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Effects of Maternal Intake of Nicotine on Fetal and Newborn Rats.* (29419)

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Administration of nicotine to pregnant animals has been shown to produce capillary damage in the placenta in dogs(1), skeletal defects of the offspring and decrease of litter size in mice(2), vertebral anomalies in chicks (3), and postponement of the appearance of the first litter(4) and lighter weight offspring in rats(5). Administration of nicotine to nursing rat mothers is associated with a poor neonatal weight gain(5) and an increase in neonatal mortality(5,6). Geller(7) gave pregnant rats nicotine doses less than 15% of those used by Nishimura and Nakai in mice (2) and produced no fetal abnormalities. This difference may be explained on the basis of dosage and species differences; however, the current status of this problem is unsettled.

Acute administration of nicotine results in a rise in serum free fatty acids in dogs and humans(8). Smokers, when compared with non-smokers, have been shown to have increased serum lipoproteins(9) and cholesterol (9,10,11) and to produce babies of lighter weight(12). The effect of nicotine on fetal lipids has not been investigated.

In this study, a nicotine solution in place of water was given to rats throughout pregnancy and up to the time of weaning in order to determine its effect on fetal and neonatal growth and mortality. Measurements of neonatal brain and liver lipid and cholesterol content were made as a preliminary investigation of the effect of nicotine on fetal lipid metabolism.

Materials and methods. Two experiments were carried out using virgin Sprague-Dawley female rats which were mated to males of the same strain. The body weight at the start of pregnancy ranged from 237 to 305 g. Conception was dated from the time of the appearance of the vaginal plug. The experimental groups were given a solution of .05 mg/ml nicotine as the sole source of drinking water from time of conception throughout pregnancy and the control animals were given distilled water. The solution and water were given *ad lib* in graduated drinking tubes and daily intake was recorded. All animals were given Purina Lab Chow *ad lib*.

In Experiment I, 8 rats given nicotine and 9 control rats were studied. Three of the experimental animals received nicotine only for the last 14 days, 6 days and 4 days of pregnancy respectively. Within a few hours of birth the newborn rats were weighed and then killed by decapitation. The brains and livers were promptly dissected free of other tissue, weighed, and frozen. Lipid and nitro-

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gen determinations were performed on pooled brains and livers for each litter. The tissues were homogenized with 50 ml of 2:1 chloroform-methanol per g of tissue in a micro Waring blendor. An aliquot of uniformly mixed tissue homogenate was removed for nitrogen analysis. The remainder was centrifuged; the supernatant was left in contact with distilled water overnight and total lipid and cholesterol were then determined. The mother rats were killed at the time of the dissections of the neonates in order to determine the number of uterine patches and absorbing fetuses.

In Experiment II, 8 rats were given the nicotine solution, with 5 controls. After the litters were delivered the nicotine group remained on the nicotine solution. All the young and maternal animals were killed with ether 21 to 22 days after delivery. Only body weight was recorded.

Results. Newborn rat (Exp. I). Nicotine intake. Five nicotine and 5 control females had daily intake records for the entire period of gestation. Average intake of water by the controls was 125 ml/kg/day. Average intake of nicotine solution was 53 ml/kg/day or the equivalent of 2.7 mg nicotine/kg/day. The body weight used in the calculation was the 10th day weight interpolated from the weekly body weight measurements.

Maternal weight gain. All rats showed a weight gain during pregnancy. One nicotine female had only one weight recorded. The other nicotine rats showed weight curves comparable to the controls in slope.

Fetal and neonatal mortality. On the basis of the count of uterine patches the average number of rats conceived was 13.3 in the nicotine group and 12.0 in the control group. Only one absorbing fetus was found in the nicotine rat group; 10 were seen in the control group, but 6 of the 10 were found in a single rat. There were 23 newborns eaten or found dead in the nicotine group and 18 in the control group. There was little difference in the total number of absorbed, dead or eaten rats between the two groups.

Newborn body, liver and brain weight. Average weight of the nicotine newborns was 5.5 g and of the control newborns, 6.5 g. An analysis of variance showed significantly greater variation in the nicotine body weight with a p value of less than 0.0005. The t test applied to the 2 means showed that the probability of lack of identity was greater than 0.9995. Average brain weight expressed as percent of body weight was increased slightly in the nicotine newborns, 4.4% compared with 4.0% in the controls. The liver weight as percent of body weight was practically the same in the 2 groups, 4.3% in nicotine rats and 4.4% in controls.

Fetal brain and liver lipid and nitrogen content. These data are expressed in the form of ratios and are shown in Table I. The arithmetic and geometric mean values of the ratios did not differ significantly between nicotine and control groups. On the other hand, an analysis of variance showed a significantly greater variation in the livers of nicotine animals in respect to lipid/tissue, lipid/nitrogen, and cholesterol/lipid ratios. Liver nitrogen/tissue ratio and brain ratios of nicotine animals did not show significant variability. The analysis of variation was carried out on logarithms of the ratios for more rigorous scrutiny.

The litter in the control group with 6 fetuses absorbed out of 12 conceived had a pattern of extreme results in the lipid ratios. Brain cholesterol/lipid ratio was 0.016, as contrasted to the mean of 0.163 for the control group. This value was not included in the calculations of statistical significance because of the magnitude of its deviation.

In the 3 nicotine rats which received the nicotine solution for less than the entire term of pregnancy, the neonatal body weights and chemical values on tissue analysis fell well within the extremes of variation found for the entire nicotine group.

Weanling rats (Exp. II). Mortality. The number born was not determined. At time of sacrifice there were 74 young in 8 nicotine litters or 9.25 rats per litter. In the control group there were 42 young in 5 litters or an average of 8.4 rats per litter. There was no apparent increase in mortality due to nicotine.

General condition. The nicotine weanlings had rougher fur but they showed no apparent

TABLE I. Lipid, Cholesterol, and Nitrogen Content of Brain and Liver with Tests of Variance.

Ratio (mg/mg)	Tissue	Exp group	No. of litters	Range	Geometric mean	95% confidence limits	F
lipid/tissue	Brain	Nicotine	8	.0242– .0273	.0259	.0249– .0269	1.71
		Control	8	.0254– .0285	.0263	.0255– .0271	
	Liver	Nicotine	8	.0182– .0416	.0258	.0209– .0317	12.0 **
		Control	9	.0219– .0274	.0248	.0235– .0262	
lipid/N	Brain	Nicotine	8	1.60 –1.96	1.79	1.69 –1.91	2.44
		Control	8	1.91 –2.11	1.96	1.89 –2.04	
	Liver	Nicotine	8	.80 –1.48	.988	.831 –1.17	8.28**
		Control	9	.80 –1.04	.880	.833 – .930	
chol/lipid	Brain	Nicotine	8	.155 – .175	.162	.154 – .169	1.63
		Control	7	.156 – .175	.163	.157 – .170	
	Liver	Nicotine	8	.082 – .255	.151	.117 – .194	5.11*
		Control	9	.136 – .215	.162	.146 – .179	
N/tissue	Brain	Nicotine	8	.0135– .0156	.0144	.0137– .0152	1.01
		Control	9	.0122– .0149	.0133	.0127– .0139	
	Liver	Nicotine	8	.0227– .0289	.0261	.0246– .0278	1.60
		Control	9	.0260– .0314	.0282	.0270– .0295	

$F = \frac{S_1^2}{S_2^2}$ where S_1 and S_2 are the standard deviations of log values.

Asterisks indicate significance: * $p < .05$ ** $p < .005$

weakness or ataxia and appeared to cling to the teats as vigorously as did controls.

Body weight in weanling rat. The mean body weight for nicotine weanlings was 32.6 g and for control weanlings, 52.8 g. Mean body weights for each litter varied in the nicotine group from 19.6 g to 51.3 g and in controls from 47.0 g to 67.5 g. The probability that these means are different is greater than 0.9995 when the *t* test is used.

Comment. These experiments show that exposure to nicotine during pregnancy causes a significant reduction in weight in the newborn and weanling rat. The mean values of the various lipid ratios in the brains of nicotine and control rats and also those of the livers in the two groups do not differ significantly. There is, however, a significant difference in variability of the lipid ratios in the case of the liver. No differences in mortality were observed between nicotine and control groups either at birth or at weaning.

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