Pituitary Growth Hormone and Luteinizing Hormone Content After Various Nursing Intensities* (33374)

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Prolactin (1, 2), adrenocorticotropic hormone (ACTH) (3), thyroid-stimulating hormone (4), and growth hormone (GH) (5) are decreased in the anterior pituitary after an overnight non-nursing period with subsequent acute nursing stimulation. Rats normally nurse their litters approximately every hour (6), and we have recently investigated the role of various intensities of chronic nursing stimulation in the secretion of prolactin and ACTH (7, 8). Increased chronic nursing increased pituitary prolactin, but ACTH remained relatively unchanged. The increased nursing intensity also increased mammary cell numbers (DNA) and secretory activity (RNA and litter weight gain). Since GH has been implicated in the regulation of lactation (9), the first objective of the present experiment was to relate changes in mammary nucleic acid content and litter weight gain with changes in GH content of pituitaries after various chronic nursing intensities.

Total gonadotropin content of pituitaries of castrated rats bearing ovarian autografts was reduced proportionally to the size of the nursing litter (10). Furthermore, luteinizing hormone (LH) content of rat pituitaries (11) and plasma (12) was reduced during lactation. Thus, a second objective of this study was to determine the LH content of pituitaries of rats subjected to various intensities of chronic nursing.

Materials and Methods. Litter size of primiparous Sprague-Dawley rats was adjusted to either 0, 2, 6, or 12 pups per mother rat on day 3 of lactation. All rats were maintained in individual cages for the duration of the experiment. Litter weight gain from day 7 to 16 after parturition was used as an index of lactational performance. Vaginal smears were recorded daily. All rats were decapitated on day 16 of lactation between 8 and 10 a.m. and anterior pituitary glands removed, weighed, and stored at —20°.

All pituitaries within groups were pooled (to provide sufficient tissue for the GH assay) and homogenized. The GH assay method of Greenspan *et al.* (13) was used except that two dose levels of pituitary homogenates (2 and 12 mg per rat per 4 days) were compared with two dose levels of NIH-GH-B9[1] (20 and 120 μg per rat). At least four and usually six hypophysectomized rats (Hormone Assay Labs, Chicago, Ill.) were used at each dose level. Pituitary LH content was estimated in duplicate at each of two dose levels (0.4 and 1.6 mg) by the ovarian ascorbic acid depletion method (14) and potency was estimated from two dose levels (0.4 and 1.6 μg) of NIH-LH-S9.[1] Five assay rats were used at each dose level. Potency, standard error of potency, and indices of precision were calculated according to the methods of Bliss (15).

At autopsy the six abdominal-inguinal mammary glands were removed and stored in 0.25 *M* sucrose at —20°. Nucleic acids were determined as previously described (16).

Results and Discussion. Increasing litter size from 0 to 2, 6, or 12 pups produced consecutive decreases in GH content of the anterior pituitary of 37, 67, and 82% respectively (Table I). The decrease in GH between the 0-pup and 12-pup nursing intensity was significant ($p<0.05$). Nursing intensity did not significantly ($p>0.05$) alter pituitary weight. In contrast to pituitary GH content, each increase in litter size resulted in increased ($p<0.01$) mammary DNA, RNA, and litter weight gain (Table II). These increases in mammary nucleic acids and in

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TABLE I. Growth Hormone (GH) and Luteinizing Hormone (LH) Content of Anterior Pituitaries of Rats Nursing Different Size Litters.

Litter size	No. rats	Av pituitary wt (mg)	Pituitary GH[a] (μg/mg)	Pituitary LH[a,b] (μg/mg)
0	20	9.92	45.0 ± 5.0	0.72 ± 0.20
2	19	10.34	28.4 ± 4.7	0.92 ± 0.19
6	20	9.42	14.8 ± 4.4	0.76 ± 0.20
12	21	10.55	7.9 ± 4.3[c]	0.60 ± 0.20

[a] Mean potency ± standard error of assay. Standards for GH and LH assays were NIH-GH-B9 and NIH-LH-S9 respectively. Average lambdas of GH and LH assays were 0.38 and 0.17 respectively.

[b] Means not significantly different ($p>0.05$).

[c] Significantly less ($p<0.05$) than 0-pup nursing intensity group.

litter weight gains with more intense nursing support our previous observations (7). These data alone do not allow one to determine whether amounts of GH in the pituitary reflect increased release or decreased synthesis of the hormone because exogenous administration of GH does not stimulate litter weight gain in rats (9). But the evidence that acute suckling increased release of GH from the pituitary (5) implies that the first alternative is the correct one for the present study.

Rats weaned on day 3 of lactation began their estrous cycles an average of 6.7 days later (range 3 to 11 days). The distribution of intervals from weaning to first estrus was biphasic with averages of 4.7 and 10.2 days. This biphasic distribution of the interval from weaning to first estrus is similar to that observed by Rothchild (10). On day 16 of lactation 4, 4, 3, and 9 mother rats that had been weaned on day 3 were in proestrus, estrus, metestrus, and diestrus respectively. Thus, the number of animals in each stage was close to that expected for rats with 5-day estrous cycles. Ten of 20 rats nursing 2 pups commenced to cycle on days 15 and 16 of lactation. At day 16 of lactation 2, 6, and 12 rats were in proestrus, estrus, and diestrus respectively. Rats nursing 6 or 12 pups never began estrous cycles before day 16 of lactation.

Pituitary LH content did not differ significantly ($p>0.05$) among the various nursing intensity regimens (Table I). There was a tendency, however, for the pituitary LH of the lactating rats to decrease with increasing nursing intensity. No direct conclusions concerning the release of LH from the pituitary can be drawn between the rats that had or had not begun estrous cycles because assays were conducted on pituitaries pooled across all stages of the estrous cycle of the 0-and 2-pup nursing intensities. Minaguchi and Meites (11) reported that suckling decreased pituitary LH compared with normally cycling controls, whereas we observed no significant difference in pituitary LH between lactating rats and rats that had been weaned on day 3 of lactation and subsequently com-

TABLE II. Nucleic Acid Content of Mammary Glands and Litter Weight Gain of Rats Nursing Different Size Litters.

Litter size	No. rats	Total DNA[a] (mg)	Total RNA[a] (mg)	Litter wt. gain[a] (g)
0	12	6.2 ± 1.0	7.5 ± 0.8	—
2	19	19.6 ± 0.8	67.1 ± 4.8	32.3 ± 3.2
6	20	23.0 ± 0.7	116.7 ± 6.2	75.5 ± 6.3
12	21	26.2 ± 1.0	138.4 ± 5.2	118.0 ± 7.0

[a] Mean and standard error of mean. All means significantly different ($p<0.01$) from each other.

menced their estrous cycles. In addition to the type of controls used in the two experiments, there may have been differences in stages of the estrous cycle at the time of killing of the rats.

Summary. Increasing the litter size of rats from 0 to 2, 6, or 12 pups progressively decreased ($p<0.05$) pituitary growth hormone (GH) content, whereas mammary development (DNA) and metabolic activity (RNA and litter weight gain) progressively increased ($p<0.01$). Nursing intensity did not significantly alter ($p>0.05$) LH content of the pituitary, although LH tended to decrease in the lactating rats with increasing nursing intensity. All non-nursed and one-half of the 2-pup nursing intensity group began their estrous cycles before day 16 of lactation, but rats nursing 6 or 12 pups did not.

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Bilirubin Production in Endotoxin-Treated or Tumor-Bearing Rats (33375)

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Treatment of rats with endotoxins has been used in this laboratory as a model system for studying abnormalities in the metabolism of iron similar to those that occur during tumor growth and various infections (1–3). These investigations have indicated that the decrease in the concentration of plasma iron after the injection of endotoxin is primarily due to an inhibited return of iron to the plasma from recently destroyed erythrocytes. Such a block in the release of iron from the reticuloendothelial system (RES) has been suggested to contribute to the decrease in plasma iron observed in dogs with sterile inflammations (4) and in patients with cancer (5).

The mechanism by which iron from recently destroyed erythrocytes was prevented from returning to the plasma is not known. If the block occurs at some point prior to the release of iron from heme, then this iron might also be prevented from entering the ferritin storage pool. Although endotoxin has been shown to favor the movement of iron from