The Influence of Lactational Intensity and Exogenous Prolactin on Serum Thyroxine Levels in the Rat (36062)

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Using a "direct output" method, thyroid hormone secretion rate (TSR) was demonstrated to be markedly reduced in the rat during lactation (1, 2). This was postulated to be due to a relative iodine deficiency resulting from the large losses of iodine occurring in milk. A subsequent report (3) indicated that lactating rats with reduced TSR also showed a comparable magnitude of reduction in serum thyroxine (T_4).

Therefore, it was of interest in the present study to establish if there is a relationship between serum T_4 levels and lactational intensity in the rat and to determine if a euthyroid function is maintained during lactation by excess iodine supplemented in the diet. Furthermore, the effects of prolactin on thyroid function were investigated in view of recent suggestions that prolactin may inhibit thyroid activity in the rat (4, 5) and amphibians (6). Radioimmunoassay techniques show that prolactin is elevated in the cow and goat during lactation (7) and that rat serum prolactin increases are directly correlated to the intensity of suckling stimuli (8).

The present experiments attempt to resolve the question of the relative contribution that prolactin secretion vs iodine loss in milk can make to a reduction in circulating thyroid hormones during lactation.

Materials and Methods. In all present experiments female Sprague-Dawley rats (Spartan Research Farms Inc., Haslett, MI) were used. The prebreeding weights were 200–220 g. Animal room temperature was maintained at 76 ± 2°F and lights were on 14 hr daily. With certain rats, litter sizes were adjusted on the day of birth to 0, 3, 6, and 12 pups to provide a gradation in lactational intensity.

The diet prepared in the feed mixing plant at the Michigan State University Department of Animal Husbandry, comprised two-thirds corn and one-third soybean oil meal (50% protein) plus complete vitamin and mineral salt premix supplements except that KI was added at the laboratory in the amount of 1.0 μg of I/g of feed. This diet was established previously as providing an adequate iodine intake for nonlactating rats (9). Rats receiving this diet without iodine supplementation develop enlarged thyroids within 2 weeks. In one experiment, the amount of KI added was increased to 10.0 μg of I/g of feed in one group of rats. To prevent loss of iodine from the diet due to sublimation, appropriate amounts of KI were added to fresh batches of feed every third day after first discarding the remainder of the previous batch from the feeding trays.

In the prolactin study, nonlactating rats received subcutaneous injections of either 0.9% NaCl solution or 1.0 mg of NIH-P-S8 ovine prolactin[2] (28.38 IU/mg) twice daily for 15 days. Prolactin was dissolved in 0.9% NaCl solution to a concentration of 10 mg/ml.

Blood was collected by heart puncture after either 15 days of prolactin administration or 14–16 days of lactation. Within 3 hr of withdrawal, the blood samples (7–8 ml) were centrifuged. The extracted serum could be stored at −20° for several months, if necessary, with no measurable loss in T_4 content.

Serum T_4 was assayed by an adaptation of

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[2] NIH-P-S8 prolactin was kindly provided by the Endocrinology Study Section, National Institutes of Health.

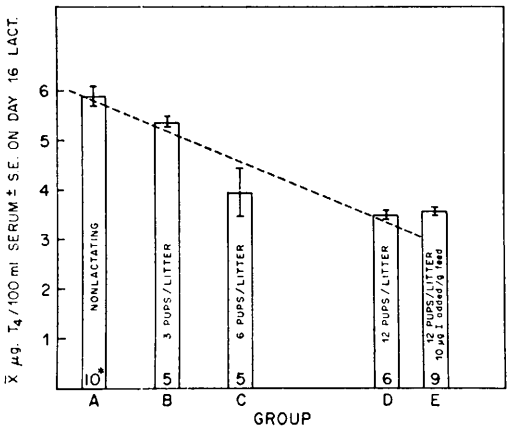

FIG. 1. Rat serum thyroxine vs lactational intensity and elevated iodine intake: * No. of rats per group; groups A–D: $r = -0.83$, $p<.001$, 1 μg of I added/g of feed; difference between groups: A vs B ($p<.05$); A vs C ($p<.01$); A vs D ($p<.001$); B vs C ($p<.02$); B vs D ($p<.001$); C vs D ($p>.30$); D vs E ($p>.30$).

the Tetrasorb-125[3] method as described previously (10). In principal, this is a competitive protein binding technique which was first described by Ekins (11) and later modified by Murphy and Jachan (12). The analysis employs an alcoholic extract of serum T_4 which will compete with and displace a quantity of labeled T_4 from a standard thyroxine-binding globulin (TBG) solution, the displaced labeled T_4 being bound subsequently to an anion exchange resin. Calibration of the assay requires comparing the displacement results of T_4 extracted from serum with results obtained using known aliquots of highly purified L-T_4 from which a standard curve may be plotted. The sensitivity of the assay was statistically accurate ($p<.05$) to within 0.5 μg/100 ml.

Results. Figure 1 illustrates levels of serum T_4 in the rat at several levels of lactational intensity, as well as elevated iodine intake. As shown in groups A, B, C, and D, as the number of pups per litter is increased from 0 to 3, 6, and 12 respectively, serum T_4 is significantly reduced. A high inverse correlation exists between serum T_4 and lactational intensity ($r = -0.83$, $p<.001$). Even with only 3 pups/litter the serum T_4 of a nursing rat is significantly reduced ($p<.05$) below that of nonlactating controls. When the amount of iodine added to the diet was increased 10-fold (group E) there was no significant increase ($p>.30$) in serum T_4 compared to that of similar rats receiving 1.0 μg of I/g of feed (group D).

[3] Abbott Radiopharmaceuticals, North Chicago, IL.

TABLE I. Effect of Prolactin Injections on Serum Thyroxine in the Adult Female Rat.[a]

Saline-injected rats		Prolactin-injected rats (1 mg of prolactin[b] injected subcutaneously twice daily for 15 days)	
Rat no.	T_4 (μg/100 ml of serum)	Rat no.	T_4 (μg/100 ml of serum)
1	6.17	12	6.28
2	4.95	13	5.53
3	5.80	14	5.53
4	5.85	15	6.39
5	6.02	16	5.85
6	7.03	17	5.54
7	6.17	18	5.73
8	6.17	19	6.71
9	5.32	20	6.28
10	6.81	21	6.17
$\bar{X}$	6.03		6.00
SE	0.19		0.13
N	10		10

[a] All rats received 1.0 μg of I^- added/g of feed.
[b] NIH-P-S8 ovine prolactin 28.38 IU/mg (10 mg of prolactin/1.0 ml of saline).

Table I shows that exogenous ovine prolactin had no significant effect on serum T_4 in the nonlactating rat ($p>.90$).

Discussion. Previous investigations, employing a "direct output" measurement of TSR, have indicated that the normal level of dietary iodine necessary for euthyroid function in a nonlactating rat is 1.0 μg I^-/g of feed (9). The same method and diet, when employed for the lactating rat, reveals that TSR is reduced to 50% of the values for nonlactating rats (1, 2). Other rats, lactating at the same intensity and maintained on the same diet as those in the TSR studies, also show a reduction in serum T_4 to about 50% of the values for nonlactating rats (3). The reductions in both TSR and serum T_4 were postulated to be due to a relative iodine deficiency resulting from the large losses of iodine occurring in milk. The present study

shows that rat serum T_4 levels are progressively reduced as lactational intensity increases, and that the heavily lactating rat either does not respond to iodine therapy by increasing T_4 production or requires an iodide supplement far in excess of the elevated level provided in the present investigation.

The results of the present study would indicate that exogenous ovine prolactin, at the high level administered, has no influence on thyroid function when evaluated by serum T_4 measurements. Since the duration of prolactin administration was nearly as long as the rat lactation period, it is unlikely that endogenous prolactin secretion, associated with lactation, would be a directly contributing factor to the reduction in serum T_4. Rat serum prolactin increases which are directly correlated to increases in suckling stimuli (8) would not appear to influence the progressive decrease in rat serum T_4 observed in the present study as lactational intensity increases. Thus these data would not support a conclusion that concomitant increases in serum prolactin might exert a progressively greater suppression on thyroid function.

These conclusions are at variance with those of earlier reports (4–6) suggesting a prolactin inhibitory effect on thyroid-stimulating hormone (TSH) and T_4. However, aside from possible differences between mammals and amphibians, the use of thyroidal ^{131}I uptake as an index of thyroid activity is questionable since both hyper- or hypothyroid function can be accompanied by either high or low ^{131}I uptake. Furthermore, Chen and Meites (13) have recently concluded that T_4 acts directly on the anterior pituitary to stimulate an increase in prolactin production and that a lack of T_4, when thiouracil is administered, will depress prolactin secretion. This reduction in prolactin secretion in the absence of adequate T_4 may partially explain why lactation can be enhanced by the administration of thyroactive substances in ruminants (14) and other species (15). If prolactin were an antithyroid agent as suggested by others (4–6), then it would be inhibiting its own production via suppression of the thyroid.

The evidence to date would suggest that the primary reason for a reduction in serum T_4 during lactation is the mammary diversion of iodine coupled perhaps with a possible decrease in total serum thyroid-binding protein. The latter could be due to the increased demand for protein synthesis during lactation. There is also the possibility that high endogenous prolactin secretion could indirectly exert an antithyroid effect by enhancing lactation, thereby increasing the mammary drain on iodine supplies which ultimately would further decrease serum T_4

Summary. Rat serum T_4 levels were inversely correlated with lactational intensity, and the markedly reduced serum T_4 observed in high-lactating rats could not be counteracted with 10 times the normal dietary iodine.

Exogenous ovine prolactin (2 mg/day for 15 days), when administered to nonlactating rats, had no effect on serum T_4. It was concluded that high serum prolactin, associated with nursing, was not a factor directly contributing to the reduction of serum T_4 observed during lactation.

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