Effect of Graded Levels of Lactogenic Hormone upon Mammary Gland Growth and Lactation in Rats.* (31547)

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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 intensity of milk secretion. 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.

In an early study, 1 mg lactogenic hormone (LGH) had no effect on lactation from day 7 to day 14(1). The present study is an extension concerning the effect of graded levels of LGH upon the secretion of milk by the rat through days 14 to 20.

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 animals were kept under standardized conditions of temperature and light (78 ± 1°F; 14 hours light and 10 hours darkness). On day 1 of lactation, litters were adjusted to 8 young and on day 4 reduced to 6 young. Ovine lactogenic hormone (LGH)‡ was diluted in normal physiological saline. From days 7 to 19 of lactation, the dams were injected subcutaneously every 12 hours as follows: 1) 18 controls were injected with physiological saline, 2) 18 rats were injected with 1 mg of LGH/day, 3) 17 rats were injected with 2 mg of LGH/day, and 4) 18 rats were injected with 3 mg of LGH/day. On days 14, 16, 18 and 20 of lactation, 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§ was injected subcutaneously into the dams (both experimental and control groups) immediately before nursing and a second unit after 15 minutes of nursing to aid in complete milk removal. On day 20 of lactation the dams were killed after nursing; 6 posterior mammary glands were removed and DNA was determined by the Webb and Levy method(2). Ribonucleic acid (RNA) was determined by measuring the total nucleic acids (TNA) and obtaining the difference between TNA and DNA. The method for measuring TNA was to boil the dried, fat-free tissue (DFFT) in 5% trichloroacetic acid according to Schneider(3). Quantitation of TNA in the supernatant was then obtained by comparing the optical density at 268.5 mμ for maximum RNA and DNA absorption with appropriate standards of 40 to 200 μg(4). In this way, an estimate of TNA was obtained by assuming a ratio of 1:1 between RNA and DNA. The RNA was then calculated by subtracting DNA estimations obtained by color reaction procedure previously described(2) from the TNA estimations. The values of RNA obtained in this way were similar to those obtained by separate digestion of the tissue with 1 N KOH for 5 hours and measurements of the RNA in 5% perchloric acid solution at 260 mμ wavelength. The amount of DNA/100 g bw is used as an index of extent of mammary gland growth and the amount of RNA/100 g bw or RNA/DNA ratio was used as an index of mammary gland protein synthetic activity during lactation.

Results. Lactating rats injected with 1 mg

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‡ Lactogenic hormone, ovine, NIH-P-S7, mean potency 24.3 I.U./mg. Kindly supplied by NIH, Bethesda, Md.
§ Oxytocin was kindly supplied by Armour-Baldwin Laboratories, Omaha, Nebr.

TABLE I. Effect of Lactogenic Hormone on Milk Yield and Litter Weight Gains in Normal Rats from Day 7 to 19 of Lactation.

Treatment	No of rats	Milk yield after 10 hr separation*(g)† Day 14	Day 16	Day 18	Day 20	Litter weight gain (g)† Day 14	Day 16	Day 18	Day 20
		1	3	6	10	14		17	19
Control	18	10.33 ± .73	13.33 ± .72	13.94 ± .74	10.94 ± .83	172.22 ± 4.55	193.44 ± 5.02	205.06 ± 6.14	226.83 ± 6.98
				7	11				
1 mg LGH	18	10.11 ± .52	14.11 ± .72	15.89 ± .86	16.33 ± 1.25	175.80 ± 3.84	187.89 ± 4.36	199.11 ± 1.90	218.78 ± 6.87
			4	8	12	15		18	20
2 mg LGH	17	11.65 ± .86	15.35 ± .73	17.94 ± 1.26	20.59 ± 1.34	195.76 ± 4.84	204.82 ± 5.77	222.82 ± 7.19	249.41 ± 7.84
		2	5	9	13	16			
3 mg LGH	18	13.72 ± .85	16.78 ± .87	20.44 ± .94	19.89 ± 1.09	189.00 ± 4.91	198.11 ± 5.56	217.67 ± 7.13	240.67 ± 8.25

* All animals were given 1 USP unit of oxytocin before nursing and another USP unit after 15 min of nursing.

† Mean ± standard error.

LGH = Lactogenic hormone injected one-half of the dose every 12 hr.

Level of significance of milk yields and litter weight gain after lactogenic hormone injection compared with controls.

3 *vs* 4, 6 *vs* 7, 17-18 <0.05; 19 *vs* 20 <0.02; 14 *vs* 16 <0.01; 6 *vs* 8 <0.005; 1 *vs* 2, 3 *vs* 5 <0.001; 6 *vs* 9, 10 *vs* 11, 12, 13 >0.001; 14 *vs* 15 >0.001.

Litter weight gain.

14 *vs* 15 >0.001; 17 *vs* 18 <0.05; 19 *vs* 20 <0.02; 14 *vs* 16 <0.01.

% increase compared with controls.

Milk yield. At 1 mg level 7)14, 11)49; at 2 mg level 4)15, 8)29, 12)88; at 3 mg level 2)33, 5)26, 9)47, 13)82.

Litter weight gain. At 2 mg level 15)11, 18)9, 20)11; at 3 mg level 16)10.

TABLE II. Effect of Lactogenic Hormone upon Mammary Gland DNA and RNA in Lactating Rats.

Treatment	No. of rats	Final mean bw (g)	Mean DFFT (mg)	DNA (μg/mg DFFT) mean ± S.E.	Total DNA (mg/100 g bw) mean ± S.E.	% increase over control	Total RNA (mg/100 g bw) mean ± S.E.	% increase over control	RNA/DNA mean ± S.E.	% increase over control
							1		5	
Control	13	286	979.70	30.32 ± 1.26	10.19 ± .31		16.17 ± .95		1.60 ± .10	
							2		6	
1 mg LGH	18	276	1100.55	25.52 ± .35	10.27 ± .35	.79	19.58 ± 1.25	21.08	1.91 ± .10	19.38
							3		7	
2 " "	17	320	1312.50	27.40 ± .71	11.22 ± .34	10.11	19.22 ± 1.53	18.86	1.73 ± .14	8.13
							4		8	
3 " "	18	300	1245.30	26.54 ± .63	11.15 ± .50	9.42	23.06 ± 1.58	42.61	2.10 ± .15	31.25

bw = body wt; DFFT = dry, fat free tissue; S.E. = standard error of mean; LGH = lactogenic hormone.

Level of significance of total RNA and RNA/DNA ratio after lactogenic hormone injection compared with controls respectively.

1 *vs* 2 <0.02; 1 *vs* 3 <0.05; 1 *vs* 4 >0.001; 5 *vs* 6 <0.2; 5 *vs* 7 <0.5; 5 *vs* 8 <0.01.

LGH failed to respond to the hormone on days 14 and 16 of lactation but significantly increased milk yield on days 18 and 20 (Table I). Two mg LGH stimulated a significant increase in milk yield except on day 14 of lactation. The administration of 3 mg LGH stimulated a highly significant increase in milk yield in comparison with the controls from days 14 to 20.

At 1 mg level, lactogenic hormone increased milk yield 14% on day 18 and 49% on day 20; at 2 mg level milk yield increased 15% on day 16, 29% on day 18 and 88% on day 20; at the 3 mg level milk yield increased 33% on day 14, 26% on day 16, 47% on day 18 and 82% on day 20 in comparison with the control group.

The mean litter weights of the rats treated with 1 mg were slightly lower than the controls. At 2 mg level mean litter weights were significantly increased on days 14, 18 and 20 compared with the control. At 3 mg level the mean litter weights were slightly higher than control values.

The mean DNA and RNA of the control group on day 20 were 10.19 $\pm$ 0.31 mg and 16.17 $\pm$ 0.95 mg/100 g bw. The mean DNA and RNA increased in animals treated with 1 mg LGH 1 and 21%; 2 mg LGH 10 and 19%; 3 mg LGH 9 and 43%, respectively (Table II).

In the control group, the RNA/DNA ratio was 1.60 $\pm$ 0.10. The protein synthetic activity per cell (RNA/DNA) increased in rats treated with 1, 2 and 3 mg LGH, 1.91 $\pm$ 0.10, 1.73 $\pm$ 0.14 and 2.10 $\pm$ 0.15, respectively.

Discussion. In a series of studies from this laboratory the effect of a number of hormones on milk secretion has been determined. It was shown first that exogenous oxytocin administered at the time of nursing increased milk removal(5). In subsequent studies, oxytocin was injected to insure complete milk removal, then growth hormone (GH)(5), L-thyroxine (L-T_4)(6), lactogenic hormone (LGH)(7), parathyroid hormone (PTH)(8), insulin(9), corticosterone and aldosterone (10), separately, and a combination of GH + L-T_4 + LGH + PTH(11) were administered. All except LGH(7) stimulated increased milk yields.

In the present study it was observed that the injection of 1 mg LGH from days 7 to 19 of lactation did not increase milk yield on days 14 and 16 but significantly increased the yield on days 18 and 20. Injection of 2 mg LGH increased milk yield significantly on days 16, 18 and 20. Treatment with 3 mg LGH increased milk yield highly significantly at all test periods. At 2 mg LGH level the rats secreted 20.6 g of milk on day 20, which is the highest level ever attained in our laboratory with a single hormone treatment.

The mean litter weight did not increase proportionately according to increases in milk yield. Litter weight gain is not a good index of intense milk yield in rats(12).

The DNA at the end of the lactation period was not significantly altered. This indicates that LGH did not stimulate cell multiplication during lactation. The increased yield of milk was thus due to increased secretion of milk per cell. RNA, which is a measure of protein synthesis, was significantly increased in the treated rats. It thus provides further evidence for the increase in milk yield observed.

Summary. Lactogenic hormone was administered subcutaneously at 1, 2 or 3 mg to lactating rats each day during days 7 to 19. Milk yields were obtained after a 10-hour isolation period of dam from pups on days 14, 16, 18 and 20 of lactation. One mg of lactogenic hormone significantly increased yield on days 18 and 20; 2 mg on days 16, 18 and 20; and 3 mg on days 14, 16, 18 and 20. Maximum stimulation occurred on day 20 with 2 mg and 3 mg of hormone, with 88 and 82% increase over controls, respectively. Determination of deoxyribo- and ribonucleic acids of mammary glands from lactators sacrificed on day 20 revealed no significant increase in DNA but significant increases in RNA after hormone treatment. The data strongly suggest that maximal levels of litter weight size and mammary gland size are limiting in ability to estimate milk secretion potential, while milk yields and RNA values reflect secretory status beyond these limits. Lactogenic hormone at 2 or 3 mg per day stimulates increased lactation more

than any other single hormone treatment attempted to date.

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The Source of Synovial Fluid Alkaline Phosphatase.* (31548)

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Most of the proteins in synovial fluid have been identified with those of serum(1,2). Because of this, as well as similarities in the distribution of electrolytes and other constituents, synovial fluid is often considered to be an ultrafiltrate of serum(3). The passage of serum proteins into the synovial cavity is, however, a selective process which excludes such proteins as macroglobulins and blood clotting factors(2,4). It is doubtful that all synovial fluid proteins are derived from serum. For example, the observation that the specific activity of alkaline phosphatase in bovine synovial fluid is often 100 times higher than in serum(3) would lead one to conclude that the synovial fluid enzyme is not derived from serum. Histological evidence supports the view that the phosphatase is a product of the surrounding connective tissues(5).

Electrophoresis(6-9), sucrose density gradient ultracentrifugation(10) and protein fractionation techniques(11) were used in an effort to establish a relationship of bovine synovial fluid phosphatase with a comparable serum or connective tissue enzyme. The investigations reported here illustrate the use of agar-gel electrophoresis and sucrose density gradient centrifugation for the separation of alkaline phosphatases. Complications resulting from isolation procedures and the breakdown of enzyme and tissue extracts are discussed because of their importance to the general problem of enzyme technology.

Methods. Samples of synovial fluid, free of contamination with blood, were obtained from the astragalotibial joints of yearling heifers or steers.‡ After clarifying the fluid by centrifugation at 1000 × *g* for 45 minutes, hyaluronidase§ (2 mg per 10 ml of fluid) was added to depolymerize the hyaluronic acid. The depolymerized solution was used for subsequent experiments.

The alkaline phosphatase of synovial fluid was purified by a combination of $(NH_4)_2SO_4$ fractionation and ion-exchange chromatography on DEAE- and TEAE-celluloses‖(11). This scheme could not be successfully applied to serum probably because it is a more complex mixture of proteins and contains phosphatases derived from a number of tissues (12).

Because of the low specific activity of alka-

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‡ Standard Beef Co., Detroit, Mich.

§ Nutritional Biochemicals Corp., Cleveland, Ohio (approx. 300 U.S.P. U/mg).

‖ Bio-rad Laboratories, New York.