Prevention of Self-Licking on Mammary Gland Development in Pregnant Rats[1,2] (35575)

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The suckling stimulus is necessary for the maintenance of lactation and mammary gland growth in the postpartum rat (1). The role a similar sensory stimulus has in the development of the mammary gland of the pregnant rat is not fully understood. Early studies have shown that sucking will induce mammary development and lactogenesis in virgin rats (2). Birch (3) noted that one of the behavioral patterns of the pregnant rat was increased self licking of the external genitalia. Self-licking of the mammary line and genitalia is greatest during the second half of pregnancy, and if self-stimulation is inhibited mammary development is reduced by 50% in the pregnant rat (4, 5). Recently, Grosvenor and Mena (6) found that self-stimulation is not important to normal lactation of the rat.

The purpose of this study was to determine if normal mammary gland growth, as determined chemically, is in part regulated by self-stimulation and to consider the possibility that stress may be involved in retarded mammary development.

Material and Methods. Sixty Sprague-Dowley-Rolfsmeyer female albino rats, weighing between 200 and 250 g, were divided into 5 equal groups. Each rat was housed in an individual cage, at a temperature of 25.6 ± 1° and was provided with Purina laboratory chow and water *ad libitum.* The daily illumination cycle incorporated 14 hr of light and 10 hr of darkness. The day spermatozoa were observed in the vaginal smear was designated as day 0 of pregnancy. A rubber collar (5) was placed around the neck of each rat, either on day 1 or day 12 of pregnancy.

One group of 12 animals served as the control for day 12 of pregnancy when the animals were sacrificed. A second group of 12 wore the collar for the first 12 days of pregnancy and then was sacrificed. A third group wore the collar throughout pregnancy and was sacrificed on day 20, while a fourth group wore the collar from day 12 to day 20 of pregnancy. The fifth group of 12 animals was not collared and was sacrificed on day 20 of pregnancy, serving as the control for the third and fourth groups.

Animals were observed frequently during the collared period to see that consumption of food and water was not impaired by the collar.

Immediately after an animal was sacrificed by an overdose of ether, six abdominal-inguinal mammary glands, adrenal glands, uteri, 20-day placentas and 20-day fetuses were removed and weighed. Mammary glands were frozen and later analyzed for DNA content (7) and RNA content (8). Values were expressed on total or unit body weight basis.

Data obtained from the two groups sacrificed on day 12 of pregnancy were analyzed statistically by Student's t test and data from the three groups sacrificed on day 20 of pregnancy were analyzed by analysis of variance and least significant difference (9).

Results. The group which wore the collar for the first 12 days of pregnancy had significantly less ($p < .05$) wet and dried fat-free mammary gland tissue on day 12 than did the normal pregnant group autopsied on day 12, but the DNA and RNA contents of the mammary glands were not statistically different between the two groups (Table I).

Groups which wore the collar from days 1 to 20 or from days 12 to 20 of pregnancy and were sacrificed on day 20 had significantly

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reduced wet and dried fat-free tissue (DFFT) weights of mammary glands compared to the normal day 20 pregnant control group ($p < .05$). Quantities of DNA and RNA (expressed either on a total or unit body weight basis) in the mammary glands were also significantly reduced in both collared groups from the control day 20 group ($p < .05$). The ratios of RNA and DNA were not different statistically between collared and control groups autopsied on day 12 of pregnancy, but the ratios of the two collared groups sacrificed on day 20 were significantly lower ($p < .05$) than their respective control.

Adrenal gland weights (mg/100 g of body wt) were significantly higher in the collared group autopsied on day 12 of pregnancy than in the respective control group, being 19.9 ± 0.9 and 14.2 ± 0.9, respectively ($p < .05$). On day 20 of pregnancy, the adrenal weights (mg/100 g of body wt) were 17.1 ± 1.2 in the group collared from days 1 to 20; 14.6 ± 0.6 in those collared from days 12 to 20; and 10.1 ± 0.6 in the day 20 control group; the two collared groups had significantly heavier adrenals than the control group ($p < .05$). When adrenal weights were expressed as milligrams per 100 g of body weight with weights of uteri and contents subtracted from the body weights, the differences persisted with the group collared for 20 days having adrenals of 20.4 ± 1.4, the group collared from days 12 to 20 having adrenals of 17.5 ± 0.7, and the control day 20 group having adrenals of 12.2 ± 0.7; the latter were significantly less than the collared groups ($p < .05$).

Wet weights of the uteri, placentas or fetuses, as well as numbers of fetuses, were not significantly different between the collared and control groups on day 12 or among the collared and control groups on day 20 of pregnancy, however.

Discussion. Data from this experiment of collared pregnant rats suggest that normal mammary gland development may be regulated, in part, by a reproductive behavioral stimulus arising during pregnancy. It would appear that this stimulus is more effective during the latter half of pregnancy. No retardation of mammary development was

TABLE I. Mammary Gland DNA and RNA in Normal and Collared Pregnant Rats.

			Mammary gland		DNA		RNA		RNA/DNA ratio
Group	No. of rats	Body wt (g)	Wet wt (g/100 g of body wt)	DFFT[a] (mg)	(mg)	(mg/100 g of body wt)	(mg)	(mg/100 g of body wt)	
i 12-Day pregnant	12	261 ± 4.0[b]	2.2 ± 0.2[c]	378 ± 19[c]	11.6 ± 0.51	4.2 ± 0.23	9.9 ± 0.58	3.6 ± 0.49	0.85
ii 12-Day pregnant w/collar	12	258 ± 3.8	1.9 ± 0.1	335 ± 12	10.9 ± 0.58	4.2 ± 0.21	8.1 ± 0.49	3.1 ± 0.20	0.74
iii 20-Day pregnant w/collar from days 1–20	12	317 ± 6.20	2.2 ± 0.1	571 ± 22	18.8 ± 1.18	5.9 ± 0.31	16.3 ± 0.40	5.1 ± 0.14	0.86
iv 20-Day pregnant w/collar from days 12–20	12	320 ± 9.10	2.2 ± 0.1	656 ± 33	19.5 ± 1.37	6.0 ± 0.43	15.7 ± 0.66	4.9 ± 0.23	0.81
v 20-Day pregnant	12	327 ± 6.10	2.5 ± 0.1[d]	793 ± 28[d]	25.9 ± 1.02[d]	8.0 ± 0.21[d]	25.6 ± 0.53[d]	7.9 ± 0.31[d]	0.98

[a] DFFT = dried fat-free tissue.

[b] Mean ± SE.

[c] Series I, analyzed by Student's t, $p < .05$.

[d] Series II, analyzed by analysis of variance–LSD, $p < .05$ for 20-day control.

found at midpregnancy, but when inhibition of self-licking was continued until day 20 of pregnancy a significant reduction of 37% was noted. These findings differ slightly from those of Roth and Rosenblatt (5) who found a 28% reduction at midterm and a 54% reduction at day 22 of pregnancy. Their study relied upon the quantitation of whole mounts of the mammary glands while the present study made use of the chemical measurement of DNA for quantitation.

A previous study in this laboratory (10) indicated that mammary gland development during the latter half of pregnancy was not significantly reduced on day 20 after hypophysectomy on day 12. Perhaps a local stimulation upon the mammary gland parenchyma enhances the stimulatory action of hormones required for optimal mammary gland growth regardless of whether the hormones originate from the pituitary gland or the placenta.

Collaring the rat soon after ovulation and prior to implantation of embryos had no detrimental effects upon reproduction as evidenced by the fact that no rats aborted and all had as many fetuses as did the control animals. These observations are in agreement with those of Roth and Rosenblatt (5) and Schnurer (11). The evaluation of collaring as a detrimental restraint was undertaken in several other ways, one of which was body weight change. Pare' (12) showed that a loss of body weight was a reliable measure of a stress response. The weights of collared animals were slightly but not significantly less than controls in this study. It was observed that the rats were able to eat pelleted food in a nearly normal fashion. However, increased adrenal weight in collared groups suggested that the treatment was stressful and that the secretion of adrenal corticoids was increased.

It is concluded that the retardation in mammary gland development resulting from collaring was not caused primarily by the increased adrenal function because no other signs of stress such as reduced body weight or pregnancy wastage were apparent. We believe that the suppressive action of collaring was due primarily to the withdrawal of the local stimulation upon the mammary gland that is normally provided by self-licking.

Summary. A rubber collar was placed around the neck of rats on day 1 or 12 of pregnancy to restrain the animal from self-licking its mammary glands. Content of mammary gland DNA and RNA in rats sacrificed on day 12 was not statistically different between collared and control groups. However, nucleic acid contents of mammary glands of rats collared from days 1 to 20 and days 12 to 20 and autopsied on day 20 of pregnancy were significantly less ($p < .05$) than the controls. Body, uteri, placenta, and fetus weights and fetus numbers were not altered by collaring. Adrenal weights were increased as a result of the treatment however ($p < .05$). It is postulated that direct stimulation by self-licking enhances the action of endocrines for optimal mammary growth in rats.

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