

ship between hormones and vitamin D in adult animals.

*Summary.* Mature male quail remained in a good physical condition even though no vitamin D was given for a year. In contrast, high mortality occurred in the vitamin D deficient females, even though calcium balance was not different from that observed in the male quail. It was suggested that the requirement of adult male birds for vitamin D is either very low or non-existent. Injections of testosterone and estradiol into vitamin D deficient hens did not influence egg production, but significantly improved bone ash of tibia, sternum, and femur. Alleviation of debility caused by the vitamin D deficiency was observed in laying hens when testosterone was

injected.

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### Effect of Maternal Nicotine Intake on Fetal Weight and Length in Rats.\* (31944)

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We have reported that addition of nicotine to the drinking water of pregnant rats resulted in lighter weight offspring and greater variability in liver lipid content than in control rats given distilled water(1). It was noted that the water intake was lower in the case of the pregnant rats drinking nicotine solution. Food intake was not measured, but the weight gain during the pregnancy was comparable to that of controls. To determine whether the fetal changes resulted from nicotine itself or decreased food intake, a different experimental design was sought in order to give nicotine in multiple small doses throughout the pregnancy with minimal disturbance or trauma to the gravid rat and, at the same time, to maintain a normal food intake.

After testing several types of feeding and drinking patterns, it was found that nicotine-

diet mixtures were acceptable to the rats at concentrations that permit comparison with the results of our previously reported experiments. In this paper we present the results of experiments in which pregnant rats were maintained on normal food and water intake and yet took in nicotine in amounts comparable to the earlier study. Fetal weight and length were determined on the 20th day of gestation.

*Materials and methods.* Sprague-Dawley derived rats were used throughout. Virgin females were mated and time of conception was marked from the appearance of the vaginal plug. Distilled water was given *ad lib* and daily intake was recorded. The food was finely ground Purina Mouse Breeder Chow given *ad lib* in non-spillable food cups. Food intake was measured at 2-3-day intervals. Nicotine was added to food by injecting a solution containing 25 mg/ml (through a 25 gauge needle) into the powdered food while mixing. The mixture was then stirred mechanically for 2 hours. Diets were freshly prepared weekly and stored at 3-4°C.

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On the morning that the vaginal plug was noted the female was placed in an individual cage. Food and water intake was recorded from that time. Alternate rats received food with nicotine throughout the study. Body weight was recorded on days 1, 8, 15, and 20.

TABLE I

Nicotine concentration (mg/g food)	Group	No. of litters	(1)			Fetal length at 20 days (cm)
			Food intake (g/kg b.w./day)	Water intake (ml/kg b.w./day)	Nicotine intake (mg/kg b.w./day)	
.05	Nicotine	12	51 ± 4.5	162 ± 92.2	2.55	3.54 ± .098
	Control	12	49 ± 10.9	157 ± 51.6	0	3.52 ± .100
.10	Nicotine	11	46 ± 6.9	119 ± 74.2	4.60	3.51 ± .166
	Control	12	59 ± 7.1	126 ± 24.6	0	3.51 ± .312

Data expressed as mean ± S.D.

(1) Calculation based on average of maternal weights on days 1 and 20.

(2) Calculated from food intake column.

(3) Calculated from litter averages.

TABLE II

Nicotine concentration (mg/g food)	Group	Avg No. of live fetuses per litter	Total No. of absorbing fetuses
.05	Nicotine	13.1	4
	Control	11.4	2
.10	Nicotine	11.8	1
	Control	12.3	0

On the 20th day the rats were killed with ether fumes and the fetuses were removed, dissected free of fetal membranes, and laid on absorbent gauze to blot dry. They were then counted and weighed together by litters. After weighing the fetuses were placed in 10% formalin for 24 hours and then measured for the maximum length (crown-rump) with vernier calipers.

*Results.* Table I shows the data obtained with 2 different nicotine concentrations, 0.05 mg/g and 0.10 mg/g of food. Food and water intake was not disturbed on the 0.05 mg/g concentration although the nicotine intake was at a level of 2.55 mg/kg/day. On the higher nicotine concentration there was a slight but significant lowering of food intake. Water intake remained normal. The average nicotine intake on the higher level was 4.60 mg/kg/day. There was no change in fetal weight or length on either concentration. There appeared to be no effect on the numbers of live and absorbing fetuses (Table II).

Fig. 1 shows the weight curves as the averages of each group of rats on days 1, 8, 15, and 20. A lag in gain is seen in the rats on the 0.10 mg/g diet, but by the 15th day these animals gained to nearly their expected level of weight.

*Comment.* In our previous report nicotine intake averaged 2.7 mg nicotine/kg/day. This was calculated on the basis of the weight at the 10th day of gestation. The values shown in this paper differ in that they are based on the average of maternal weights on days 1 and 20. The nicotine intake values calculated by the latter method would be somewhat lower than if based on the 10th day weight because of the geometric increase in maternal weight (Fig. 1). Using the first and last day weight avoids some of the effect that would be caused by the lag in accepting the diet

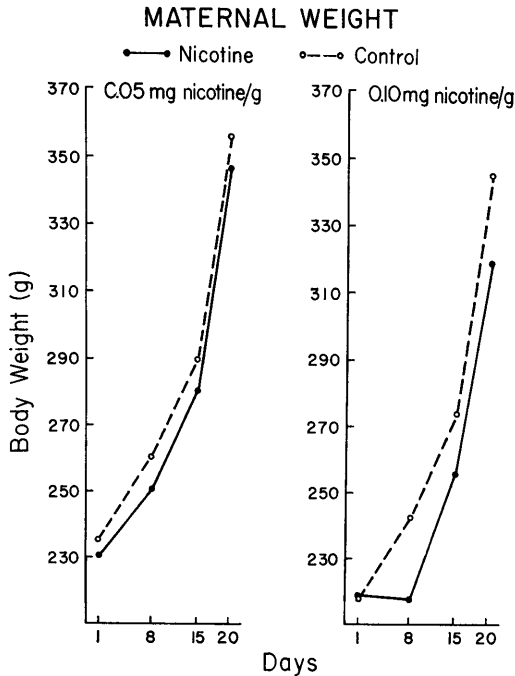


FIG. 1.

by the group on the 0.10 mg/g diet. This is reflected in the delay in maternal weight gain in this group (Fig. 1). The nicotine intake of the 0.05 mg/g group, 2.55 mg/kg/day, thus is nearly the same as that in our earlier report in which the rats received nicotine in their drinking water in a concentration of 0.05 mg/ml. The nicotine intake of the 0.10 mg/g group was much higher.

The absence of effect on fetal weight and length indicates that nicotine *per se* was not responsible for the low newborn weight in the earlier report. In view of the decrease in water intake it is likely that food intake was decreased as well. Chow and Lee showed that the maternal weight gain might remain normal in the face of food restriction in pregnant rats(3).

It has been well established that decreased birth weight is related to smoking during pregnancy in the human(3,4,5). Our observations cannot be related to these at present because of the species difference, the different method of administering nicotine, and the possibility that some other factor in tobacco smoke than nicotine may influence fetal development. One can suggest from our findings that diet should be controlled in evaluating the effect of smoking on fetal growth.

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### Rubella Virus Complement-Fixing Antigen: Sedimentable and Non-sedimentable Antigenic Components. (31945)

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In studies on rubella virus and rubella virus infections several serological methods have been developed. Some of these methods include the neutralization test by the direct inhibition of CPE in tissue culture(1), the inhibition of CPE from a challenge virus by the interference technique(2-4), the fluores-

cent antibody test(5), and recently the complement-fixation (CF) test(6-9). Each of these tests has its inherent difficulties: notably, the time required for the neutralization test, the complexities of the FA test, and the high costs and low sensitivity of the CF test. Two CF antigen preparations have been