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Effect of Alloxan on Lactation and Replacement Therapy with Insulin in the Rat.* (30395)

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The influence of graded levels of insulin upon the secretion of milk by the rat from day 14 to 20 of lactation has been reported (1). It was shown that daily injection of 3 units of protamine zinc insulin into rats of the Sprague-Dawley-Rolfsmeyer strain increased milk yield 38% on day 14, 25% on day 16, 26% on day 18 and 79% on day 20. It was suggested that insulin plays a role in the lactation process and that the rate of secretion of insulin in this group of rats was less than optimal.

It has been shown that injection of alloxan at a level of 15 mg/100 g body weight (bw) induces a chronic state of diabetes in our strain of rats(2). It was observed during the 13th to 18th days after the injection that feed consumption increased over 50% above the previous level.

The object of the present study was to determine the effect of an alloxan induced deficiency of insulin upon the milk secretion of rats and, if milk secretion was reduced, to determine the level of insulin which would restore lactation to normal or above.

Materials and methods. Fifty lactating rats of the Sprague-Dawley-Rolfsmeyer strain were housed in individual cages, fed Purina lab chow and water *ad libitum*. On day 4 of lactation the litters were reduced to 6, and a single intraperitoneal injection of alloxan (reagent) at a level of 15 mg/100 g bw was administered to induce a chronic state of diabetes(3). Twenty rats treated with alloxan were used as alloxan-treated controls. One-half of the controls were put into metabolism cages to measure feed consumption. Protamine zinc insulin suspension (USP) (Lilly†) was diluted in alkaline saline solution. From day 5 to 19 of lactation the dams were injected subcutaneously, once daily, approximately at the same time as follows: (1) 20 controls were injected with alkaline saline, (2) 20 rats were injected with 2 units of insulin; 5 rats of this group were put into metabolism cages to measure feed consumption, and (3) 10 rats were treated with 3 units of insulin. Milk yields were estimated as previously described(1). On day 20 the dams were killed after nursing, the 6 posterior mammary glands were removed and DNA was determined as described previously (4). The amount of DNA/100 g bw was used as an index of the extent of mammary gland growth.

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Results. When chronic alloxan diabetes was induced in rats on day 4 of lactation, milk yield on day 14 of lactation was reduced 16%, on day 16, 8%, and on day 18, 9%. On day 20 of lactation milk yield of the group on alloxan exceeded the normal controls by 5%. The mean litter weight of the normal control group was 211 g compared to a litter weight of the alloxan treated dams of 180 g (Table I).

When replacement therapy with 2 units of insulin/day was administered to chronic alloxan diabetic lactating rats, milk yield was increased 45% on day 14, 21% on day 16, 22% on day 18, and 61% on day 20 compared to similar rats treated with alloxan alone. Of the group of 20 rats, five died with hypoglycemia symptoms. The mean litter weight of this group was 196 g on day 20.

The mean mammary gland DNA of the alloxan-treated group on day 20 was 10.36 ± 0.56 mg/100 g bw whereas the glands of the rats receiving 2 units of insulin showed a mean DNA of 9.57 ± 0.46 mg/100 g bw, a non-significant difference.

The 10 alloxan-treated rats given 3 units of insulin for replacement therapy all died of hypoglycemia symptoms within a day or two on treatment.

The feed consumption of normal lactating rats has been presented(5). It has been shown also that the feed consumption of alloxan-treated non-lactating rats was first reduced, then increased markedly(2). In the present experiment, the alloxan-treated lactating rats showed greater feed consumption up to the 15th day of lactation in comparison with the control group but consumed the same amount per 100 g bw during the final period (Table II). The group receiving 2 units of insulin was intermediate in feed consumption during the first 15 days between controls and alloxan-treated animals but was similar during the final period.

Discussion. The role of insulin in milk secretion was studied in the present experiment by observing the effect of chronic induction of diabetes by administration of 15 mg of alloxan/100 g bw to rats on day 4 of lactation. While it is not known to what extent the beta cells of the pancreas were

TABLE I. Effect of Alloxan and Replacement Therapy of Rats from Day 5 to 20 of Lactation and Litter Weight Gains.

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	Normal control	9.88 ±.51	11.58 ±.60	12.67 ± .67	8.60 ± .53	177.54 ± 5.65	183.29 ± 6.07	193.00 ± 6.77	211.71 ± 8.17‡
20	Treated with alloxan control	8.30 ±.73[1]	10.70 ±.87[3]	11.55 ± 1.10	9.05 ± 1.03[5]	167.65 ± 6.55	170.20 ± 9.21	173.95 ± 8.52	179.75 ±10.20
15	Treated with alloxan + 2 units insulin	12.07 ±.78[2]	12.93 ±.91[4]	13.93 ± 1.17	14.60 ± 1.43[6]	161.33 ± 6.02	171.53 ± 5.94	180.00 ± 6.56	196.40 ± 8.34
17	Normal, 2 units insulin	12.06 ±.65	12.24 ±.86	12.76 ± 1.04	11.41 ± 1.51	164.41 ± 4.72	172.35 ± 6.02	184.59 ± 2.10	203.53 ± 7.27‡
17	Normal, 3 units insulin	13.59 ±.76	14.47 ±.62	15.94 ± .66	15.35 ± .84	177.71 ± 1.86	192.88 ± 6.54	209.41 ± .80	223.71 ± 7.65‡

* All animals were given 1 USP unit of oxytocin before nursing and another USP unit after 15 min of nursing. † Standard error of mean.
‡ Data from Kumaresan & Turner(1).
Level of significance of milk yields compared with alloxan (control) and treated with alloxan + insulin. [1-2] $P < .001$; [3-4] $P < .05$; [5-6] $P > .005$.
% Compared at normal controls and treated with alloxan controls: Day 14, −16%; Day 16, −8%; Day 18, −9%; Day 20, +5%.
% Increase compared at alloxan treated and alloxan plus insulin treated group. Milk Yield—Day 14, 45%; Day 16, 21%; Day 18, 22%; Day 20, 61%. Litter Wt Gain—Day 14, −4%; Day 18, 3%; Day 20, 9%.

TABLE II. Effect of Lactation on Feed Consumption.

Kind of treatment	Lactation (days)	No. of animals	Feed consumption: Per day (g)	Feed consumption: Per 100 g bw (g)	Increase (%)	Reference
Normal feed consumption	1– 6	34	17.3	6.4		Anderson & Turner, 1963
	7–15	34	29.7	10.7	72	*Idem*
	15–20	34	35.1	13.0	103	"
Alloxan treated	1– 6	10	24.8	8.4		
	7–15	10	36.0	12.0	45	
	15–20	10	40.2	13.0	62	
Alloxan + 2 units of insulin	1– 6	5	21.1	7.4		
	7–15	5	32.7	11.9	61	
	15–20	5	36.2	12.9	75	

destroyed and insulin secretion inhibited, it has been shown that chronic diabetes is induced and feed consumption is increased(2).

Lactation was most markedly influenced on day 14 by alloxan treatment but by day 20 of lactation had returned to a normal level. These data seem to indicate that the presence of low levels of insulin associated with high levels of blood glucose and an increased feed intake do not have a markedly depressing effect on the lactation process.

That insulin does have a positive role in the lactation process was again shown by replacement therapy. When 2 units of insulin/day were administered to the alloxan-treated rats, milk yield was increased on day 14 by 45%, on day 16 by 21%, on day 18 by 22% and on day 20 by 61% compared to the untreated rats. It is interesting to note that their milk yield slightly exceeded the milk yield of normal rats administered 2 units of insulin (Table I).

In normal lactating rats, injection of 3 units of insulin caused the death of 3 of 20 animals due to hypoglycemic symptoms(1). In the present experiment this level of insulin caused the death of all 10 animals so treated. This observation was surprising at the time but in the light of our observations it may have been due to the immediate injection of insulin after the alloxan treatment. For a day or two after treatment, feed consumption was greatly reduced but then increased rapidly so that during the first 6 days feed consumption was greater than in control lactating rats (Table II). It is suggested that if insulin replacement therapy had been delayed for a few days or had been given in increasing amounts, administration of 3 units of insulin would not have resulted fatally.

Summary. Chronic diabetes was induced in rats on day 4 of lactation by intraperitoneal injection of 15 mg/100 g body weight of alloxan. The reduction in insulin secretion caused a reduction of milk secretion of 16% on day 14, of 8% on day 16, and 9% on day 18. On day 20 milk secretion was 5% above the control group. When 2 units of insulin were administered daily, milk secretion increased 45% on day 14, 21% on day 16, 22% on day 18, and 61% on day 20 in comparison with the group treated with alloxan. Their milk production slightly exceeded the production of normal animals injected with 2 units of insulin. The DNA content of the mammary glands of the 2 groups was not significantly different.

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