Restoration of Pituitary Lactogen Released in Response to Suckling* (33921)

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Reece and Turner (1) provided the first direct evidence that suckling generated stimuli cause a release of anterior pituitary lactogen (LTH). They observed a decrease in pituitary lactogen concentration when lactating rats were reunited with their litters for a 3-hr period following a 12-hr interval of nonsuckling. Subsequent investigations have provided evidence which suggests that 2 min (2) or 30 min (3–6) of suckling, following 10-hr isolation, were sufficient to induce a considerable fall in pituitary lactogen content of the rat. The rate of restoration of pituitary lactogen following its suckling induced discharge was investigated by Grosvenor and Turner (3). They showed that approximately one-half the presuckling level of pituitary lactogen was restored 2.5 hr postsuckling, but complete restoration was not accomplished even 9.5 hr postsuckling. Suckling-generated stimuli originating at the teat, appear to terminate in the hypothalamus (7). How the hypothalamus sequentially influences pituitary lactogen levels, however, remains obscure. Based on reduced quantities of prolactin inhibiting factor (PIF) in the hypothalami of chronically suckled rats, it has been suggested (8, 9) that suckling stimulates lactogen release by depressing the synthesis or release of PIF. This idea is not supported by others (10, 11) who have observed PIF levels in lactating rats which are not dissimilar from those of nonpregnant females. The present study was undertaken to establish the rate of lactogen restoration following its suckling-induced discharge during early lactation and to consider the necessity of the hypothalamic influence in this process.

Materials and Methods. Seventy primiparous lactating rats of the hooded Norway strain were housed in individual cages under conditions of controlled temperature (70 ± 5°F) and lighting (12 hr light daily). The diet consisted of Purina laboratory chow and water *ad libitum*. On the first or second day of lactation litters were reduced to six and when 3 or 4 days old were isolated from their mothers for 10 hr (11 p.m.–9 a.m.). Following this isolation period maternal and litter weights were recorded. Ten rats were immediately sacrificed by decapitation while all remaining rats were permitted to suckle their litters for exactly 2 hr from the time suckling was initiated. Special care was taken to avoid disturbing the nest in handling mothers or litters. When the nests were disturbed the mothers repaired them when reunited with their litters and this delayed initiation of suckling. Following 2 hr of suckling, litters were again isolated from their mothers and weighed. Only rats which had successfully suckled, as determined by little weight gain and visual observation of milk in the litters' stomachs, were used. These rats were then sacrificed 0, 1, 2, 4, 8, or 10 hr following the suckling period (10/group). Anterior pituitaries (AP) were removed, weighed individually, and frozen until assayed for lactogen. Anterior pituitaries from each group were pooled and assayed for lactogen by the method of Reece and Turner (12). Anterior pituitaries were ground in an agate mortar, suspended in distilled water, and assayed against a lactogen standard[2] in 12 pigeons/group. The anterior pituitary suspension (0.05 ml/day) was injected intradermally over the right crop gland of 6 pigeons and 2 μg of

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[2] Ovine prolactin (20 IU/mg) received as a gift from the Endocrinology Study Section, NIH.

TABLE I. Repletion of Anterior Pituitary (AP) Lactogen (LTH) *in Vivo* Following 2 hr of Suckling.[a]

Time (hr)	Body wt (g)	AP wt (mg)	LTH[e] (IU/AP)	LTH[e] (IU/100 mg of AP)
Presuckled	238.4 ± 6.2	10.50 ± 0.38	0.87 ± 0.08	8.26 ± 0.84
0[b]	234.1 ±12.0	10.45 ± 0.58	0.10 ± 0.10[c]	0.92 ± 0.01[c]
1[b]	230.0 ± 6.0	10.29 ± 0.31	0.31 ± 0.02[c]	2.99 ± 0.23[c]
2[b]	243.4 ± 6.8	10.62 ± 0.68	0.50 ± 0.03[c]	4.74 ± 0.29[c]
4[b]	238.4 ± 9.8	10.12 ± 0.42	0.65 ± 0.06[d]	6.43 ± 0.54[d]
8[b]	243.6 ± 4.5	10.44 ± 0.31	0.78 ± 0.08	7.50 ± 0.76
10[b]	237.0 ± 7.3	9.95 ± 0.29	0.89 ± 0.07	8.92 ± 0.69

[a] Values are the means ± standard error of means.
[b] Time following 2-hr suckling interval.
[c] Significantly less than the comparable presuckled value: [c] $p < 0.01$; [d] $p < 0.05$.

lactogen standard (0.042 IU), in the same volume, were injected over the left crop gland for 4 days. The procedure was reversed in the remaining 6 pigeons to minimize that component of the experimental error due to side to side variation. The pigeons were sacrificed on the fifth day, the crop glands were removed and visually rated by two observers for degree of proliferation. These data were analyzed using analysis of variance, and planned comparisons of treatment means were accomplished using the least significant difference (13).

To determine whether the anterior pituitary would replete lactogen when removed from hypothalamic control, an additional 20 rats were prepared as previously described and sacrificed immediately following 2 hr of suckling. Anterior pituitaries were aseptically removed and divided into approximately equal halves which were weighed individually. One-half anterior pituitary from each of two rats (one anterior pituitary equivalent) was placed in a 25-ml culture flask and cultured for 2 hr according to the techniques described by Talwalker *et al.* (14). The corresponding halves served as controls and were frozen immediately. The lactogen content of cultured anterior pituitary halves were compared directly with the uncultured control halves by injecting them over the crop glands of 12 pigeons. Statistical analysis of these data was accomplished using the *t* test for paired observations.

Results. Two hr of suckling, following 10 hr of nonsuckling, significantly reduced anterior pituitary lactogen content (IU/AP) from an initial high level of 0.87 for the presuckled group, to a low level of 0.10 for rats which were sacrificed immediately following suckling. This represents an 88.5% reduction in lactogen content. Lactogen restoration occurred rapidly during the early postsuckling periods, reaching 0.31 and 0.50 IU/AP at the end of the first and second 1-hr intervals, respectively, and this increase was linear. Thereafter, lactogen accumulation by the anterior pituitary proceeded at a much reduced rate. By the end of the fourth hour of the postsuckling interval, the lactogen content of the anterior pituitary had reached 0.65 IU/AP. The average anterior pituitary lactogen content of rats sacrificed 8- or 10-hr postsuckling (0.78 and 0.89 IU/AP, respectively) was not significantly different from the comparable average for the presuckled group. Therefore, complete lactogen restoration was accomplished by the anterior pituitary *in vivo* within 8 hr after its release by 2 hr of suckling. Summarized data are presented in Table I and the rate of restoration of lactogen content by the anterior pituitary *in vivo* is depicted in Fig. 1.

In the *in vitro* experiment the average lactogen content of anterior pituitary halves from the precultured and postcultured groups was 3.17 and 2.39 Reece-Turner units, respectively, and the difference was not significant.

Discussion. The present study demon-

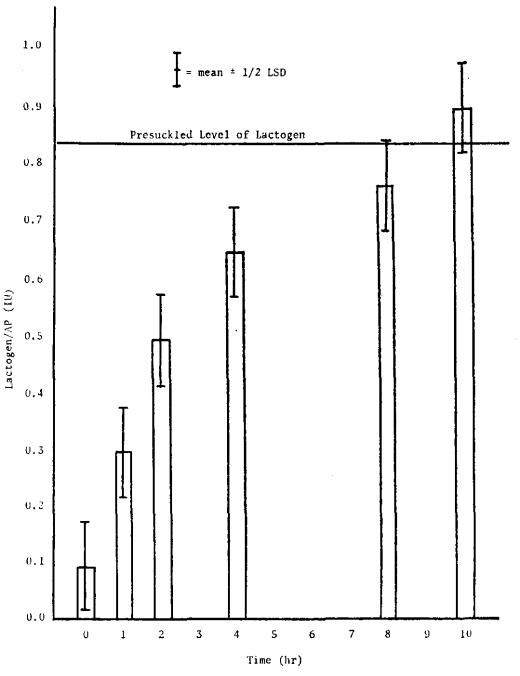

FIG. 1. Restoration of anterior pituitary lactogen after release in response to suckling.

strates that 2 hr of suckling, following 10 hr of nonsuckling, effectively reduces anterior pituitary lactogen content. Other workers have reported similar decreases in pituitary lactogen content in rats which were removed from their litters for 10–12 hr, then suckled for 3 hr (1), 0.5 hr (3–7) or 2 min (2). Grosvenor and Turner (3) reported that pituitary lactogen content was about ½ the presuckled level 2.5 hr postsuckling but complete restoration was not achieved even 9.5 hr following suckling. The results of the present investigation also demonstrate rapid restoration of pituitary lactogen during the early postsuckling interval, however; complete lactogen restoration was observed as early as the eighth hour postsuckling. Lactating rats suckle their young frequently during lactation, therefore, anterior pituitary lactogen values reported here do not represent those usually found in anterior pituitaries of lactating rats removed without reference to the time of suckling.

In contrast to the anterior pituitary *in vivo*, the anterior pituitary *in vitro* does not replete lactogen stores previously depleted by suckling stimuli. This result would suggest that a hypothalamic influence is necessary to permit lactogen reaccumulation. Talwalker *et al.* (14) demonstrated the presence of an agent, provisionally termed prolactin inhibiting factor, in the hypothalamus of rats which inhibited the synthesis and release of anterior pituitary lactogen. Subsequent investigations (8, 9) indicated that chronic suckling during postpartum lactation was accompanied by a marked depression in hypothalamic PIF content. This result was interpreted as evidence that suckling promotes the release and presumably the synthesis of lactogen by chronically inhibiting the production of PIF during lactation (8). This view is not supported by present results, for (a) lactogen was not chronically released but in response to the suckling stimulus, which would suggest that during intervals of nonsuckling, PIF or some other inhibitory agent(s) must be present to prevent the release of lactogen (B) the inhibitory agent(s) cannot inhibit both synthesis and release *in vivo* for synthesis proceeded apace while release was prevented during periods of nonsuckling; and (c) lactogen buildup, following its suckling-induced discharge, did not occur *in vitro* which suggests that some factor(s) must be present in the hypothalamus which allows buildup to proceed. Other workers (10, 11) have presented evidence that PIF is indeed present in the hypothalamus of the lactating rat.

The decreased rate of lactogen synthesis, as the nonsuckling interval increased, suggests that lactogen accumulation, in the acidophil cells of the pituitary, may be the factor responsible for controlling lactogen synthesis. The release of lactogen in response to suckling may be precipitated by an inhibition of PIF synthesis or release or a block of PIF action.

Recently it was reported that 2 min of suckling was as effective as 30 min in evoking the discharge of anterior pituitary lactogen and suggested that the ample lactogen remaining in the pituitary following suckling may be, at least temporarily, nonreleaseable (10). No detection of a significant release of lactogen *in vitro*, in the present investigation, supports this idea.

Summary. The effects of the suckling stimulus upon release and subsequent repletion of anterior pituitary (AP) lactogen were investigated in hooded Norway rats on the third or fourth day of lactation. Two hr of suckling resulted in an 88.5% reduction in lactogen content. The AP lactogen content gradually increased during the postsuckling interval with restoration to presuckling levels after 8 hr. To determine whether lactogen repletion would occur *in vitro*, AP were obtained from rats immediately following 2 hr of suckling, and cultured for 2 hr. In contrast to the results obtained *in vivo*, lactogen repletion did not occur *in vitro*.

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The Interaction of a Hydrophobic Probe with Human Blood Cells* (33922)

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Many biologic properties of mammalian cells are dependent upon the conformational features of macromolecular components of the cell membranes and cell contents. Such biologic properties include contact inhibition (1), viral adsorption (2), and antigen recognition (3). Since hydrophobic interactions play a key role in the maintenance of specific macromolecular conformations on and within cells, a study of the interaction between hydrophobic probes and mammalian cells can provide direct information on the nature of cell receptors and recognition sites. Arylaminonaphthalenesulfonates are useful as probes for hydrophobic regions on macromolecules (4–6). Members of this class of compounds show enhanced fluorescence quantum yield when located in a hydrophobic environment. We here report an extension of the use of an arylaminonaphthalenesulfonate to the study of hydrophobic regions of mammalian cells.

Materials and Methods. The interaction between 1,8-anilinonaphthalenesulfonate (ANS) and human peripheral blood cells was investigated. Leukocytes and erythrocytes were prepared from heparinized blood by sedimentation in 1% gelatin (7). The leukocytes were washed four times by centrifugation in 0.05 *M* phosphate–0.15 *M* NaCl, pH 7.2 (PBS) and resuspended in PBS or in tissue culture medium F-12 (Grand Island Biological Co.) at a concentration of 1×10^6

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