Effect of a Combination of Lactogenic, Growth, Thyroid, and Parathyroid Hormones on Lactation in Rats.* (29204)

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It is generally accepted that milk secretion is initiated and maintained by the lactogenic hormone. However, a number of other hormones may influence the rate of intensity of milk secretion and oxytocin can influence the completeness of milk removal at the time of milking or nursing. It has been suggested that the great variation in milk yield of mammals is due in part to variation in the secretion rate of those hormones which influence the intensity of milk secretion. If one or more hormones is secreted in less than optimal amounts, the milk yield would be limited by the extent of the deficiency. An increase in milk yield resulting from administration of a hormone suggests suboptimal secretion of that hormone. The extension of the concept would involve the administration of a series of hormones, each of which had shown, individually, a stimulating effect upon milk secretion in normal animals.

In initiating this series of experiments, it was considered important to insure complete milk removal at nursing time. It was shown that the injection of oxytocin to rats of the Sprague-Dawley-Rolfsmeyer strain on day 14 of lactation just prior to the return of the litter to the dams, after a 10-hour separation period, increased the milk yield by 50%(1). This increase in milk yield was not considered to be due to a stimulation of milk secretion but represented more complete removal of milk present in the mammary glands. In subsequent experiments, oxytocin was administered at a level of 1 USP unit at the beginning of the nursing period.

When growth hormone (1 mg/day) was administered from day 7 to 14, milk yield was increased 61% above the controls(1), with growth hormone and thyroxine, milk yield was increased 75%(2) and with growth hormone, thyroxine and lactogenic hormone, 84%(3) above the control milk yield. The influence of the parathyroid hormone in thyroparathyroidectomized(4,5) and normal rats (6) and its effect on calcium concentration of milk in rats(7,8) has been presented. The object of the present study was to extend the previous observation on the effect of a combination of hormones to include the parathyroid hormone.

Materials and methods. Lactating rats of the Sprague-Dawley-Rolfsmeyer strain were housed in individual cages, fed Purina Lab Chow and water *ad libitum*. The animal room was maintained at a temperature of 78 ± 1°F. On day 4 post-partum, litters were reduced to 6 young and daily weights of dams and litters were taken from day 7 to 20. From day 7 to 20 post-partum, 30 dams were injected subcutaneously, once daily, with 30 USP units parathyroid hormone (PTH)‡/100 g BW, 1 mg lactogenic hormone (LGH)§/animal, 1 mg growth hormone (GH)§/animal and 3 μg L-thyroxine (T_4)/100 g BW. The control animals were divided into 3 groups; 10 animals were uninjected, 11 animals received 0.3 ml/100 g BW of a solution of 1.6 g glycerin and 0.2 g phenol in 100 ml of water. This volume injected subcutaneously was equal to the volume of 30 USP units PTH/100 g BW. The third group of 8 animals was injected subcutaneously with 0.3 ml/100 g BW of the solution received by the second group plus 0.2 ml/animal of 0.9% NaCl in 0.1 N NaOH and an additional 0.1 ml/100 g BW of the same solution. The alkaline NaCl served as vehicle for the hor-

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TABLE I. Effects of Hormones on Milk Yield and Litter Weight Gains in Normal Rats from Day 7 to 20 of Lactation.

No. of animals	Treatment	Milk yield after 10 hr separation* (g)†				Litter wt gain (g)†			
		Day 14	Day 16	Day 18	Day 20	Day 14	Day 16	Day 18	Day 20
29	Controls	6.9 ±.6	8.9 ±.7	9.9 ±.6	8.2 ±.6	73.9 ± 3.7	76.1 ± 4.4	83.9 ± 5.7	94.8 ± 6.3
30	GH, LGH, PTH, T_4	13.3 ±.6[1]	14.5 ±.7[2]	16.0 ±.5[3]	16.3 ±.9[4]	80.7 ± 3.5[5]	85.2 ± 3.5[6]	103.1 ± 3.9[7]	119.8 ± 6.2[8]

* All animals given 1 USP unit oxytocin before nursing.
† Mean ± standard error.
GH = growth hormone; LGH = lactogenic hormone; PTH = parathyroid hormone; T_4 = L-thyroxine.
Level of significance of milk yields after hormones addition compared with controls: 1-4: $P < .005$.
% increase compared with controls: *Milk yield:* 1) 92%, 2) 62%, 3) 52%, 4) 99%. *Litter weight gain:* 5) 9%, 6) 12%, 7) 23%, 8) 26%.

mones LGH 0.1 ml/1 mg, GH 0.1 ml/mg and T_4 0.1 ml/3 μg. Since these 3 groups of control animals did not show statistically significant differences in their response, they were grouped together. On days 14, 16, 18 and 20 post-partum, milk yields were estimated from the increases of litter weights during a 30-minute nursing period following 10 hours of isolation from the mother. One USP unit of oxytocin (OXT) was injected subcutaneously into the dams (both experimental and control groups) immediately before nursing to aid in complete milk removal. On day 20 of lactation dams were killed, 6 posterior mammary glands were removed and DNA was determined as previously described (9). The amount of DNA/100 g BW was used as index of extent of mammary gland development.

Results. Administration of 30 USP units PTH/100 g BW, 1 mg LGH, 1 mg GH and 3 μg T_4/100 g BW once daily into normal lactating rats increased milk production 92% on day 14 and 99% on day 20 ($P<0.005$, Table I). Total DNA/100 g BW of mammary glands of the experimental group was significantly greater than that of the control (Control 9.3 ± 0.3 and experimental group 10.5 ± 0.3 mg/100 g BW with $P<0.005$). It was also shown that gains in litter weight of the experimental animals were higher than in controls (Table I).

Discussion. The role of the parathyroid hormone in the secretion of milk has been demonstrated by the increase in milk yield of rats in synergism with lactogenic, growth, and thyroid hormones. It has been shown that 30 USP units of PTH/100 g BW in conjunction with 3 μg T_4/100 g BW/day restored lactation to a normal level in thyroparathyroidectomized rats(4). It was shown, further, that this level of PTH stimulated significant increases in milk yield when administered to normal rats(6).

In the present experiment, addition of exogenous PTA to the combination of GH, LGH, and T_4 increased milk secretion on day 14 from 84% to 92% above the controls. During the advance of lactation, milk secretion continued to increase so that on day 20, milk

yield had increased 99% above that of the control group.

With each additional exogenous hormone administered there has been a progressive increase in milk yield. It is suggested that these observations prove that each of these hormones play a role in the milk secretion process. In those animals in which one or more of these hormones is secreted in lesser amounts, their lactation will be limited by the extent of the deficiency. It should be pointed out that the extent of mammary gland growth during pregnancy and lactation also varies considerably so that rats with optimal secretion rates of all the hormones would still show variation in milk yield due to differences in the amount of secretory tissue.

In the past, litter weight gains have been used as the index of milk yield as compared to milk test periods used in the present experiments. This former index is quite satisfactory for measuring deficiencies in milk secretion. As a measure of milk yield above normal, litter weight gains are less satisfactory. It has been shown(1) that litter weight gain was related to the estimated milk yield up to a point but higher milk yields were not related to further gains. Two factors may be involved. During the experimental period from days 7 to 20 the endogenous supply of oxytocin released at each nursing period may be limited so that complete milk removal is not attained, as compared to the milking test periods on days 14, 16, 18 and 20 employing oxytocin. Second, the capacity of the litter to grow in an environment of excessive food supply may be limited. An increase of about 25% in litter weight gains may be the extent of the genetic capacity for growth of which the young are capable.

Normally, milk yield increases for a period with the advance of the lactation period, then begins a gradual decline. The decline in milk yield is believed to be due in part to a decline in rate of hormone secretion and in part to loss of epithelial cells due to involution. It will be noted in the normal group, maximum milk yield occurred on day 18, with a decline on day 20. The experimental group continued to increase in milk yield to the 20th day.

It was observed that the DNA/100 g BW of the experimental group was significantly greater than that of the control group. It is possible that the hormones administered stimulated increased growth of the mammary glands during lactation(10) or tended to prevent glandular involution toward the end of the lactation period.

Summary. The effect of 30 USP units of PTH/100 g BW in conjunction with 1 mg LGH/animal, 1 mg GH/animal and 3 μg T_4/100 g BW on the milk yield of normal rats was determined by daily administration of the hormones from day 7 to 20 postpartum with determination of milk yield after 10 hr separation period on days 14, 16, 18 and 20. One USP unit of OXT was injected at each period to aid in complete milk removal. Administration of the hormones increased milk yield 99% and litter weight 26% over the control group on day 20. The DNA content of mammary glands of experimental animals at day 20 was significantly greater than that of controls, suggesting that treatment either stimulated increased growth of the gland or prevented involution.

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