Effect of Hypophysectomy During Late Pregnancy Upon Mammary Gland Growth in the Rat (37654)

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The role anterior pituitary hormones play in mammogenesis and lactogenesis has been studied extensively in both control and experimental animals. These studies have demonstrated that all the anterior pituitary hormones are either directly or indirectly involved in mammary gland development and secretion (1–3).

The effect of hypophysectomy on mammary gland development during pregnancy, in rats and mice, has been studied by several investigators (4–6), with the overall conclusion being that the anterior pituitary was necessary during the first half of gestation, but was dispensible during the second half. The most frequent explanation given for continued mammogenesis in animals hypophysectomized after midpregnancy was that hormones elaborated by the placenta were capable of substituting for the hormones of the anterior pituitary. Both mammotropic and luteotropic properties have previously been demonstrated in the rat placenta (7).

Since previous studies involving hypophysectomy after midpregnancy have employed morphological techniques in the assessment of mammary gland development, it was thought desirable to repeat these studies, substituting chemical determination of nucleic acid content as the means of assessing mammary gland proliferation.

Methods and Materials. All data were obtained from primiparous Sprague–Dawley–Rolfsmeyer rats killed on Day 20 of pregnancy. Group I (10 animals) served as normal, pregnant controls. Animals in Group II (17 animals) were hypophysectomized on Day 14 of pregnancy. All hypophysectomies were performed by the transauricular method on a model H-200 Hoffman-Reiter hypophysectomy instrument. The completeness of each operation was checked by histologically examining serial sections through the sella turcica. The sections were stained with Harris' hematoxylin, Schiff's leuco-fuchsin (basic) and Orange G. Group III (11 animals) consisted of 90-day-old, virgin females. This group was used to establish levels of nucleic acids in mammary glands of virgin animals.

On the day of sacrifice, the six abdominal-inguinal mammary glands were removed and immediately frozen. Frozen tissue was minced and defatted in 2:1 (v/v) chloroform–methanol for 24 hr, then in ether for 24 hr. The tissue was then dried, weighed and ground to a fine powder. Total nucleic acid was extracted from 25 mg samples of ground tissue with hot trichloroacetic acid (TCA), as described by Schneider (8). Deoxyribonucleic acid (DNA) content was estimated by the diphenylamine method of Burton (9), and ribonucleic acid (RNA) content by the orcinol procedure (10).

Wet weights for each of the following organs were also obtained at dissection of animals in Groups I and II: (a) intact uterus (uterus + placenta + pups), (b) uterus, (c) placenta, (d) ovaries, and (e) adrenal glands.

Results. Six animals in Group II were found to have incomplete hypophysectomies. Data for these rats were discarded. There was a significant difference ($p < 0.005$) in mammary gland DNA of pregnant control and Day 14 hypophysectomized animals on Day 20 of pregnancy (Table I). The average milligrams of DNA/100 g body weight was 7.08 for pregnant control and 6.11 for hypophysectomized animals. Values for total

TABLE I. Mammary Gland Nucleic Acid Content.

	No. of rats	Body wt[a] (g)	DFFT[b] (mg)	Total DNA (mg)	Total RNA (mg)	DNA (mg/100 g of body wt)	RNA/DNA
I (pregnant controls)	10	259	519 ± 12[c]	18.0 ± 0.38	17.8 ± 1.80	7.08 ± 0.10	0.98 ± 0.02
II (Day 14 hypophysectomized)	11	234	358 ± 5[c]	15.2 ± 0.63[c]	12.9 ± 0.31[c]	6.11 ± 0.24[d]	0.89 ± 0.06
III (virgin controls)	11	225	274 ± 12	4.57 ± 0.17	8.03 ± 0.22	1.93 ± 0.06	1.86 ± 0.08

[a] Corrected for weight of fetuses.
[b] Dry fat-free tissue.
[c] Mean and SE.
[d] Significantly lower than pregnant controls: $p < 0.005$; [c] $p < 0.001$.

DNA and total RNA were significantly ($p < 0.001$) reduced in hypophysectomized animals. There was no significant difference between the RNA:DNA ratios of the two groups. Mammary glands of virgin animals contained 1.93 mg DNA/100 g body weight; the RNA:DNA ratio was 1.86.

Wet weights of intact uterus, uterus, and placenta of pregnant control and hypophysectomized animals revealed no significant difference (Table II). The placenta and fetal membranes of the animals in control and hypophysectomized groups were observed to be well developed and intact at sacrifice. The mean value for adrenal wet weights of pregnant controls (65.53 mg) was significantly higher ($p < 0.001$) than the corresponding value (43.71 mg) for hypophysectomized animals. Also the mean value for ovarian wet weights of pregnant controls (120.58 mg) was significantly higher ($p < 0.001$) than the mean for ovarian wet weights of the hypophysectomized animals (96.8 mg).

Discussion. DNA was expressed as DNA/100 g body weight to eliminate any variation in mammary gland size due to body weight or connective tissue content of the gland. Connective tissue content has been demonstrated to remain constant throughout pregnancy and lactation (11), therefore, any change in DNA content in the mammary gland is interpreted as a change in parenchymal cell numbers. The fact that mammary gland total RNA and total DNA were proportionally reduced in hypophysectomized animals is reflected in the RNA:DNA ratios of the pregnant control and hypophysectomized groups. In this case the protein synthetic capacities of the mammary gland cells on Day 20 of pregnancy appear to be the same in the Day 14 hypophysectomized and pregnant control groups.

Data presented for DNA/100 g body weight and RNA:DNA ratios of mammary gland tissues are consistent with data previously published in the literature (12). Comparison of our data with this earlier data suggests that mammary gland proliferation stopped soon after operation in the Day 14 hypophysectomized rats. These findings are in contrast to prior reports, based on morphological studies, which contended that

TABLE II. Wet Weights of Organs from Normal and Hypophysectomized Pregnant Rats.

	Pregnant controls	Pregnant + Day 14 hypophysectomized
No. of rats:	10	11
Organs		
Intact uterus[a] (g)	55.33 ± 1.69[b]	52.09 ± 7.62
Uterus (g)	3.10 ± 0.09	3.11 ± 0.21
Placenta (g)	5.46 ± 0.21	5.13 ± 0.34
Ovaries (mg)	120.58 ± 4.65	96.80 ± 3.91[c]
Adrenals (mg)	69.53 ± 9.89	43.71 ± 2.25[c]

[a] Intact uterus includes uterus + pups + placenta.

[b] Mean and SE.

[c] Significantly lower than pregnant controls $p < 0.001$.

mammary gland proliferation continued in rats and mice which were hypophysectomized after midpregnancy (4–6).

There was no statistical difference between body weights of pregnant control and hypophysectomized animals. This seems to suggest that the differences observed in mammary gland DNA/100 g body weight between the two groups was, indeed, due to the effect of hypophysectomy and not to any decrease in food intake.

Failure of the mammary gland to involute following hypophysectomy after midpregnancy may be due to mammotropic influence from the placenta. Comparison of placental wet weights of experimental and pregnant control animals suggests that hypophysectomy on Day 14 of pregnancy had little effect on the development of the placenta. Similar findings have been reported for rats hypophysectomized on Day 12 and sacrificed on Day 20 of pregnancy (13). Whether this placental influence is capable of maintaining mammary gland proliferation in the absence of the anterior pituitary hormones is questionable, since potent mammotropic activity has been reported only in Day 12 placentae (14) and in Day 12 pregnant rat serum (15). Also of particular interest is that placental mammotropin is apparently structurally different from pituitary prolactin, since placental mammotropin demonstrates only weak stimulation of the pigeon crop-sac (14).

It remains possible that absence of continued mammary gland proliferation observed in our experimental animals could be due to a decrease in circulating growth promoting substances, since growth promoting activity has not been demonstrated in the rat placenta (7). Another possible explanation could be that normal synergistic relationships among the hormones of the anterior pituitary, ovaries and placenta have been disrupted. These aspects were not investigated.

Decline in the wet weights of the ovaries and adrenal glands of Day 14 hypophysectomized animals is offered as proof of the effectiveness of hypophysectomy. What effect these lower wet weights had on the fate of the mammary glands of the hypophysectomized animals is unclear.

Data presented strongly suggest that the anterior pituitary gland hormones are necessary for the continued development of the mammary glands, throughout pregnancy in the rat.

Summary. The DNA concentration of mammary glands of rats hypophysectomized on Day 14 and sacrificed on Day 20 of pregnancy was significantly lower than the DNA concentration of pregnant controls sacrificed on Day 20. The RNA:DNA ratios of the hypophysectomized and control groups were not significantly different. There was a significant decrease in the wet weights of ovaries and adrenal glands in hypophysectomized animals when compared to the weights of these organs in control animals.

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