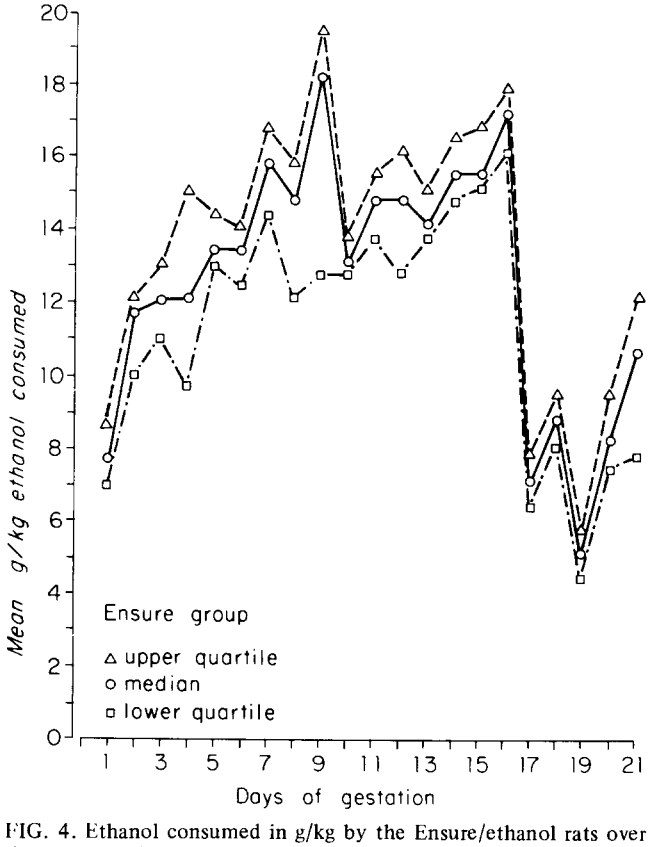

FIG. 4. Ethanol consumed in g/kg by the Ensure/ethanol rats over the gestational period. Medians and upper and lower quartiles are plotted.

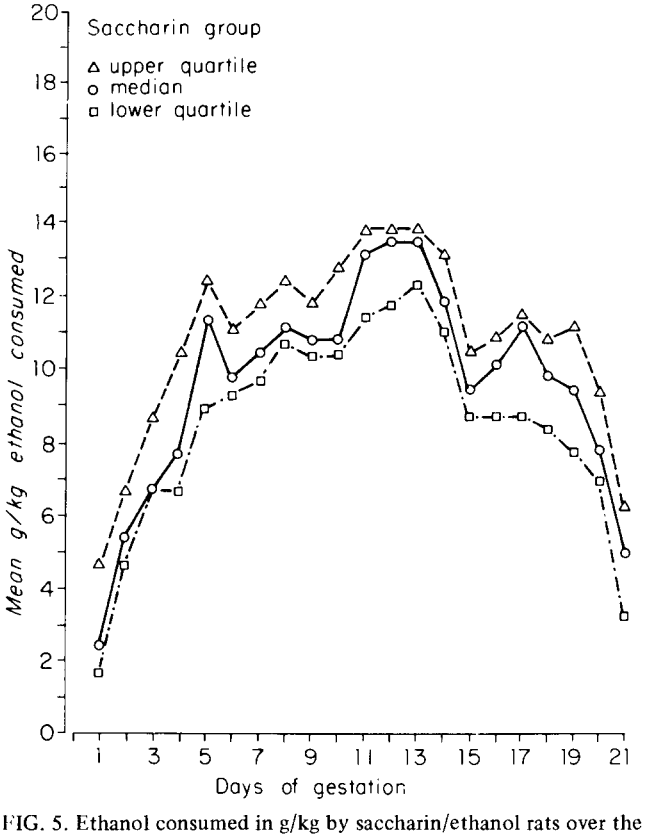

FIG. 5. Ethanol consumed in g/kg by saccharin/ethanol rats over the gestational period. Medians and upper and lower quartiles are plotted.

be traumatic to gravid animals. Their offspring birth weights and litter size did not differ, however. The two rats which received the Ensure without ethanol consumed significantly more of it and gained 2–4 times the weight of the two Ensure/ethanol groups. The litter size and offspring weights were also apparently greater.

Table 1 details the several methods which have been utilized in this laboratory in studies of the survival, development and subsequent function of rat offspring whose dams received ethanol for a selected time period during the gestational/nursing period.

DISCUSSION

Another study [8] administered ethanol concentrations of 8.1–9.7% in Metrecal from 10–30 days to non-gravid rats. The mean ethanol consumption was 15.3 g/kg with a marked periodicity in evidence with periods of abstinence occurring every 7–12 days during which period the consumption dropped below 8 g/kg/day. The pregnant rats in the current study did not exhibit this voluntary abstinence, even though ethanol consumption remained at a high level until the last trimester. See Figs. 4 and 5. However, there is no a priori reason to assume that

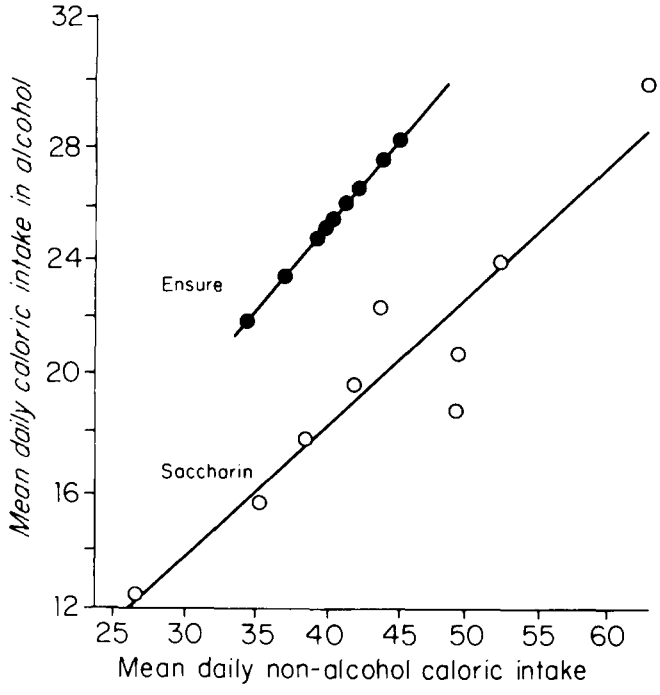

FIG. 6. Least-squares fit of daily non-ethanol by daily ethanol caloric intake.

consumption patterns of pregnant rats would be identical to non-pregnant animals.

Although developmental and behavioral differences in offspring have been found utilizing the saccharin/ethanol solution herein described, it is apparent that neither of the oral intake methods utilized here in nonfood-deprived rats results in the high, sustained blood ethanol levels necessary for the withdrawal syndrome to occur. An excellent review of alcohol addiction in animals and the accompanying withdrawal syndrome can be found in [13].

The investigators who have observed the withdrawal syndrome in rats have employed procedures which either initially, or subsequently, resulted in severe weight loss [2, 5, 8] or have used procedures which allow administration 3–4 times daily, e.g., intubation [7,14]. Neither of these procedures should be used with pregnant rats since the weight loss may result in fetal under-nutrition and the intubation several times a day may be stressful. Not only is it necessary to maintain a daily alcohol intake of 11–15 g/kg for the withdrawal symptoms to appear [14] but a high blood ethanol level must apparently also be maintained over the day in order for such symptoms to be made manifest. Episodic peaking of the BAL once or twice daily is not a sufficient condition [18]. The results of the current study support this contention. If it is assumed the 100 mg/100 ml blood alcohol level must be maintained as a prerequisite for the appearance of withdrawal symptoms, the lack of such symptoms was to have been expected in the current study. The Ensure group when they met the BAL criterion did so only once a day, usually at 7 a.m. or 1 a.m., and the saccharin solution group less often, usually at 7 a.m.

Although the pattern of food consumption for the two alcohol groups began low, then rose to its highest point in trimester two to drop again just prior to birth, the pattern for the two animals who received plain Ensure continued to rise throughout the gestational period. No other studies have been found which have examined the pattern of caloric intake as a concomitant of alcohol intake during pregnancy. It is obvious from Fig. 6 that the Ensure group exhibited less variability and consumed considerably more ethanol as a percentage of total calories over the 21-day gestational period. The variability of the saccharin solution group as compared with the Ensure rats is also evident in Fig. 6.

The points for the Ensure rats fall on a straight line since the percentage of ethanol to Ensure is a constant. It is interesting that the saccharin solution rats exhibit far more variability in their total caloric intake, but that the slope of the two lines is almost parallel. This means that the saccharin solution rats tended to maintain the same proportional increase of ethanol calories to food calories even though these proportions were not fixed as they were for the liquid diet group.

The more intesting and inexplicable result is the sharp drop in intake on Day 17 of gestation by the Ensure group depicted in Fig. 4. This was found in all of the nine rats, and is not in evidence in the saccharin solution group. One possible explanation is a voluntary period of abstinence which occurred later and returned to baseline more slowly than the phenomenon described previously [8]. Both groups tended to increase consumption over the first two trimesters and then drop during the third trimester. The three Ensure/ethanol rats which did not have blood withdrawn followed the same pattern as the other alcohol groups and dropped consumption during the third trimester, so the withdrawal of blood apparently was not a factor. The liquid diet group than increased consumption again to some extent on Days 19–21. These intergroup differences over the last 5–7 days of gestation can not be explained by the length of gestation since all rats in all groups delivered on Day 24, counting the day of arrival as Day 1.

A comparison of the two oral methods of ethanol administration to gravid rats revealed the following similarities and differences.

Similarities

1. Total caloric intake varied for both the liquid diet and aqueous solution groups with the greatest consumption occurring during the second trimester (Fig. 2).
2. Food consumption patterns varied as a function of trimester for both treatments with the greatest consumption occurring during the second trimester.
3. Both groups exhibited uneven circadian BAL patterns with ethanol consumption occurring primarily at night.
4. BAL scores did not vary as a function of successive withdrawals for either group, i.e., blood ethanol levels later in pregnancy did not differ from those taken early in pregnancy.
5. Proportional weight gain over the 21-day gestational period was equal for both groups, although the Ensure group averaged 20 g heavier on Day 1 of pregnancy (Fig. 3).
6. Live litter size and weight was the same for both ethanol groups (Table 1).

Differences

1. Ethanol consumption patterns differed as a function of trimester for the Ensure but not for the saccharin solution group (Fig. 1).

2. The saccharin solution group exhibited more variability in their total daily caloric intake, total daily caloric ethanol intake, and BAL readings at 7. a.m., 1 p.m. and 7 p.m., but not at 1 a. m. where the two groups did not differ.

3. The mean percentage of ethanol consumed daily was higher for the liquid diet than for the saccharin solution rats (38% vs 31%).

4. The mean daily g/kg of ethanol consumed was greater for the Ensure than for the saccharin solution groups 11.9 g/kg vs 9.7 g/kg (Figs. 4 and 5).

If BAL determinations are made they should probably be performed on rats whose offspring will not be utilized in later behavioral studies, since the greater weight gain over pregnancy by the Ensure/ethanol rats which did not have blood taken is an indication of trauma incurred. Isocaloric pair-feeding for the control Ensure rats is indicated if the 10% concentration of ethanol is used since the control rats on ad lib feeding gained 2–4 times as much weight as the Ensure/alcohol rats.

It would appear that the Ensure as a vehicle for ethanol may have some advantages to gravid rats over the aqueous solution, assuming that the total diet is as adequate as the Purina chow diet. The decreased variability in intake among rats and the greater amount of maternal ethanol consumed would be advantageous if the effects on offspring were being studied. There are some indications from pilot studies in our laboratory that the Ensure offspring may not do as well as the saccharin offspring if the enriched Ensure diet is continued through the nursing period. Studies are being conducted to determine the effects of Ensure during the nursing period on subsequent offspring behavior.

REFERENCES

1. Bradley, J. V. *Distribution-Free Statistical Tests.* Englewood Cliffs, NJ: Prentice-Hall, 1968, 126 pp.
2. Branchey, M., G. Rauscher and B. Kissin. Modifications in the response to alcohol following the establishment of physical dependence. *Psychopharmacologia* **22:** 314–322, 1971.
3. Chernoff, G. A mouse model of the fetal alcohol syndrome. *Teratology* **11:** 14A, 1975.
4. Fabre, L. F., R. W. Farmer, M. K. Roach, G. E. Fritchies and W. M. McIssac. Biochemical and metabolic aspects of alcoholism. *Ann. N.Y. Acad. Sci.* **215:** 346–355, 1973.
5. Falk, J. L., H. H. Samson and G. Winger. Behavioral maintenance of high blood ethanol and physical dependence in the rat. *Science* **177:** 811–813, 1972.
6. Freund, G. Alcohol consumption and its circadian distribution in mice. *J. Nutr.* **100:** 30–36, 1970.
7. Hunt, W. A. Changes in neuro-excitability of alcohol-dependent rats undergoing withdrawal as measured by the pentylenetetrazole seizure threshold. *Neuropharmacology* **12:** 1097–1102, 1973.
8. Hunter, B. E., J. N. Riley, D. W. Walker and G. Freund. Ethanol dependence in the rat: A parametric analysis. *Pharmac. Biochem. Behav.* **3:** 619–629, 1975.
9. Jones, K. L. and D. W. Smith. Recognition of the fetal alcohol syndrome in early infancy. *Lancet* **2:** 999–1001, 1973.
10. Kalant, H. Absorption, diffusion, distribution and elimination of ethanol: Effects on biological membranes. In: *The Biology of Alcoholism,* edited by B. Kissen and H. Begleiter. New York: Plenum Press, 1971, pp. 1–62.
11. Kesaniemi, Y. A. Metabolism of ethanol and acetaldehyde in intact rats during pregnancy. *Biochem. Pharmac.* **23:** 1157–1162, 1974.
12. Martin, J. C., D. C. Martin, G. Sigman and B. Radow. Offspring survival, development and operant performance following maternal ethanol administration. *Devl Psychobiol.* **10:** 435–446, 1977.
13. Mello, N. K. A review of methods to induce alcohol addiction in animals. *Pharmac. Biochem. Behav.* **1:** 98–101, 1973.
14. Mucha, R. F., J. P. J. Pinel and R. H. V. Oot. Simple method for producing an alcohol withdrawal syndrome in rats. *Pharmac. Biochem. Behav.* **3:** 765–769, 1975.
15. Pilström, L., E. Fellenius and K–H. Kiessling. The use of semi-synthetic liquid diet for the supply of ethanol to rats and its effect on lysosomal enzyme activities in the liver. *Br. J. Nutr.* **29:** 297–306, 1973.
16. Randall, C. L., W. J. Taylor and D. W. Walker. Ethanol-induced malformations in mice. *Alcoholism: clin. exp. Res.* **1:** 219–224, 1977.
17. Rawat, A. K. Effect of maternal ethanol consumption on fetal hepatic metabolism in the rat. *Ann. N.Y. Acad. Sci.* **273:** 175–187. 1976.
18. Samson, H. H. and J. L. Falk. Pattern of blood ethanol elevation and the development of physical dependence. *Pharmac. Biochem. Behav.* **3:** 1119–1123, 1975.
19. Timm, N. H. *Multivariate Analysis with Application in Education and Psychology.* Monterey, Calif.: Brooks/Cole Publishing Co., 1975, pp. 490–511.
20. Tze, W. J. and M. Lee. Adverse effects of maternal alcohol consumption on pregnancy and foetal growth in rats. *Nature* **257:** 479–480, 1975.
21. Ulleland, C., R. P. Wennberg, R. P. Igo and N. J. Smith. The offspring of alcoholic mothers. *Pediat. Res.* **4:** 474, 1970.
22. Winer, B. J. *Statistical Principles in Experimental Design.* New York: McGraw-Hill, 1962.