Effect of Pregnancy on Feed Consumption and Mammary Gland Growth in Rats* (33467)

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The neural control of voluntary food intake has been studied for many years (1). Little attention has been devoted to a study of the role of the endocrine glands and their hormones and to the effect of reproduction and lactation on food intake. In a preliminary study in this laboratory, wide variation in voluntary food consumption in Sprague-Dawley-Rolfsmeyer female mature rats was observed (2). It was suggested that the differences observed might be due, in part, to differences in their normal hormone secretion rates. A number of studies have been reported on the effect of removal of various endocrine glands and destruction of B-cells by alloxan and replacement therapy and the effect of various hormones in normal animals (3–6).

In a continuation of these studies, the effect of pregnancy on food consumption is reported. In addition, the extent of mammary gland growth as measured by DNA was estimated at the end of pregnancy and related to food consumption and weight gain of the dams.

Materials and Methods. Eighteen nulliparous mature female rats of the Sprague-Dawley-Rolfsmeyer strain weighing a mean of 229 g were placed in metabolism cages constructed so as to prevent coprophagy and feed wastage. Temperature was maintained at 78 ± 1°F and the rats were artificially illuminated during daylight hours. Purina lab chow with an energy value of 4.41 cal/g and 23.4% total protein was fed during a preliminary period of 8 days to determine the mean daily feed consumption of each animal in the group.

Five females and two breeding male rats were put together in a cage and sperm were checked every morning by the vaginal smear method. The rats found with sperm were considered in the zero day of pregnancy and again were put into metabolism cages individually to measure feed consumption during pregnancy. The rats were weighed daily; feed was fed in excess each day; and the residue was weighed back the following morning.

The pregnant animals were sacrificed on day 20; 6 abdominal-inguinal glands were removed; and DNA was estimated by the method of Webb and Levy (7). The body and horns of the uterus were removed with fetuses and counted and weighed on a precision balance. The mean DNA/100 g of body wt. was computed on the basis of the initial body weight (244 g) and on the net weight gain at the end of pregnancy (288 g). The feed consumption per day during pregnancy was based on the mean value for the 6-day intervals and the total body weight at these intervals.

A group of 24 comparable rats weighing a mean of 240 g were maintained as controls. Their mean feed consumption was determined at intervals comparable to the pregnant group. These animals gained 6 g during the period comparable to pregnancy (Table I).

Results. During the preliminary control period, the rats weighing 229 g consumed a mean of 13.88 g/day and 6.08 g/100 g of body wt. (Table I). During the first 6 days of pregnancy daily feed consumption increased to 16.8 g/day a highly significant increase of 21% over the control period. From days 7–13 mean daily feed consumption increased to 18.47 g, an increase of 33%, and during days 14–20 a further increase to 21.07 g/day, an increase of 52%.

The rats weighing a mean of 244 g on day 1

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TABLE I. Effect of Pregnancy on Feed Consumption of Rats.

Condition and time (days)	No. of animals	Body wt. (g)	Feed consumption (g) Per day[b]	SE	Per 100 g of body wt.	SE	Increase /day (%)	Increase/100 g of body wt. (%)
Before breeding								
8	18	229	13.88[c]	± 0.24	6.08	± 0.10		
Pregnancy								
1–6	18	262	16.80[d]	± 0.39	6.40	± 0.01	21	5.26
7–13	18	279	18.47[e]	± 0.40	6.62	± 0.08	33	8.88
14–20	18	318	21.07[f]	± 0.59	6.64	± 0.10	52	9.21
Control group								
1–6	24	240	13.34		5.56			
7–13	24	241	14.31		5.94			
14–20	24	246	13.53		5.50			

[a] SE = standard error of mean.
[b] Levels of significance: [c–d, e, f]. $p < .001$.

of pregnancy consumed a mean of 14.46 g/day and 5.85 g/100 g of body wt. On day 20 of pregnancy, after correcting for fetus weight, rats weighing 288 g consumed 24.61 g and 8.60 g/100 g of body wt., a highly significant increase of 70% in feed consumption between days 1 and 20 of pregnancy.

The rats increased in body weight a mean of 104 g during pregnancy. Of this increase 60 g was due to the fetuses and 44 g to net body weight increase.

The 24 control rats weighing a mean of 240 g consumed a mean of 13.34 g/day and 5.56 g/100 g of body wt. during days 1–6 and changed insignificantly in feed consumption during the 20-day period comparable to pregnancy. Their body weight gain was 6 g compared to a net gain of 44 g of the pregnant group.

When based on the net weight of the dams at the end of pregnancy (288 g), the mean DNA was 6.50 mg/100 g of body wt. (Table II). However, when based on the initial body weight (244 g) the mean DNA was 7.62 mg/100 g of body wt. The mean DNA based on the initial body weight was quite similar to the DNA of two previous groups of pregnant rats (8, 9) but was lower when based on the net final weight.

The mean DNA of the mammary glands of the 18 rats at day 20 of pregnancy was 6.50 ± 0.22 mg/100 g of body wt. While the number of the rats in each group was limited, it was interesting to note that as the daily food consumption of the individual rats increased there was a graded increase in DNA of their mammary glands. Similarly, as the body weight of the rats increased during pregnancy there was a graded increase in DNA (Table III).

Discussion. These data indicate that a marked increase in daily food consumption occurs during pregnancy (70%). This increase is due, in part, to the nutrients required by the growing fetuses and in part to the stimulus for growth of the mothers. It will be noted that there was a net increase in growth of the dams of about 2 g/day. It has been reported that a similar increase in body weight of plateaued female rats can be stimulated by the daily injection of 1 mg of bovine growth hormone (10). In a previous study (11) ovariectomized rats injected daily with ovarian hormones plus 2 mg of bovine growth hormone gained 44 g in 20 days. These data would suggest that pregnancy might stimulate increased secretion of growth hormone. However, Schalch and Reichlin (12) reported that GH levels in the rat were not elevated during pregnancy or lactation.

TABLE II. Normal Growth of the Mammary Gland at End of Pregnancy.[a]

No. of animals	Days of pregnancy	Body wt. (mean, g)		Wt. increase due to pregnancy (mean, g)	Fetal wt. (mean, g)	DNA (μg/mg) DFFT (mean ± SE, μg)	DFFT (mean, mg)	Total DNA (mean ± SE, mg)	DNA/100 g of body wt. (mean ± SE, mg)
		Initial	Final						
19	20					32.24 ± 0.80	749	24.71 ± 1.30	7.63 ± 0.39[b]
49	20	237	333	38	38	32.02 ± 1.96	550	17.61 ± 0.45	7.41 ± 0.18[c]
18	20	244	348	44	60	31.40 ± 0.77	600	18.60 ± 0.81	6.50 ± 0.22[d] 7.62 ± 0.22[e]

[a] Abbrev.: DFFT = dry, fat-free tissue; SE = standard error of mean.
[b] Griffith and Turner (8).
[c] Kumaresan and Turner (9).
[d] Based on net final weight (288 g).
[e] Based on initial body weight (244 g).

Marked variation occurs in the growth of the mammary glands of individual rats during pregnancy. Similarly, variation in individual food intake has been observed. If rats eating more have a higher secretion rate of important hormones, then these higher secretion rates might also favorably influence mammary gland growth. While the number of animals in each group was limited, there was observed a gradual increase in DNA with increasing food consumption (Table III).

While the mean increase in body weight of the dams was 44 g during pregnancy, considerable variation in body weight gains was observed also. To determine whether there was a relation between body weight gains and mammary gland growth, the rats were grouped on this basis. It was observed that there was a graded increase in DNA/100g body wt. of the rats showing increasing body weight gains (Table III).

Summary. Normal voluntary food intake of 18 rats was determined prior to and during pregnancy. Before conception the rats weighing 229 g consumed a mean of 13.88 g/day. During days 1–6 of pregnancy, the rats weighing 262 g consumed 16.80 g/day, an increase of 21%. During days 7–13 body weight increased to 279 g and food consumption to 18.47 g/day, an increase of 33%, and during days 14–20 body weight increased to 318 g and food consumption to 21.07 g/day, an increase of 52%. Between days 1 and 20 of pregnancy, food consumption increased a highly significant 70%. During pregnancy the dams increased 104 g; of this 60 g was due to fetal growth and 44 g to an increased weight of the dams. The control group increased only 6 g during 20 days.

The mean total DNA of the mammary glands at day 20 of pregnancy was 18.60 mg. This is 7.62 mg/100 g of body wt. when based on the initial body weight and 6.50 mg/100 g of body wt. when based on the net weight of the dams at the end of pregnancy. It was observed that the rats consuming increased amounts of food per day and those showing increasing net body weight gains during pregnancy showed graded increases in mammary gland DNA.

TABLE III. Effect of Increasing Food Consumption and Body Weight Gains on Mammary Gland Growth.

Mean daily food consumption during pregnancy (g)	No. of animals	Total DNA (mg/100 g of body wt.; mean)	Increase of body wt. (g)	No. of animals	Total DNA (mg/100 g of body wt.; mean)
17	5	6.02	under 29	4	5.81
18	4	6.17	30–39	5	6.43
19	4	6.33	40–49	5	6.55
20	1	6.69	50–59	2	6.79
21	3	8.05	60–69	2	7.88
22	—	—	Total	18	6.50
23	—	—			
24	1	6.13			
Total	18	6.50			

1. Anand, B. K., Physiol. Rev. **41**, 677 (1961).
2. Grossie, J. and Turner, C. W., Proc. Soc. Exptl. Biol. Med. **107**, 520 (1961).
3. Hahn, D. W., Ishibashi, T., and Turner, C. W., Proc. Soc. Exptl. Biol. Med. **119**, 1238 (1965).
4. Grossie, J. and Turner, C. W., Proc. Soc. Exptl. Biol. Med. **118**, 25 (1965).
5. Kumaresan, P. and Turner, C. W., Proc. Soc. Exptl. Biol. Med. **122**, 526 (1966).
6. Kumaresan, P. and Turner, C. W., Proc. Soc. Exptl. Biol. Med. **120**, 828 (1965).
7. Webb, J. M. and Levy, N. B., J. Biol. Chem. **213**, 107 (1955).
8. Griffith, D. R. and Turner, C. W., Proc. Soc. Exptl. Biol. Med. **106**, 448 (1961).
9. Kumaresan, P. and Turner, C. W., Endocrinology **76**, 396 (1966).
10. Papkoff, H. and Li, C. H., Methods Hormone Res. **2**, 671 (1962).
11. Kumaresan, P. and Turner, C. W., J. Dairy Sci. **43**, 592 (1965).
12. Schalch, D. S. and Reichlin, S., Endocrinology **79**, 275 (1966).

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Enzymatic Determination of Small Quantities of Hydrogen Peroxide* (33468)

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Avi-Dor *et al.* described a method for the determination of minute amounts of H_2O_2 that is based on the use of horseradish peroxide and hydrogen donors. With this they measured the H_2O_2 formed by purified D-amino acid oxidase from D-alanine and O_2 (1). However, the detection of the small amounts of H_2O_2 formed during such oxidative dehydrogenation is complicated by decomposition of the H_2O_2 by catalase or by further oxidation of the metabolites. To avoid these problems, Keilin and Hartree (2) attempted direct estimations of H_2O_2 based on the oxidation of ethanol by H_2O_2 in the presence of an excess of catalase. This reaction proceeds more rapidly than the catalytic decomposition of H_2O_2. Measurements of O_2 consumption in their system have established that certain enzymes [mammalian L-amino acid oxidase (3) and L-α-hydroxy acid oxidases (4)] do produce H_2O_2 during the reaction. The work herein reported described the pho-

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