Effects of Endogenous Thyrocalcitonin and Vitamin D_3 on Milk Yield in Parathyroidectomized and Thyroparathyroidectomized Rats[1]

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Removal of both parathyroid and thyroid glands was shown to result in pronounced milk reduction, tetany and loss of pups if the operation was performed in early lactation in the rat (1, 2). Replacement therapy was successful when L-thyroxine (L-T_4) at 3 μg/100 g of body wt/day and a synthetic vitamin material AT-10, at 187.5 μg/day were injected. In the same study the authors reported that successful replacement therapy was also achieved by injection of 3 μg/100 g of body wt/day of L-T_4 and 40 USP units of parathyroid hormone (PTH), twice daily, starting immediately after the operation on day 7 of lactation in the rats. Djojosoebagio and Turner (3) administered calciferol (Vitamin D_2) at a level of 0.2 mg/100 g of body wt/day in conjunction with 3 μg of L-T_4/100 g of body wt/day in thyroparathyroidectomized (TPTX) lactating rats from the day of operation (day 4) to day 19 and found that milk yields on days 14, 16, 18, and 20 were not significantly different from the milk yield of a group of sham-operated rats, but were lower than the yield in a larger group of control animals. In another study, (4) it was reported that 3.5 μg of L-T_4/100 g of body wt/day in conjunction with 3 $\times$ 20 USP units of PTH/day in TPTX lactating rats (operated on day 4) resulted in successful therapy in 10 rats. Srivastava and Turner (5) reported that they also obtained successful replacement therapy in TPTX rats with 3 μg of L-T_4/100 g of body wt/day and Vitamin D_3 at the level of 8000 IU or 0.2 mg/100 g of body wt/day. The present study was designed to investigate whether or not endogenous thyrocalcitonin (TCT) plays a significant role in milk yield in rats and if vitamin D_3 at 8000 IU/day is sufficient replacement therapy in the absence of the parathyroid glands.

Materials and Methods. Thirty-three primiparous lactating rats of the Sprague-Dawley-Rolfsmeyer strain were placed in individual litter cages with Purina Lab Chow and tap water *ad libitum* in an animal room maintained at 25.6 + 1° with 14 hr of artificial light. On day 4 of lactation, litters were reduced to 6 pups for each dam. The dams were operated upon on day 6 of lactation; and replacement therapy was continued each day to day 19 of lactation. The litters were weighed on days 6, 14, 16, 18, and 20. Two different methods were used to measure the milk production. One was by the difference of litter weight before and after suckling which followed a 10-hr isolation from the dams. One USP unit of oxytocin (Armour-Baldwin, pop) was injected immediately before, and another unit 15 min after, nursing for a total period of 30 min to aid in removal of milk (4). A second method was based on the difference of the mother's body weight before and after nursing. The balance was sensitive to 0.1 g (Dial-O-Gram, Ohaus Scale Corp, Union, N.J.). The experimental groups and treatments are shown in Table I.

Parathyroidectomy and thyroparathyroidectomy were done as described by Hirsch *et al.* (6). The L-T_4 was dissolved in normal saline with a few drops of 1 *N* NaOH

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TABLE I. Experimental Groups.

Group	No. of animals	Operation[a]	Treatments
A	5	Normal control	None
B	5	Sham operated	Alkaline saline and sesame oil
C	7	PTX	None
D	5	PTX	8000 IU of Vit. D_3/day
E	5	TPTX	None
F	6	TPTX	3 μg of L-T_4/100 g of body wt/day and 8000 IU of Vit. D_3/day

[a] PTX = parathyroidectomized; TPTX = thyroparathyroidectomized.

solution. Group means of dam weight, litter weight, and milk production were compared statistically by analysis of variance and the least significant difference (LSD) test (7).

Results. Crystalline vitamin D_3, given at a level of 8000 IU/day (1 IU = 0.25 μg of pure crystalline vitamin; 8000 IU = 0.2 mg/day) in conjunction with 3 μg of L-T_4/100 g of body wt/day given subcutaneously (sc) to TPTX, and 8000 IU of Vitamin D_3/day to PTX lactating rats was incapable of replacing the parathyroid gland function in maintaining normal milk yield when compared to the normal control (A) and sham-operated control (B) group. Milk yields in groups C, D, E, and F were significantly depressed ($p<0.05$) when compared to groups A and B on days 14 through 20. Group B had a nonsignificantly lower milk yield on days 14 through 20 when compared to group A. Group C was significantly less than group D on days 18 and 20 only, while group E was on all 4 days (Table II). Litter weights on day 20 of lactation were significantly lower ($p<0.05$) in groups C, D, E and F than in groups A and B (Table III). Analysis of variance of milk production showed that the two methods for determining milk production were not significantly different, where as groups, days, and groups–times–days interaction were all significantly different sources of variation ($p<0.05$). Similarly, litter weights showed significant differences between groups and days–times–groups interaction. Dams in groups C, E and F showed loss of body weight as compared to groups A, B, but the losses of body weight were not significantly different in any of the groups based on analysis of variance. One fatal tetany and three nonfatal tetany attacks were observed in groups E and C.

Discussion. PTH influence on calcium

TABLE II. Milk Production at Different Days of Lactation.[a]

	Groups and treatments	No. of animals	Mean ± SE (g) Day: 14	16	18	20
A	Normal control	5	6.9 ± 0.47	8.5 ± 0.75	11.1 ± 0.27	8.4 ± 0.32
B	Sham operated, saline and sesame oil daily from day 6	5	6.1 ± 0.21	7.3 ± 0.14	9.7 ± 0.27	6.0 ± 0.19
C	PTX, without treatment	7	5.3 ± 0.66	4.4 ± 0.84[b]	4.2 ± 0.88[b]	3.6 ± 1.10[b]
D	PTX, 8000 IU of Vit. D_3/day	5	3.16 ± 0.17[b]	5.4 ± 0.33[b]	6.8 ± 0.47[b]	5.1 ± 0.25[c]
E	TPTX, without treatment	5	1.96 ± 0.54[b]	2.2 ± 0.76[b]	2.65 ± 0.64[b]	2.55 ± 0.55[b]
F	TPTX, 3 μg of L-T_4/100 g of body wt/day; 8000 IU of Vit. D_3/day	6	4.78 ± 0.52[b]	5.2 ± 0.41[b]	6.7 ± 0.44[b]	5.55 ± 0.37[c]

[a] Based on difference of body wt of 6 pups before and after suckling.

[b] Significantly different from normal control and sham-operated group ($p<0.05$) by least significant difference (LSD) test.

[c] Significantly different from normal control ($p<0.05$).

metabolism was demonstrated many years ago. Most striking of the changes resulting from parathyroid gland extirpation was tetany. In lactating rats, the removal of the parathyroid glands resulted in marked reduction of milk production (8, 9). Vitamin D has been implicated as a factor in calcium metabolism primarily because of its action on the intestinal mucosa enabling the cells to transport calcium from the lumen of the gut into the blood. In addition to the action on intestinal absorption of calcium, vitamin D has been suggested by Carlsson (12) to facilitate mobilization of calcium from bone. Arnaund *et al.* (13) reported that presence of vitamin D in physiological doses was necessary for the mobilization of calcium and phosphate from bone by PTH. However, vitamin D was not necessary as a co-participant in the action of PTH on the renal tubule.

In the present study vitamin D_3 at a level of 8000 IU/day ameliorated the symptoms of PTX but did not completely replace PTH in maintaining milk production. PTX alone was less severe and took a longer time to manifest a detrimental effect upon milk production than TPTX. Thyroxine was reported to play a significant role in calcium metabolism by altering bone turnover via a direct action on bone cells and by increasing the size of the calcium compartment in the body (14). Adams and Jowsey (15) also reported that thyroxine induced hyperthyroidism caused an increase in bone turnover, an increase in serum calcium level and a decrease in plasma protein concentration of both intact and parathyroidectomized dogs. They suggested that the increased bone resorption in response to thyroxine was independent of the parathyroid glands. This action of thyroxine might explain the fact that in our experiment endogenous thyroxine helped to combat the absence of PTH to some extent in the PTX group and exogenous thyroxine acted similarly in the TPTX group. However, complete replacement of PTH in both PTX and TPTX groups with 8000 IU of Vitamin D_3/day was not achieved as far as milk production was concerned. We presume that PTH is probably necessary for the lactating

TABLE III. Litter Weight at Different Days of Lactation.

	Groups and treatment	No. of animals	Body wt of 6 pups (g; mean ± SE) Day: 6	14	16	18	20
A	Normal control	5	87.5 ± 2.5	140.5 ± 13.6	147.4 ± 13.9	150.7 ± 13.9	155.2 ± 15.1
B	Sham operated, saline and sesame oil daily from day 6	5	79.8 ± 7.2	100.2 ± 7.2[a]	109.6 ± 4.0[a]	125.4 ± 4.8[a]	137.7 ± 4.4[a]
C	PTX, without treatment	7	81.1 ± 7.2	114.7 ± 8.9	99.9 ± 19.1	101.8 ± 19.1[b]	96.6 ± 26.4[b]
D	PTX, 8000 IU of Vit. D_3/day	5	79.8 ± 5.3	83.9 ± 5.3[b]	89.2 ± 5.4[b]	96.8 ± 5.4[b]	106.3 ± 5.1[b]
E	TPTX, without treatment	5	72.3 ± 3.4	80.1 ± 21.4[b]	71.9 ± 22.9[b]	71.9 ± 22.9[b]	31.6 ± 19.6[b]
F	TPTX, 3 μg of L-T_4/100 g of body wt/day; 8000 IU of Vit. D_3/day	6	84.8 ± 5.5	93.4 ± 12.8	101.8 ± 16.5	99.2 ± 16.2[b]	107.1 ± 15.8[b]

[a] Significantly different from normal control ($p<0.05$) by least significant difference (LSD) test.
[b] Significantly ($p<0.05$) different when compared to normal control and sham-operated group by LSD.

mammary glands of rats for optimum milk secretion. McLean and Urist (16) also suggested that PTH influences the secretion of calcium by the lactating mammary glands in rats.

Both PTH and TCT are probably essential for maintaining normal serum calcium level (10, 11). Sammon *et al.* (17) and Bronner *et al.* (18) suggested that TCT has a derivative-like control that refines further the action of PTH on bone, PTH being the primary regulator of blood calcium. In our previous study (19) we found that thyroid gland content of TCT reaches a maximum on day 10 of lactation in the same strain of rats used in this study. This indirectly supports the concept that TCT is not discharged during lactation when the parathyroid gland is hyperactive to meet the increased demand of calcium in milk. The interpretation reached from that study was that the TCT was not required to stabilize the calcium level from going too high because the demand of milk synthesis prevented the calcium from reaching a level high enough to trigger the release of TCT. Based on that study and the present study it is suggested that TCT is not a critical factor for maintenance of lactation in rats.

Summary. Thirty-three lactating rats were divided into 6 groups: (A) normal control, (B) sham-operated control, (C) parathyroidectomized (PTX), (D) PTX plus 8000 IU of vitamin D_3/day, (E) thyroparathyroidectomized (TPTX), (F) TPTX plus 3 μg of L-T_4/100 g of body wt/day plus 8000 IU of vitamin D_3/day. Milk production in (A) and (B) were not significantly different but were higher ($p<0.05$) than other four groups. Group C was significantly less than D on days 18 and 20 only, while E was significantly less than F in all 4 days. PTX was less severe on milk production than TPTX and took a longer time to manifest the detrimental effects. Vitamin D_3 at 8000 IU/day ameliorated the symptoms of PTX but did not completely replace PTH for normal lactation. PTH seems essential for lactating mammary glands for normal lactation in rats. Endogenous TCT seems not essential for milk production. Thyroxine probably has some calcium mobilizing effect similar to PTH.

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