Adrenal Function During Prolonged Lactation[1] (34909)

W. W. THATCHER[2] AND H. A. TUCKER

Animal Reproduction Laboratory, Department of Dairy, Michigan State University, East Lansing, Michigan 48823

Adrenal secretions may be limiting to milk synthesis during advanced lactation in the rat. This view is supported by previous reports from our laboratory which suggest that pituitary ACTH declines during advanced lactation (1) and glucocorticoid supplementation (2, 3) at this time, will prevent declines in mammary gland nucleic acid and litter weight gain. Various indirect measurements such as adrenal weight (4), cholesterol (5), ascorbic acid concentration (6), and peripheral plasma levels of corticoids (7) have been used to estimate changes in adrenal cortical function in lactating rats. The objective of the present study was to characterize directly changes in adrenal cortical function with advancing lactation and to determine if these changes are associated with lactational performance. Resting levels of plasma and adrenal corticosterone and plasma corticoid binding globulin (CBG) activity were measured in rats killed at 16, 24, and 32 days of lactation. Changes in adrenal activity were compared with milk production and mammary gland nucleic acid measurements.

Materials and Methods. Primiparous Sprague-Dawley rats were maintained at 24° with 14 hr of light daily. On day 3 of lactation, the rats were weighed, thoracic teats were ligated and litter size was adjusted to six. At this time rats were assigned to one of three groups containing 20 rats each to be killed either on day 16, 24, or 32 of lactation. At day 16 and again on day 24 of lactation 8-day-old replacement litters were presented to mother rats to maintain an intense suckling stimulus. Litter weights were recorded at days 8 and 16 of age for all litters. Cumulative litter weight gains were calculated from days 8 to 16, days 16 to 24 and from days 24 to 32 of lactation. Final body weights of lactating rats were recorded at time of killing, and 6 abdominal-inguinal mammary glands were analyzed for nucleic acid content as previously described (8).

Routine maintenance of the rat room and experimental manipulations were carried out between the hours of 1 and 4 p.m. daily. All experimental animals were killed, between 8:00 and 8:30 a.m., by decapitation within 10 sec from the time the cage was first handled. Systemic blood was collected in heparinized beakers from the decapitated trunk, centrifuged, and the plasma was assayed for corticosterone by the fluorometric technique of Silber *et al.* (9) as modified by Guillemin *et al.* (10). Adrenal glands were trimmed and frozen in 2 ml of 0.85% NaCl in 20% ethanol until assayed for corticosterone by the fluorometric technique of Moncloa *et al.* (11). Estimates of CBG activity as described by Doe *et al.* (12) were made on plasma samples pooled within treatments.

An additional group of 20 virgin rats was killed in metestrus to determine if the experimental procedures resulted in resting levels of corticosterone and to compare the levels in nonlactating rats with those of lactating rats.

Results. The cumulative litter weight gain (LWG) for the 8 days prior to sacrifice was less ($p<0.01$) in the 32-day treatment group than the LWG responses of the 16- and 24-day treatment groups (Table I). Total mammary gland DNA did not change significantly ($p>0.05$) between day 16 (32.2 mg)

[1] Michigan Agricultural Experiment Station Journal Article No. 5033. This research was supported in part by U. S. Public Health Service Grant AM-09200.

[2] NIH predoctoral fellow (1-F-GM-39, 138-01). Present address: Department of Dairy Science, University of Florida, Gainesville, Florida 32601.

TABLE I. Nucleic Acid Content of Mammary Glands and Litter Weight Gain of Intensely[a] Suckled Rats During Extended Lactation.

	Days of lactation[b]		
	16	24	32
Litter wt gain (g)[c]	79.3 ± 6.4	82.7 ± 3.9	30.6 ± 2.9
Total DNA (mg)	32.2 ± 1.2	31.0 ± 1.0	25.5 ± 0.9
Total RNA (mg)	172.9 ± 9.4	140.5 ± 8.6	92.7 ± 5.7
RNA/DNA	5.3 ± 0.1	4.5 ± 0.2	3.6 ± 0.2

[a] Litters 16 days old replaced with 8-day-old foster litters.
[b] Mean and SE of mean.
[c] Cumulative litter weight gains were recorded between days 8 and 16 for all litters.

and 24 (31.0 mg), but declined ($p<0.01$) to 25.5 mg by day 32 of lactation. Mammary RNA content declined linearly ($p<0.01$) from days 16 to 32 of lactation. Mammary RNA/DNA ratios were 5.3, 4.5, and 3.6, respectively. The changes in ratio paralleled very closely total RNA changes. These results indicated that lactational performance declined during the advancing stages of lactation.

A linear decline ($p<0.01$) in corticosterone content per two adrenals (Table II) was observed between day 16 (2.0 μg), day 24 (1.7 μg) and day 32 (1.4 μg) of lactation. The adrenal corticosterone content at day 32 of lactation was not significantly different ($p>0.05$) from that found in adrenals from virgin rats (1.2 μg). A linear decline ($p<0.05$) was detected in adrenal corticosterone concentration from 3.4 μg at day 16 to 2.6 μg on day 32 of lactation. Adrenal weights did not change significantly ($p>0.05$) with advancing stages of lactation.

In contrast, peripheral plasma levels of corticosterone (Table II) did not reflect the decrease in either adrenal corticosterone content or concentration. Plasma corticosterone concentrations (μg/100 ml) were not significantly different ($p>0.05$) among stages of lactation at day 16 (18.8), day 24 (18.3), and day 32 (16.0) of lactation. The quantities of corticosterone bound to CBG per 100 ml of rat plasma were 63, 58, and 75 μg at day 16, 24, and 32 of lactation, respectively. Since all measurements of CBG activity were determined on pooled plasma samples within treatments, there is no estimate of variation among animals within treatments.

The correlation coefficients of adrenal corticosterone content with mammary nucleic acids and LWG are summarized in Table III. The within stage correlation coefficients between total adrenal corticosterone content and mammary gland RNA (0.60), DNA

TABLE II. Adrenal and Plasma Corticosterone Levels, Plasma Corticosteroid Binding Globulin Activity and Adrenal Weights in Intensely[a] Suckled Rats During Extended Lactation.

		Days of lactation[b]		
	Virgin rats[b]	16	24	32
Corticosterone				
(μg/2 adrenals)	1.2 ± 0.1	2.0 ± 0.3	1.7 ± 0.1	1.4 ± 0.1
(μg/100 mg of adrenal)	2.2 ± 0.1	3.4 ± 0.4	3.0 ± 0.2	2.6 ± 0.2
(μg/100 ml of plasma)	10.6 ± 0.9	18.8 ± 3.0	18.3 ± 1.7	16.0 ± 1.6
(μg bound/100 ml of plasma)[c]	—	63	58	75
Adrenal wt (mg)	49.9 ± 2.2	57.2 ± 2.0	54.3 ± 1.5	53.2 ± 1.8

[a] Litters 16 days old replaced with 8-day-old foster litters.
[b] Mean and SE of mean.
[c] Pooled plasma samples within treatments.

TABLE III. Correlation Coefficients of Mammary Gland Nucleic Acid Content and Litter Weight Gains with Adrenal Corticosterone Content of Intensely[a] Suckled Rats During Extended Lactation.

Stage of lactation	Correlation coefficients		
	RNA	DNA	LWG
16	0.60[b]	0.59[b]	0.44[c]
24	0.26	0.11	—0.02
32	0.43	0.29	0.35
Pooled within stage of lactation	0.45[b]	0.35[b]	0.27
Total correlation	0.55[b]	0.48[b]	0.41[b]

[a] Litters 16 days old replaced with 8-day-old foster litters.

[b] Correlation coefficients significantly different from zero ($p < 0.01$); [c] ($p < 0.05$).

(0.59), and LWG (0.44) were significant ($p<0.01$) at day 16 of lactation. Pooled within stage of lactation correlation coefficients were 0.45 ($p<0.01$) for RNA and 0.35 ($p<0.01$) for DNA. The total correlations for RNA, DNA, and LWG, disregarding stage of lactation, were 0.55, 0.48, and 0.41, respectively ($p<0.01$).

Discussion. The mean resting level of plasma corticosterone (10.6 μg/100 ml) for virgin rats killed at metestrus was in close agreement with resting levels reported by Guillemin *et al.* (13). These results suggested that the management procedures used in this experiment resulted in "resting" levels of plasma corticosterone.

Holzbauer (14) observed that the quantity of adrenal cortical hormones in the adrenal gland was related to the secretory activity of the adrenal. Consequently, the results of the present study suggested that corticosterone secretion increases markedly from the nonlactating state to early lactation. However, as lactation advanced, the secretion rates of corticosterone declined to the point at 32 days where they were comparable to those found at metestrus.

During lactation the resting levels of plasma corticosterone were about 62% greater than levels at metestrus. This agrees with other reports (7). However, there were no significant changes in peripheral plasma levels of corticosterone that reflected a decrease in adrenal secretion rate as lactation advanced. If the adrenal secretion rate of corticosterone is greater at day 16 of lactation, then the increased amount of secreted corticosterone may be utilized by the body target tissues. The mammary gland is a tissue that has an apparent corticoid requirement to initiate and maintain lactation (2, 15, 16). Consequently, the mammary tissue (as well as other tissues) may utilize the additional corticoid secreted at the peak of lactation. This may explain why we failed to detect a significant decrease in circulating levels of corticosterone with advancing lactation.

The within-stage correlation coefficients between total adrenal corticosterone content and mammary gland RNA, DNA, and LWG were largest at day 16 of lactation, a time when adrenal corticosterone content was greatest and the mammary gland was stimulated maximally. Decreased adrenal secretion at days 24 and 32 of lactation may be associated with a general decrease in the responsiveness of the mammary tissue. Such a decrease in mammary gland responsiveness may account for the absence of significant within-stage correlations between adrenal corticosterone content and the mammary gland traits measured at these two stages of lactation. Since the pooled and total correlations were significant, the declines in adrenal corticosterone content may be related to the decrease in mammary gland function as lactation advanced.

Gala and Westphal (17) reported that CBG activity is depressed during lactation, but at four days postweaning it rises to a level comparable to that found in the virgin rat. In the present experiment, higher CBG activity at day 32 of lactation, as compared with days 16 or 24, may reflect an increase in binding activity during advanced lactation. If the CBG activity is greater at day 32 of lactation, then the amount of free plasma corticosterone may be less regardless of the peripheral blood levels.

Adrenal cortical secretions may be rate limiting to milk synthesis during prolonged lactation because pituitary ACTH (1) and adrenal corticosterone content decrease during this time. Furthermore, CBG activity of

the plasma increases, (although total circulating levels of corticosterone did not decrease significantly) and declines in lactational performance can be prevented with exogenous corticoids (2, 3).

Summary. Adrenal corticosterone content and concentration decreased linearly from days 16 to 32 of lactation. Peripheral plasma concentrations of corticosterone in lactating rats were higher than the resting levels measured in virgin rats. However, corticosterone concentrations in plasma did not change significantly between days 16 and 32 of lactation. Rats killed at day 32 of lactation had a greater plasma corticoid binding globulin (CBG) activity than rats killed at days 16 and 24. Adrenal corticosterone content was correlated significantly with mammary gland RNA, DNA, and litter weight gain. The data support the concept that adrenal secretions may be rate limiting to milk synthesis during prolonged lactation.

1. Thatcher, W. W., and Tucker, H. A., Proc. Soc. Exp. Biol. Med. **128**, 46 (1968).

2. Thatcher, W. W., and Tucker, H. A., Endocrinology **86**, 237 (1970).

3. Thatcher, W. W., and Tucker, H. A., Proc. Soc. Exp. Biol. Med. **134**, 705 (1970).

4. Bearn, J. C., Gould, R. P., and Jones, H. E. H., Acta Anat. **41**, 273 (1960).

5. Poulton, B. R., and Reece, R. P., Endocrinology **61**, 217 (1957).

6. Anderson, R. R., and Turner, C. W., Endocrinology **70**, 796 (1962).

7. Voogt, J. L., Sar, M., and Meites, J., Amer. J. Physiol. **216**, 655 (1969).

8. Tucker, H. A., Proc. Soc. Exp. Biol. Med. **116**, 218 (1964).

9. Silber, R. H., Busch, R. D., and Oslapas, R., Clin. Chem. **4**, 278 (1958).

10. Guillemin, R. G., Clayton, G. W., Lipscomb, H. S., and Smith, J. D., J. Lab. Clin. Med. **53**, 830 (1959).

11. Moncloa, F., Peron, F. G., and Dorfman, R. I., Endocrinology **65**, 717 (1959).

12. Doe, R. P., Fernandez, R., and Seal, U. S., J. Clin. Endocrinol. **24**, 1029 (1964).

13. Guillemin, R., Clayton, G. W., Smith, J. D., and Lipscomb, H. S., Endocrinology **63**, 349 (1958).

14. Holzbauer, M., J. Physiol. (London) **139**, 294 (1957).

15. Meites, J., Hopkins, T. F., and Talwalker, P. K., Endocrinology **73**, 261 (1963).

16. Tucker, H. A., and Meites, J., J. Dairy Sci. **48**, 403 (1965).

17. Gala, R. R., and Westphal, U., Endocrinology **76**, 1079 (1965).

Received Mar. 31, 1970. P.S.E.B.M., 1970, Vol. 134.