Influence of Acute and Chronic Suckling on Plasma Corticosterone and Mammary Nucleic Acids[1] (35317)

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(Introduced by H. D. Hafs)
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The stimulus of suckling or milking affects the rate of release of several homones including adrenocorticotropin (ACTH) (1). Based on observations of thymus involution during intense suckling in rats, Gregoire (2) suggested that stimuli generated by suckling promote the release of ACTH. This hypothesis was confirmed by Denamur *et al.* (3), who demonstrated a marked depletion of pituitary ACTH in goats and sheep 30 min after milking. More recently Voogt *et al.* (4) demonstrated that 0.5 hr of suckling following 12 hr nonsuckling resulted in decreased pituitary ACTH and increased plasma corticosterone in rats.

Rats suckle their young almost continually during early lactation and accumulation of milk during periods of nonsuckling could adversely affect milk secretion. Yokoyana and Ota (5) reported that daily removal of milk from the mammary gland by suckling is essential for maintenance of alveolar cell secretory activity. They concluded that mammary engorgement might play a role in retarding secretory activity. McNaught (6) demonstrated decreased metabolic activity of the mammary gland of rats 8 hr after removal of their litters.

The objective of the present investigation was to evaluate the acute and chronic effects of suckling on adrenal function and mammary nucleic acids.

Materials and Methods. Sixty primiparous Sprague-Dawley rats[2] were housed in individual cages under controlled temperature (70 ± 5°F) and lighting (14 hr light daily). On the second day of lactation the thoracic teats were ligated and rats were assigned at random to a 2 × 3 factorial arrangement of treatments in a completely randomized design. The factorial arrangement consisted of two litter sizes and three suckling regimes. Litter size was adjusted to 2 or 6 pups on day 2 of lactation. On day 7 of lactation litters were either: (i) retained with their mother; (ii) isolated for 12.5 hr; or (iii) isolated for 12.0 hr then reunited with their mother for 0.5 hr of suckling. Litters in the latter group were weighed following isolation and again after the suckling period. We used only litters that had successfully suckled as evidenced by an increase in litter weight and milk in the litters' stomachs.

Following the treatment period, all rats were sacrificed by decapitation and the trunk blood was collected in 12 × 75-mm tubes containing 0.5-mg of ethylenediaminetetraacetic acid. Blood plasma was obtained by centrifugation (2500*g* at 4°) and assayed for corticosterone by the fluorometic technique [Silber *et al.* (7)], modified by Thatcher (8). The mammary gland, ovary, adrenal, and anterior pituitary were removed and weighed. Adrenal glands were immediately homogenized in cold 2.5% metaphosphoric acid and stored at −20° until assayed for ascorbic acid (9). Mammary glands were stored in 0.25 *M* sucrose at −20° until analyzed for nucleic acid content (10).

Results. Orthogonal contrasts revealed no significant effect ($p>0.05$) of litter size or acute suckling on body or anterior pituitary weight (Table I). Altering the suckling period on day 7 of lactation did not significantly ($p>0.05$) change average weight of ovaries from rats suckling either 2 or 6 pup litters. Therefore, ovarian weight within litter size was averaged across suckling periods. The

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[2] Obtained from Spartan Research Laboratories, Haslett, Michigan.

TABLE I. Influence of Number of Young and Suckling Stimulus on Body, Anterior Pituitary (AP) and Ovarian Weight.[a]

Litter size	No. of rats	Suckling period		Wt		
		Nonsuckling	Suckling	Body (g)	AP (mg)	Ovaries (mg)
2	10	0.0	12.5	229.8 ± 4.7	10.2 ± 0.3	77.6 ± 2.6
	10	12.5	0.0	222.5 ± 6.7	9.2 ± 0.3	72.0 ± 3.1
	10	12.0	0.5	232.6 ± 4.6	9.5 ± 0.4	84.7 ± 4.0
Av				228.3 ± 3.1	9.6 ± 0.2	78.1 ± 2.1[b]
6	10	0.0	12.5	221.1 ± 3.8	9.7 ± 0.4	64.7 ± 3.2
	10	12.5	0.0	234.8 ± 4.8	10.0 ± 0.4	71.6 ± 4.0
	10	12.0	0.5	238.9 ± 4.6	10.8 ± 0.4	74.6 ± 4.1
Av				231.6 ± 3.7	10.1 ± 0.2	70.3 ± 2.2

[a] Values are means ± SE for 10 rats.

[b] Greater than the comparable av for 6 pups ($p < 0.01$); interaction of litter size and suckling period not significant ($p > 0.05$).

ovaries of rats suckling 2 pups averaged 78.1 mg, which was significantly greater ($p<0.05$) than the comparable average (70.3 mg) of rats suckling 6 pups (Table I).

Restriction of litter size to 2 or 6 pups did not significantly ($p>0.05$) influence adrenal weight by day 8 of lactation (Table II). But within the group with 6 pups/litter, the average adrenal weight of rats from which litters were isolated for 12.5 hr was significantly less than the comparable average for rats subjected to 0.5 hr of suckling, after litter isolation for 12.0 hr (Table II). Plasma corticosterone of rats suckling 2 or 6 pups averaged 27.5 and 39.5 μg/100 ml, respectively, and the difference was significant ($p<0.05$). Isolation of 2- or 6-pup litters for 12.5 hr reduced plasma corticosterone 17.4 and 31.3%, respectively, relative to constantly suckled controls. However, only the latter value was significant ($p<0.05$). Twelve hr of isolation followed by 0.5 hr of suckling by 2 or 6 pups increased ($p<0.01$) plasma corticosterone 100.5 and 334.0%, respectively, relative to corresponding nonsuckled control values. The error variances associated with mean plasma corticosterone values for the several treatments were heterogenous ($p<0.05$). Here,

TABLE II. Effect of Suckling on Plasma Corticosterone (PC) and Adrenal Ascorbic Acid (AAA) Content of Rats Suckling 2 or 6 Pups.[a]

Litter size	No. of rats	Suckling period		Adrenal wt (pair) (mg)	PC (μg/100 ml)	AAA (μg/100 mg)
		Nonsuckling	Suckling			
2	10	0.0	12.5	58.1 ± 2.0	23.8 ± 3.4	493.5 ± 28.7
	10	12.5	0.0	56.2 ± 1.9	19.7 ± 1.8[c]	491.0 ± 29.0
	10	12.0	0.5	59.7 ± 2.6	39.4 ± 8.8	482.6 ± 35.4
Av				58.0 ± 1.2	27.5 ± 3.5[d]	489.0 ± 17.3
6	10	0.0	12.5	61.2 ± 2.0	29.3 ± 3.6	495.2 ± 30.8
	10	12.5	0.0	54.8 ± 2.2[b]	20.2 ± 2.7[e]	468.4 ± 30.4
	10	12.0	0.5	65.1 ± 2.4	70.0 ±14.9	440.9 ± 28.9
Av				60.4 ± 1.4	39.5 ± 6.6	468.1 ± 17.2

[a] Values are means ± SE for 10 rats.

[b] Less than 65.1 ($p < 0.05$).

[c] Less than 39.4 ($p < 0.01$).

[d] Greater than comparable av for 6-pup group ($p < 0.01$).

[e] Less than 29.3 ($p < 0.05$) and 70.0 ($p < 0.01$).

TABLE III. Effect of Suckling on Mammary Weight and Nucleic Acid Content.[a]

Litter size	No. of rats	Suckling period: Non-suckling	Suckling period: Suckling	Mammary wt (g)	Total (mg): DNA	Total (mg): RNA	RNA/DNA
2	10	0.0	12.5	7.0 ± 0.3	14.3 ± 1.3	33.9 ± 4.7	2.3 ± 0.2
	10	12.5	0.0	8.1 ± 0.5	12.3 ± 1.1	29.1 ± 3.4	2.4 ± 0.1
	10	12.0	0.5	7.6 ± 0.4	13.7 ± 1.3	28.9 ± 3.8	2.2 ± 0.2
	Av			7.6 ± 0.2[b]	13.4 ± 0.7[b]	30.6 ± 2.3[b]	2.3 ± 0.1[b]
6	10	0.0	12.5	8.4 ± 0.3[c]	22.2 ± 1.3	82.9 ± 5.8[d]	3.8 ± 0.3[e]
	10	12.5	0.0	15.8 ± 0.7	19.9 ± 0.4	62.0 ± 3.2	2.1 ± 0.1
	10	12.0	0.5	13.6 ± 0.4	19.6 ± 1.4	63.7 ± 1.4	3.2 ± 0.1
	Av			12.6 ± 0.7	20.6 ± 0.6	69.5 ± 3.4	3.4 ± 0.1

[a] Values are means ± SE for 10 rats.
[b] Less than comparable av for 6-pup group ($p < 0.01$).
[c] Less than 15.8 and 13.6 ($p < 0.01$).
[d] Greater than 62.0 and 63.7 ($p < 0.01$).
[e] Greater than 2.1 and 3.2 ($p < 0.05$).

heterogeneity of variance resulted from an increased variance associated with the means representing plasma corticosterone levels of rats suckling their young after 12.0 hr of isolation (see standard errors, Table II). Adrenal ascorbic acid content was not affected significantly ($p>0.05$) by litter size or acute suckling.

The average mammary weight of rats suckling 6-pup litters was greater ($p<0.01$) than the comparable average of rats with 2-pup litters (Table III). Removal of litters for 12.5 hr resulted in an accumulation of milk which caused increased mammary weight. But this increase was significant ($p<0.01$) only in rats suckling 6 pups. Milk removal by 2 or 6 pups during 0.5 hr suckling decreased mammary weight relative to the nonsuckled controls, but in neither case was the decrease significant ($p>0.05$). Adjusting litter size to 2 or 6 markedly influenced mammary nucleic acids by day 8 of lactation. Average mammary DNA, RNA, and RNA/DNA of rats with 6-pup litters were greater ($p<0.01$) than the corresponding averages of rats that nursed 2 pups. Within litter size groups, the acute suckling regime did not affect ($p>0.05$) total mammary DNA. The effect of acute suckling on mammary RNA and RNA/DNA ratio was dependent upon the number of pups nursed. Acute suckling did not influence total mammary RNA or RNA/DNA ratio of rats suckling 2-pup litters. In contrast, when rats nursing 6 pups had their litters removed for 12.5 hr, average mammary RNA decreased 25.1% and RNA/DNA decreased 18.5% relative to the comparable averages of constantly nursed controls. Reuniting litters (6 pups) with their mothers for 0.5 hr did not restore mammary RNA and RNA/DNA values to those of constantly suckled controls.

Discussion. The ability of the suckling stimulus to decrease ovarian weight has been previously reported (11, 12). Rothchild (11) suggested that reduced ovarian weight during lactation in the rat may result from an inhibitory effect of suckling generated stimuli on gonadotropin release.

Although chronic suckling influenced plasma corticosterone levels, the effect of acute suckling was more marked. Removal of litters for 12.5 hr decreased plasma corticosterone but not adrenal ascorbic acid of rats with 6- but not 2-pup litters. These results suggest that maintenance of plasma corticosterone at levels characteristic of lactating rats requires suckling more frequently than once every 12.5 hr. Allowing 0.5 hr of suckling after 12.0 hr of litter isolation resulted in a 1- and 3-fold increase in plasma corticosterone in rats suckling 2- and 6-pup litters, respectively.

Voogt *et al.* (4) reported a fourfold increase in plasma corticosterone by 0.5 hr of suck-

ling following 12.0 hr of nonsuckling. Heterogeneity of variance associated with mean plasma corticosterone values resulted from a failure of 4 of the 20 rats to respond to 0.5 hr of suckling. In this regard, the increase in plasma corticosterone following 0.5 hr of suckling is probably underestimated. The increase in plasma corticosterone due to 0.5 hr of suckling was dependent on the number of suckling young in the litter. The design of this experiment did not allow us to evaluate whether the effect of litter size on the plasma corticosterone peak was chronic due to preconditioning of the adrenal–pituitary axis between days 2 and 8 of lactation or acute due to the intensity of the suckling stimulus only during 0.5 hr of suckling.

Isolation of litters for 12.5 hr resulted in a significant increase in mammary weight due to milk accumulation in the glands of rats suckling 6 but not 2 pups. Similarly, 12.5 hr of nonsuckling resulted in a significant depression in the protein synthetic activity (RNA and RNA/DNA) of the mammary gland of rats nursing 6 but not 2 pups. Possibly the mammary engorgement of rats suckling 6-pup litters caused the decline in mammary secretory activity as suggested by Cross and Silver (13). Other workers (5, 6) have reported the detrimental effects of milk engorgement on mammary respiratory activity. Mammary DNA was not affected by acute suckling.

Summary. Thoracic teats of rats were ligated and litters were adjusted to 2 or 6 pups on day 2 of lactation. Rats were constantly with their litters until day 7 of lactation when they were subjected to: (i) continued suckling; (ii) 12.5 hr nonsuckling; or (iii) 12.0 hr nonsuckling then 0.5 hr suckling.

Plasma corticosterone (μg/100 ml) of rats suckling 2 or 6 pups was 23.83 and 29.31, respectively, when litters suckled continuously. It was reduced to 19.75 and 20.15, respectively, following 12.5 hr of nonsuckling. Following 12 hr nonsuckling, 0.5 hr of suckling increased plasma corticosterone to 39.44 and 70.01 μg/100 ml in rats with 2 and 6 pups, respectively. Corticosterone released in response to suckling appeared to be related to intensity of the suckling stimulus.

Rats suckling 6 pups had more mammary ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and a greater RNA/DNA ratio than rats with 2 pups. Mammary gland RNA and RNA/DNA ratio decreased after 12.5 hr nonsuckling and mammary weight increased 88.1 and 15.7%, respectively. These results suggest that the accumulation of milk can directly supress the protein synthesizing capacity of the mammary gland.

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