1. McFall, E., Magasanik, B., *J. Biol. Chem.*, 1960, v235, 2103.
2. Eaton, M. D., Scala, A. R., Jewell, M., *Cancer Research,* 1959, v19, 945.
3. Waymouth, C., *J. Nat. Cancer Inst.*, 1956, v17, 315.
4. Matthews, R. E. F., Smith, J. D., *Advances in Virus Research,* 1955, III, 49.
5. Staehelin, M., *Progress in Medical Virology,* 1959, v2, 1.
6. Hurst, E. W., Hull, R., *Pharmacol. Rev.*, 1956, v8, 226.
7. Visser, D. W., Lagerborg, D. L., Pearson, H. E., PROC. SOC. EXP. BIOL. AND MED., 1956, v93, 61.
8. Hughes, A., *Exp. Cell Res.*, 1952, v3, 108.
9. Biesele, J. J., Berger, R. E., Clarke, M., *Cancer Res.*, 1952, v12, 399.
10. Hakala, M. T., Taylor, E., *J. Biol. Chem.*, 1959, v234, 126.
11. Aronow, L., *Biochim. Biophys. Acta* 1961, v47, 184.

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Effect of Adrenalectomy and Corticoid Replacement on Mammary Gland Growth in Rats.* (27111)

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Evidence concerning the need of adrenocorticoids in normal mammary gland growth is positive but scanty. Lyons *et al.*(1) found that desoxycorticosterone at a level of 100 μg per day synergized with growth hormone and estrone in the hypophysectomized, ovariectomized, adrenalectomized rat to produce mammary growth comparable to that in the intact animal. A similar synergizing action of adrenocorticoids with growth hormone and estrone was reported by Cowie and Lyons (2).

The following experiments were conducted in adrenalectomized-ovariectomized (ADRX-OVARX) rats to determine the role of adrenocorticoids on mammary gland growth. It was felt that use of quantitative DNA index (3) of mammary growth would produce results more meaningful than those reported using qualitative or semi-quantitative morphological technics.

Materials and methods. Mature female albino rats of Sprague-Dawley-Rolfsmeyer strain weighing at least 200 g were maintained on standard laboratory pellet feed at constant temperature of 78 ± 1°F. Adrenalectomy and ovariectomy were performed in one operation. During a post-operative recovery period of 14 days, 1% saline drinking water was provided to maintain electrolyte balance. This was continued in treatment groups except those given desoxycorticosterone acetate (DOCA).

One μg estadiol benzoate (EB) and 3 mg progesterone (P) were injected subcutaneously in 0.1 ml carrier each day for 19 days. The levels and ratio were found by Moon *et al.*(3) to be optimal in rats. Carrier consisted of 10% benzyl alcohol and 90% olive oil.

Adrenocorticoids were suspended in physiological saline with aid of 5 mg gum arabic per 100 mg steroid and injected subcutaneously each day in 0.1 to 0.25 ml carrier. Prednisone (1,2-dehydrocortisone) was injected alone and in combination with EB and P at 100 μg per day. Hydrocortisone acetate (HC) was injected alone at 100 μg per day and in combination with EB and P at 100, 250, and 1000 μg per day. DOCA was injected alone and in combination with EB and P at 250 μg per day.

Animals were sacrificed on the day following the nineteenth day of injection. Six posterior mammary glands were removed and

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TABLE I. Experimental Mammary Gland Growth in Adrenalectomized-Ovariectomized Rats Injected for 19 Days Following 14-Day Post-operative Recovery Period.

Treatment	No. of animals	Body wt		DFFT (mg)	DNA (μg) /mg DFFT	Total DNA (mg)	DNA (mg)/100 g final body wt
		Initial	Final				
Virgin control	8		252	322	20.0	6.59	2.61 ± .31[1]
OVARX control*	15		274	362	23.2	8.40	3.05 ± .14[2]
OVARX†							
1 μg EB; 3 mg P	36		274	604	32.9	19.64	7.19 ± .25[3]
ADRX-OVARX							
With saline							
100 μg pred.	13	215	236	320	17.5	5.47	2.31 ± .10[4]
1 μg EB; 3 mg P	12	269	289	438	31.3	13.41	4.64 ± .24[5]
Idem + 100 μg pred.	11	268	280	566	33.2	18.49	6.63 ± .26[6]
100 μg HC	9	234	257	345	21.9	7.36	2.88 ± .18[7]
1 μg EB; 3 mg P							
Plus 100 μg HC	14	257	267	589	32.4	18.91	7.08 ± .30[8]
" 250 μg HC	21	238	254	549	32.4	17.69	6.96 ± .29[9]
" 1 mg HC	18	242	216	440	26.1	11.22	5.21 ± .30[10]
Without saline							
250 μg DOCA	7	250	252	325	23.8	7.75	3.06 ± .16[11]
1 μg EB; 3 mg P + 250 μg DOCA	16	244	271	384	29.3	11.12	4.09 ± .17[12]

Student's "t"	Probability
1 - 3, 5, 6, 8, 9, 10, 12	.01
2 - 3, 4, 5, 6, 8, 9, 10, 12	.01
5 - 3, 6, 8, 9	.01
10 - 3, 6, 8, 9	.01
11 - 12	.01

* Data from Moon *et al.*(3).
† Accumulated laboratory data(10).

frozen. Glands were extracted with hot 95% ethanol for 6 to 10 hr and with hot ether for 4 to 8 hr. Dried fat-free tissue (DFFT) was weighed and ground. A 25 mg aliquot was used to determine DNA by method of Webb and Levy(4).

Results. Mammary glands in rats sacrificed 34 days after ovariectomy had DNA content of 3.05 ± .14 mg 100 g final body weight as opposed to 2.61 ± .31 mg DNA in virgin control group. Difference in 2 groups was not significantly different (Table I). ADRX-OVARX rats receiving 100 μg prednisone for 19 days had DNA level of 2.31. This was not significantly different from virgin control group, but it was lower than castrates ($P<.01$). Group receiving 100 μg HC per day had DNA level of 2.88 ± .18 and group receiving 250 μg DOCA per day had DNA content of 3.06 ± .16 mg. Neither was significantly different from virgin or OVARX control groups. OVARX rats receiving 1 μg EB and 3 mg P for 19 days had DNA level of 7.19 ± .25 mg per 100 g body weight, while group ADRX-OVARX and given same treatment had only 4.64 ± .24 mg DNA. This difference was highly significant ($P<.01$).

Prednisone at 100 μg per day and HC at 100 or 250 μg per day, when given in conjunction with EB and P, elevated DNA to levels not significantly different from those found in OVARX animals given EB and P and whose adrenals were intact. However, 1 mg HC or 250 μg DOCA per day were not effective in aiding mammary growth of ADRX-OVARX animals given EB and P simultaneously.

Body weight gains were permitted by all treatments of adrenocorticoids except HC at 1 mg per day. In this group an average body weight loss of 26 g occurred.

Discussion. Mammary gland growth as measured by DNA content was reduced significantly in animals when adrenals were removed in spite of injection with EB and P and maintenance of electrolyte balance with saline drinking water. This implicated the

glucocorticoids as essential agents in mammary growth. This was verified when prednisone at 100 μg per day or HC at 100 and 250 μg per day were administered in addition to EB and P and saline to ADRX-OVARX rats, for mammary growth returned to level of OVARX group with intact adrenals given EB and P.

Ability of DOCA to stimulate mammary growth was not confirmed by present experiments because a low level of 250 μg per day was administered. This level was used for its ability to maintain electrolyte balance. Since DOCA has approximately one-third the mammary growth potency of progesterone (5), much greater amounts than used here would be required to confirm the mammogenic action of DOCA reported by other investigators(5,6,7,8,9). Such levels are considered to be far above physiological range.

Action of glucocorticoids is thought to be mammopoietic rather than mammogenic since no mammary growth was obtained with HC or prednisone in absence of EB and P.

HC at 1 mg per day in presence of EB and P caused reduction in body weight and mammary DNA, probably by its gluconeogenic action on body proteins in general. On the other hand, 100 or 250 μg HC per day along with EB and P increased mammary DNA and did not adversely affect body weight. These observations further support the view that action of glucocorticoids on mammary growth is a synergistic one.

Optimal level of glucocorticoids required for mammary growth in rats appears to be between 100 and 250 μg per day.

Summary. Adrenalectomized - ovariectomized (ADRX-OVARX) rats were injected with 1 μg estradiol benzoate (EB) and 3 mg progesterone (P) per day for 19 days. Groups included those maintained on 1% saline drinking water alone and with 100 μg prednisone and 100, 250, or 1000 μg hydrocortisone acetate (HC) per day and on 250 μg desoxycorticosterone acetate (DOCA) per day. Mammary growth was determined by DNA content of 6 posterior glands. Adrenocorticoids in absence of EB and P had no effect on mammary growth. Injection of EB and P plus 100 μg prednisone or 100 or 250 μg HC per day increased DNA significantly above ADRX-OVARX group receiving EB and P plus saline only ($P<.01$) and not significantly different from OVARX group receiving EB and P. Groups receiving 1 mg HC or 250 μg DOCA per day plus EB and P had DNA levels similar to ADRX-OVARX group on EB and P plus saline. The role of adrenocorticoids in mammary growth is considered to be synergistic by action on general metabolism. The term "mammopoietic" is suggested to describe the adrenocortical effect on mammary growth.

1. Lyons, W. R., Li, C. H., Johnson, R. E., *Rec. Prog. in Hormone Res.*, 1958, v14, 219.

2. Cowie, A. T., Lyons, W. R., *J. Endocrinol.*, 1959, v19, 29.

3. Moon, R. C., Griffith, D. R., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 788.

4. Webb, J. N., Levy, H. B., *J. Biol. Chem.*, 1955, v213, 107.

5. Mixner, J. P., Turner, C. W., *Endocrinology*, 1942, v30, 706.

6. Van Heuverswyn, J., Folley, S. J., Gardner, W. U., PROC. SOC. EXP. BIOL. AND MED., 1939, v41, 389.

7. Trentin, J. J., Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bull.* No. 418, 1948.

8. Leonard, S. L., Reece, R. P., *Endocrinology*, 1942, v30, 32.

9. Smith, T. C., Braverman, L. E., *ibid.*, 1953, v52, 311.

10. Accumulated laboratory data.

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