

fed animals had significantly lower K_m' values than from glucose fed animals. This was noticeable in both the whole homogenate and isolated microsomal fraction. K_m' values obtained from diabetic animals were significantly higher than those from normal animals. All other treatments increasing the liver glucose-6-phosphatase activity produced no significant changes in the kinetic properties of this enzyme.

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Aldosterone and Corticosterone in Maintenance of Lactation in Adrenalectomized Rats.*† (28231)

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Earlier investigations have established that rats adrenalectomized early in lactation are unable to lactate sufficiently to support young (1,2). Maintenance of normal lactation in rats adrenalectomized on day 4 of lactation has been accomplished with daily subcutaneous injections of 500 μ g hydrocorticosterone acetate plus 250 μ g desoxycorticosterone acetate(3). However, the predominant secretions of the adrenal cortex of the rat are aldosterone and corticosterone(4). Cowie and Tindal(5) found that 50 μ g aldosterone per day maintained lactation in adrenalectomized rats at approximately 70% of normal. Corticosterone alone or in combination with other adrenal steroids has not been tested for maintenance of lactation. This investigation was designed to determine minimal levels of these

steroids which would maintain lactation in adrenalectomized rats when injected subcutaneously each day.

Materials and methods. Pregnant Sprague-Dawley-Rolfsmeyer rats were maintained in individual litter cages with free access to pelleted feed and water. The animal room temperature was maintained at $78 \pm 1^\circ\text{F}$. A total of 105 lactating rats was used in this study. On day 4 of lactation the mothers were adrenalectomized or adrenal-ovariectomized in a single operation and litters were standardized to 6 pups. Injections were made subcutaneously each day beginning on day of operation.

Aldosterone-21-acetate† was dissolved in sesame oil and injected in a volume of 0.1 ml per day. Corticosterone-21-acetate was suspended in physiological saline with the aid of

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gum arabic in a concentration of 1 mg per 0.1 ml carrier. Mothers and pups were weighed daily.

Milk production was estimated on day 14 of lactation. The mother was isolated from her pups for 10 hours, then reunited and given 2×1 I.U. of oxytocin intraperitoneally to aid in milk removal. Loss in body weight of the dam and gain in body weight of the pups after a 30-minute period of nursing were averaged to obtain an estimate of milk production.

On the day when all pups in the litter had succumbed or on day 20 of lactation, the mother was sacrificed and autopsied to determine completeness of adrenalectomy and to remove 6 posterior mammary glands for subsequent determination of desoxyribonucleic acid (DNA). Mammary gland DNA was determined as described previously(6). Student's "t" test was used in statistical treatment of the data.

Results. Aldosterone injected daily at 12.5 μ g was unable to support lactation (Table I), while 25 μ g aldosterone per day resulted in no loss of pups. However, the pups were extremely small, and growth was curtailed sharply between days 14 and 20 of lactation. Corticosterone alone at 1 mg per day was not able to support lactation as shown by 47% mortality on day 20. Milk production on day 14 was 65% of normal while the litter growth index was 61% of normal. The combination of 12.5 μ g aldosterone and 1 mg corticosterone per day resulted in loss of 53% of the pups. However, when 25 μ g aldosterone and 1 mg corticosterone were used, the loss of pups by day 20 was only 17%. Furthermore, milk production on day 14 was 81% of normal and litter growth index was 81% of normal. Litter weights on day 14 averaged 93% of normal and 82% of normal on day 20.

In three groups of lactators both adrenal glands and ovaries were removed on day 4. One group received no treatment. By day 14 of lactation all pups were dead in this group. The group maintained on 2 mg of corticosterone per day lost only 14% of the pups to day 20 of lactation. Litter weights averaged 78 and 74% of normal on days 14 and 20, respectively, while milk production on day 14

was 60% and litter growth index was 61% of normal. The doubly-operated group which received 25 μ g aldosterone and 1 mg corticosterone had 27% pup mortality. Litter weights were 94% and 91% of normal on day 14 and 20; milk production on day 14 was 94% of normal while litter growth index was only 73% of normal.

Body weight of the dams increased generally with aldosterone treatment provided it was at least 25 μ g per day, and decreased slightly when corticosterone was given alone.

On day 20 of lactation, normal mammary glands had 11.1 mg DNA/100 g B.W. This was significantly greater than in adrenalectomized groups receiving 12.5 μ g aldosterone or 1 mg corticosterone per day and adrenal-ovariectomized group receiving no treatment.

Discussion. Previous studies using hydrocortisone acetate and desoxycorticosterone acetate to support lactation in adrenalectomized rats revealed that 250 μ g desoxycorticosterone acetate plus 500 μ g hydrocortisone acetate per day in subcutaneous injections administered once daily maintained lactation at a level similar to normal(3). The present study indicates that single daily injections of 25 μ g aldosterone-21-acetate plus 1 mg corticosterone-21-acetate maintain lactation in the adrenalectomized or adrenal-ovariectomized lactating rat at approximately 80% of normal.

Lactation in adrenalectomized rats of this strain terminates at the 12th to 15th day of lactation when adrenal extirpation is performed on day 4. If both adrenals and ovaries are removed on day 4, lactation will cease several days sooner than if the ovaries are left intact. This has been shown by Cowie (7) and repeated in this experiment. Such a finding suggests the ovaries are contributing to maintenance of lactation to a slight degree by production of steroid hormones. Possibly the progesterone of the ovary can be converted to desoxycorticosterone in the absence of the adrenal. Cowie and Tindal(5) found that 50 μ g aldosterone acetate maintained lactation at approximately 70% of normal in the adrenalectomized rat. This was based on the "litter growth index"(8). The present study suggests that 25 μ g aldosterone acetate

TABLE I. Criteria of Lactational Performance in Adrenalectomized or Adrenal-Ovariectomized Rats Receiving Daily Replacement of Aldosterone and/or Corticosterone.

Group	No. of dams	Wt of dams (g)			Wt of litter (g)			Milk prod (g) Day 14 ^b	Litter growth index ^c	Pup mortality		Total DNA (mg/100 g B.W.) Day 20 ^d
		Day 4	Day 14	Day 20	Day 4	Day 14	Day 20			Day 14	Day 20	
Normal control	12	295(12) ^a	283(12)	277(12)	50.8(12)	150.9(12)	211.9(12)	8.8(12)	11.9(12)	2	7	11.1(12)
<i>Adrx day 4 lactation</i>												
No treatment	15	259(15)	248(13)	—	53.4(15)	87.0(13)†	All dead	2.0(13)†	3.9(15)†	16	100	—
12.5 µg aldo	8	267(8)	253(5)	255(1)	52.2(8)	78.3(4)†	"	0.0(4)†	4.8(5)†	63	100	6.4(6)†
25.0 " "	5	267(5)	290(5)	295(5)	49.6(5)	128.0(5)*	135.4(5)†	5.9(5)*	7.2(5)*	0	0	10.7(5)
1 mg cortico	13	275(13)	256(12)	261(9)	55.3(13)	121.0(12)†	156.6(8)*	5.7(12)†	7.3(12)†	13	47	9.5(12)*
12.5 µg aldo	5	274(5)	272(5)	302(2)	54.6(5)	130.2(5)	154.0(3)	6.0(5)*	9.4(5)	10	53	10.3(4)
1 mg cortico	7	261(7)	285(7)	300(6)	52.7(7)	140.3(7)	173.0(6)	7.1(7)	9.6(7)*	0	17	9.7(7)
25.0 µg aldo	1	—	—	—	—	—	—	—	—	—	—	—
<i>Adrx, ovarx day 4 lactation</i>												
No treatment	17	273(17)	—	—	56.4(17)	All dead	—	—	3.2(10)†	100	—	6.9(17)†
2 mg cortico	15	258(15)	235(15)	235(13)	54.9(15)	118.3(15)†	155.9(13)†	5.2(15)†	7.3(15)†	0	14	11.4(15)
25.0 µg aldo	8	276(8)	297(8)	308(6)	57.1(8)	137.4(7)	193.0(6)	8.3(7)	8.6(8)*	20	27	9.7(8)

^a No. of dams or litters used to obtain mean.^b Dams were isolated from pups for 10 hr, then reunited and given 2 × 1 I.U. oxytocin to aid in milk removal. Loss in weight of mother and gain in weight of litter after 30 min of nursing were averaged to determine milk production.^c "Litter growth index" is defined as mean daily gain in weight per litter over the 5-day period from 6th to 11th days (Cowie and Folley, *J. Endocrinol.*, 1947, v5, 9).^d Dams were sacrificed on day 20 of lactation or day when all pups in litter had succumbed.* Significantly less than normal control ($P < .05$),
† ($P < .01$).Adrx = Adrenalectomized
Ovarx = Ovariectomized
Aldo = Aldosterone-21-Acetate
Cortico = Corticosterone-21-Acetate
D = Day of lactation

maintains lactation at 60% of normal, based on the litter growth index plus several additional indices.

It is not possible to measure a daily secretion rate of a hormone by the technique of gland extirpation and single daily injection of the hormone that is normally being secreted constantly from that gland. However, this method does provide an estimate of the relative value of the hormone and the levels required for maintenance of a particular physiological function, lactation in this case.

This study suggests that daily requirements of aldosterone and corticosterone in maintenance of normal lactation in the rat exceed 25 μ g per day for aldosterone and 1 mg per day for corticosterone. The combination of the 2 adrenal steroids acting synergistically appears to be obviously better than either hormone alone.

Summary. Lactating albino rats were adrenalectomized or adrenal-ovariectomized on day 4 of lactation and maintained on aldosterone and/or corticosterone by daily sub-

cutaneous injection until day 20. Criteria of lactational performance, including litter weights on days 14 and 20, milk production on day 14, and litter growth index, indicated that 25 μ g aldosterone acetate and 1 mg corticosterone acetate per day maintained lactation at approximately 80% of the normal level.

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Failure of Secretin or Duodenal Acidification to Inhibit Ethanol-Induced HCl Secretion by Heidenhain Pouches.* (28232)

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Acidification of duodenal contents is a potent stimulus for liberation of secretin which in turn stimulates the pancreas to secrete. Intravenous administration of commercially prepared secretin inhibits the response of Heidenhain pouches to a meal of meat(1), an inhibition not influenced by removal of the pancreas(2). Since acidification of the duodenum can inhibit gastric secretion(3), the suggestion has been made that secretin may mediate the inhibitory effects of acidification of the duodenum on gastric secretion(1). The following tests were done to determine whether acidification of the duodenum and intravenous administration of secretin have sim-

ilar effects on the gastric secretory response to ethanol.

Methods. Vagally denervated (Heidenhain) gastric pouches were prepared in 6 mongrel dogs. Two dogs underwent a second operation in which the pylorus was divided, the duodenal stump was closed, a metal cannula was placed in the duodenum, and the open end of the gastric antrum was anastomosed to the side of the upper part of the jejunum. Tests of secretory function were begun 3 or more weeks after operation when the animals were eating well and were in excellent condition.

The dogs were fasted for 24 hours before each experiment. In all tests gastric secretion was stimulated by instillation into the

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