

Effect of Graded Levels of Insulin on Lactation in the Rat.* (30198)

PERIANNA KUMARESAN AND C. W. TURNER

(With technical assistance of Mary E. Powell and C. L. Wallace)

Department of Dairy Husbandry, University of Missouri, Columbia

In a series of studies from this laboratory, the effect of a number of hormones on milk secretion and removal has been determined. It was shown first that exogenous oxytocin administered at the time of nursing increased milk removal(1). In subsequent studies, oxytocin was injected to insure complete milk removal, then growth hormone (GH)(1), thyroxine (T_4)(2), lactogenic hormone(3), and parathyroid hormone(4) were administered separately and together(5).

The present report is a continuation of this study concerning the effect of graded levels of insulin upon the secretion of milk by the rat from day 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 animal room was maintained at a temperature of $78 \pm 1^\circ\text{F}$. On day 4 postpartum, litters were reduced to 6 young. Protamine zinc insulin suspension (U.S.P.) (Lilly[†]) was diluted in distilled water. From day 7 to 19 postpartum, the dams were injected subcutaneously, once daily, approximately at the same time as follows: 1) 24 controls were injected with distilled water, 2) 16 rats were injected with 1 unit of insulin, 3) 17 rats were injected with 2 units of insulin, 4) 20 rats were injected with 3 units of insulin, 5) 10 rats were injected with 4 units of insulin, and 6) 5 rats were injected with 5 units of insulin. On days 14, 16, 18, and 20 postpartum, 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 in-

jected 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 as previously described(6). The amount of DNA/100 g bw was used as index of extent of mammary gland development.

Results. Lactating rats injected with 1 unit of insulin failed to respond to the hormone (Table I). Two units stimulated a significant increase in milk yield on days 14 and 20. The administration of 3 units stimulated a highly significant increase in milk yield in comparison with the controls from days 14 to 20. However, 3 rats of a group of 20 died of hypoglycemic symptoms. The injection of 4 units produced hypoglycemia and death in 8 out of 10 rats, and 5 units resulted in death of all rats so injected.

At the 3 unit level, insulin increased milk yield 38% on day 14, 25% on day 16, 26% on day 18, and 79% on day 20 in comparison with control group.

The mean litter weights of the control and 3 unit groups on day 14 were the same. On day 16, the insulin group had increased 5%, on day 18, 9%, and on day 20, 7% in comparison with the control group.

The mean DNA of the control group on day 20 was 9.52 ± 0.41 mg/100 g bw whereas the 3 unit insulin injected group had a mean DNA of 10.41 ± 0.41 mg/100 g bw, a non-significant increase over the control group.

Discussion. In the present study it was shown that 3 units of protamine zinc insulin administered subcutaneously daily to Sprague-Dawley-Rolfsmeyer lactating rats stimulated a highly significant increase in milk yield on days 14 to 20 of lactation. This level appears to be optimal for lactating rats although 3 of 20 rats died. Higher levels

* Contribution from Mo. Agri. Exp. Sta. Journal Series No. 2884, approved by the Director. This investigation was supported in part by a grant from USPHS.

[†] Kindly supplied by Eli Lilly and Co., Indianapolis, Ind.

[‡] Oxytocin supplied by Armour Lab., Chicago, Ill.

TABLE I. Effect of Insulin on Milk Yield and Litter Weight Gains in Normal Rats from Day 14 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
24	Control	9.88 ± .51 ¹	11.58 ± .60 ⁴	12.67 ± .67 ⁶	8.60 ± .53 ⁸	177.54 ± 5.65	183.29 ± 6.07 ¹¹	193.00 ± 6.77 ¹³	211.71 ± 8.17 ¹⁵
16	1 unit insulin	10.69 ± .79	11.19 ± .98	12.44 ± 1.11	9.81 ± .71	169.63 ± 5.08	171.38 ± .22	177.50 ± 8.29	192.88 ± 1.03
17	2 units "	12.06 ± .65 ²	12.24 ± .86	12.76 ± 1.04	11.41 ± 1.51 ⁵	164.41 ± 4.72	172.35 ± 6.02	184.59 ± 2.10	203.53 ± 7.27
17	3 units "	13.59 ± .76 ³	14.47 ± .62 ⁵	15.94 ± .66 ⁷	15.35 ± .84 ¹⁰	177.71 ± 1.86	192.88 ± 6.54 ¹²	209.41 ± .80 ¹⁴	223.71 ± 7.65 ¹⁶

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

† Mean ± standard error.

Level of significance of milk yields and litter wt gain after insulin injection compared with controls:

1 vs 2—0.02; 4 vs 5—0.005; 1 vs 3, 6 vs 7, 8 vs 10, 15 vs 16—0.001; 8 vs 9, 11 vs 12—0.05; 13 vs 14—0.01.

% Increase compared at 3 units level with control.

Milk yield: 3) 38%, 5) 25%, 7) 26%, 10) 79%. Litter wt gain: 12) 5%, 14) 9%, 16) 7%.

were not tolerated and lower levels of insulin were only slightly effective.

It has been suggested that hormones which stimulate an increase in milk yield in the rat indicate that the hormone plays a role in the normal lactation process. When the intensity of milk secretion is increased in a group of animals, it suggests that the rate of secretion of the hormone in some of the animals of the group was less than optimal.

It has now been demonstrated that GH, T₄, lactogenic and parathyroid hormones separately and together stimulate increased lactation. To this list insulin may now be added.

While insulin may stimulate increased feed intake, the major effect of insulin on milk secretion may be its effect upon cell permeability and increased availability of glucose by the mammary epithelial cells concerned in the synthesis of lactose. However, the synthesis of milk protein and fat may be accelerated also.

Summary. The daily subcutaneous injection of 3 units of protamine zinc insulin into Sprague-Dawley-Rolfsmeier lactating rats from day 7 to 19 stimulated highly significant increases in milk yield in comparison with the control group. On day 14 milk yield was increased 38%, on day 16, 25%, on day 18, 26%, and on day 20, 79%. The increase in mean litter weight in comparison with the controls was not significant. The mammary glands as measured by DNA were not significantly increased on day 20 by insulin.

1. Grosvenor, C. E., Turner, C. W., Proc. Soc. Exp. Biol. and Med., 1958, v100, 158.
2. ———, *ibid.*, 1958, v100, 162.
3. ———, *ibid.*, 1959, v101, 699.
4. Djojoseobagio, S., Turner, C. W., Endocrinology, 1964, v74, 554.
5. ———, Proc. Soc. Exp. Biol. and Med., 1954, v116, 213.
6. Griffith, D. R., Turner, C. W., *ibid.*, 1959, v102, 619.

Received February 18, 1965. P.S.E.B.M., 1965, v119.