red cells of mildly plethoric mice without producing a significant alteration in plasma iron. These findings indicate that ACTH, testosterone and T_3 stimulate erythropoiesis indirectly by increasing the elaboration of endogenous erythropoietin, or else they require a certain level of erythropoietin in the recipient animal for their erythropoietic effects.

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Relationship Among Nursing Frequency, Lactation Pituitary Prolactin, and Adrenocorticotropic Hormone Content in Rats.* (32376)

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Maintenance of mammary structure and synthesis during lactation is dependent on frequent nursing(1). Adjusting litter size to higher numbers caused significant progressive increases in mammary deoxyribonucleic acid (DNA) totaling more than 100% during the first 16 days of lactation, whereas low nursing intensities did not significantly change mammary DNA content. Greater nursing intensities correspondingly increased ribonucleic acid (RNA) and RNA/DNA values, which suggested that the mammary cells produced during lactation were functional.

Short-term suckling following a 12-hour non-nursing period caused prolactin release (2,3) which was not fully restored to pre-

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nursing levels 9 hours later(3). However, rats normally nurse their litters approximately every hour(4), thus the role of nursing in causing prolactin release under normal circumstances is not known. Recently, we have shown that increasing chronic nursing stimuli from 2 to 6 pups for a period of 2 weeks increased pituitary prolactin potency 81%, but pituitary adrenocorticotropic hormone (ACTH) potency declined 19%(5). However none of the pituitary hormone potency changes was statistically significant ($P>0.05$).

The large variation in prolactin and ACTH potency may have been caused, in part, by the fact that no attempt was made to regulate the interval between last nursing and removal of pituitary for hormone assay. The purpose of the present study was to determine the effects of known specific nursing frequencies applied for an 8-day period on mammary nucleic acids, pituitary prolactin and ACTH.

Materials and methods. Rats of the Sprague-Dawley strain were maintained at 24 ± 1°C and subjected to illumination between 5 a.m. and 7 p.m. Thoracic teats of rats were ligated and litter size adjusted to 6 pups on day 3 of lactation. Starting on day 8 of lactation 5 groups of rats were either weaned (non-nursed group), allowed access to litters one, two or four times (×) daily, or allowed *ad libitum* nursing. Nursing times for the 1×, 2× and 4× groups were equally spaced at 5 a.m., 11 a.m., 5 p.m. and 11 p.m. The nursing period was 1 hour and litter weights before and after each nursing period was recorded. Four mother rats suckled 1× per day each shared one litter and 2 mother rats suckled 2× per day each shared one litter. The 4× mothers were each nursed by a single litter. Thus, each litter was allowed 4 nursing periods daily, but at any given time ¼, ½ and all of the 1×, 2× and 4× lactating mother rats, respectively, were nursed. The total number of nursing periods between day 8 and 16 of lactation for the mother rats was 8, 16, and 32 for the 1×, 2×, and 4× groups, respectively. If a litter lost weight for 3 consecutive days a new litter was substituted. All lactating rats were decapitated 5 hours after the last nursing period on day 16. The last nursing period for 1×, 2×, and 4× groups was determined by time of day the litter was first removed on day 8 whereas one-fourth of *ad libitum* group litters were removed at each of those times listed above. One-fourth of the non-nursed group was decapitated 8 days after weaning at either 5 a.m., 11 a.m., 5 p.m., or 11 p.m.

At autopsy, anterior pituitaries were removed, weighed and stored at —20°C. Anterior pituitaries were pooled within nursing intensity in groups of 3 according to time of decapitation. Prolactin potency was assayed in White King pigeons by the method of Reece and Turner(2). Low doses of pituitary homogenate (1.0 mg) and standard NIH-P-S_6 ovine prolactin (1.0 μg)† were injected into right and left crop sac areas, respectively, of 8 birds, and high doses of pituitary homogenates (4.0 mg) and standard prolactin (4.0 μg) were injected similarly into 8 additional birds.

ACTH potency was estimated by the methof of Saffran and Schally(6). Each pooled pituitary homogenate was assayed twice at 2 dose levels (0.033 and 0.133 mg) and potency was estimated from 2 dose levels (0.003 and 0.012 units) of porcine ACTH‡ with a stated potency of 103 units per mg. Potency, standard error of potency and lambdas were calculated according to the method of Bliss(7).

Six abdominal-inguinal mammary glands were removed at autopsy, trimmed, weighed, and stored in 0.25 M sucrose at —20°C. Nucleic acid determinations were made as previously described(8).

Results and discussion. Non-nursed, 1×, 2×, 4× and *ad libitum* nursed rats averaged 6.27, 8.43, 18.50, 24.98 and 26.92 mg total mammary DNA, respectively. RNA averaged 9.64, 28.40, 77.72, 155.32, and 203.32 mg, respectively, and RNA/DNA ratio averaged 1.56, 3.84, 4.16, 6.18 and 7.56, respectively (Table I). The 4× and *ad libitum* groups contained significantly more ($P<0.01$) DNA than lower intensity groups, but were

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† Ovine prolactin (NIH-P-S_6) was supplied by Nat. Inst. Health.

‡ Porcine ACTH was obtained from Mann Research Laboratories, Inc., New York City.

TABLE I. Nucleic Acid Content of Mammary Glands of Lactating Rats Nursed at Different Frequencies.

Daily nursing frequency	No. rats	Avg body wt (g)	Avg fresh gland wt (g)	Total DNA (mg)*	Total RNA (mg)*†	RNA/DNA*
Non-nursed	11	251 ± 7.4	3.577 ± .208	6.27 ± .66‡	9.64 ± .96	1.56 ± .06‖
1×	12	243 ± 4.6	4.102 ± .216	8.43 ± .88	28.40 ± 3.62	3.84 ± .64§
2×	12	244 ± 8.4	8.696 ± .503	18.50 ± .67§	77.72 ± 5.24	4.16 ± .17§
4×	12	265 ± 4.3	14.557 ± .869	24.98 ±1.18	155.32 ±12.86	6.18 ± .36¶
Ad libitum	12	251 ± 6.8	16.884 ± .803	26.92 ± .75	203.32 ± 7.72	7.56 ± .20

* Mean and standard error of mean.
† All means significantly different from each other ($P<.01$).
‡ Significantly less than 2×, 4× and *ad libitum* groups ($P<.01$).
§ Significantly less than 4× and *ad libitum* groups ($P<.01$).
‖ Significantly less than all other groups ($P<.01$).
¶ Significantly less than *ad libitum* group ($P<.05$).

not significantly different from each other ($P>0.05$). RNA and RNA/DNA increased significantly ($P<0.01$) with each increase in nursing intensity. Thus, between-nursing intervals as short as 5 hours (4× group) did not permit maximal protein synthesis responses (*ad libitum* group) but maintained cell numbers.

Milk weights, as measured by litter weights before and after each nursing period totaled —1.3, 41.3, and 93.6 g per total nursing time for the 1×, 2× and 4× groups, respectively. Although each litter was suckled 4 times daily, mother rats nursed 1× or 2× daily did not produce sufficient milk for survival of the litter. Thus, unlike rabbits which nurse only once per day(9), the mother rat must suckle her offspring at least 4 times per day.

Relating quantitative changes in end-organ response with changes in pituitary hormone content may provide an indirect estimation of secretion of the hormone. In the present study prolactin content averaged .013, .014, .017, .017, and .027 IU/mg of pituitary with each increase in nursing frequency, respectively (Table II). *Ad libitum* nursed rate contained significantly ($P<0.05$) more prolactin than all other groups. Prolactin content per gland paralleled the prolactin content per mg of pituitary tissue (Table II). These observations, which confirm the trends of the data of our previous experiment(5), were interpreted as indicating that prolactin probably stimulated some of the increase in nucleic acid content of the mammary gland in response to increased nursing frequency. However, increasing the nursing intensity from non-nursed to *ad libitum* levels increased prolactin content only 2.1-fold whereas mammary DNA and RNA increased 4.3- and 21.1-fold, respectively. If a log-dose relationship between prolactin secretion and mammary gland response exists an even greater increase in prolactin secretion than in the target organ response would have been expected. Thus, other endocrine factors synergising with prolactin or milk removal *per se*

TABLE II. Prolactin and ACTH Content of Pituitaries of Lactating Rats Nursed at Different Frequencies.

Daily nursing frequency	Avg pituitary wt (mg)	Prolactin potency*		Avg adrenal wt (mg)	ACTH potency*	
		IU/mg	IU/gland		milliunits/mg‡	units/gland‡
Non-nursed	9.4	.013 ± .003	.122 ± .028	56.7	279.3 ± 47.8	2.63 ± .45
1×	9.0	.014 ± .004	.126 ± .036	59.8	270.3 ± 48.0	2.43 ± .43
2×	8.5	.017 ± .001	.145 ± .009	54.9	314.4 ± 44.3	2.67 ± .38
4×	8.9	.017 ± .007	.151 ± .062	58.4	277.1 ± 39.5	2.47 ± .35
Ad libitum	8.6	.027 ± .002†	.232 ± .017†	58.2	395.6 ±141.1	3.40 ±1.21

* Mean and standard error of mean.
† Significantly greater ($P<.05$) than all other nursing intensities.
‡ Means not significantly different ($P>.05$).
Average lambdas of prolactin and ACTH assays were .39 and .16, respectively.

(1), which reduces intramammary pressure, may play important roles in the mammogenic and galactopoietic responses to nursing. On the other hand, there may not be a proportional relationship between prolactin secretion rate and pituitary prolactin content.

Pituitaries contained .014, .020, .015, and .021 IU of prolactin per mg at 5 a.m., 11 a.m., 5 p.m. and 11 p.m., respectively, ($P > 0.05$). Thus, contrary to results in the virgin rats of Clark and Baker(10) prolactin concentration in lactating rats did not fluctuate according to diurnal rhythms. Nursing stimuli may have masked any diurnal changes in pituitary prolactin potency. Nursing frequency did not influence pituitary weight (Table II).

Contrary to prolactin, ACTH, although always present in the pituitary, did not appear ($P > 0.05$) to vary with nursing frequency (Table II). This confirms our previous results based on nursing intensities applied by varying litter size(5). Although nursing frequency did not alter pituitary ACTH, the present study does not rule out the possibility that basal levels of ACTH may synergize with other pituitary mammogens to maintain cell structure and metabolic activity. ACTH content averaged 258.5, 310.1, 405.7 and 255.0 milliunits/mg of pituitary at 5 a.m., 11 a.m., 5 p.m. and 11 p.m., respectively, ($P > 0.05$). Although diurnal variation in ACTH content was not statistically significant, trends of data may suggest a peak ACTH content at 5 p.m. which agrees well with the data of Critchlow *et al*(11).

Summary. Increasing the nursing frequency in rats from non-nursed levels (controls) to 1×, 2×, 4× and *ad libitum* frequencies progressively increased mammary DNA, RNA and pituitary prolactin content 4.3-, 21.1- and 2.1-fold, respectively. Pituitary ACTH content, although always detectable, was unaffected by nursing frequency.

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Studies on the Mechanism of Action of Polymyxin B On *Cholera vibrios*. (32377)

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Gan and Tjia(1) and Powell and Finkelstein(2) found that *Vibrio cholerae* are highly sensitive to polymyxin B and El Tor vibrios relatively resistant. Roy, Mrihda and Mukerjee(3) reported *V. cholerae* to be sensitive to 15 μg/ml concentration of polymyxin B in nutrient agar media whereas El Tor vibrios were resistant to this concentration.

Few and Schulman(4) working with 7 different sensitive and resistant bacteria and Newton(5) with *Pseudomonas aeruginosa* found that when polymyxin is added to washed suspensions of sensitive organisms, it causes the release of soluble constituents from the cells. These workers suggested that the bactericidal activity of polymyxin is due to its combining with and disorganizing the structure of the bacterial cell wall, which is