Synergistic Effect of Various Hormones on Experimental Mammary Gland Growth in Mice.* (28349)

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In the initial study of mammary gland growth in the female mouse using DNA as an index of growth, the extent of lobule-alveolar development on the 18th day of pregnancy was determined(1). This was followed by a study of the effect of recurring pregnancy(2). In a subsequent study on the experimental development of the mammary gland of ovariectomized mice, it was shown that 1 μg estradiol benzoate (EB) and 3 mg progesterone (P) administered for 19 days stimulated the greatest degree of gland development, however, it was only 57.6% of the DNA observed at 18 days of pregnancy (3). The synergism of various pituitary preparations with 1 μg EB has been reported also, but none of the preparations approached the DNA level at the end of pregnancy(4).

In an attempt to stimulate mammary gland growth equal to that observed at the end of pregnancy, ovariectomized and adrenalectomized mice were administered EB and P with thyroxine, hydrocortisone, Prednisone, lactogen,‡ and growth‡ hormone in various combinations.

Materials and methods. Mature female albino mice of the Swiss-Webster strain were maintained on a pelleted feed in a room maintained at 78 ± 1°F. Ovariectomized and adrenalectomized animals were given a 14 day recovery period; the latter being maintained on 1% NaCl and 100 μg Prednisone/day.

The various hormones and combinations of hormones were injected subcutaneously daily for 19 days or as indicated in Table I. EB and P were dissolved in 10% benzyl alcohol and 90% olive oil and given in a volume of 0.1 ml/day. L,thyroxine (T_4) was dissolved in alkaline saline and given in a volume of 0.01 ml/3 g BW. A new solution was prepared every third day. Hydrocortisone acetate (HCA) and Prednisone were suspended in physiological saline with aid of gum arabic and injected in 0.1 ml volume. Lactogenic hormone (LGH) was injected in 0.1 (0.5 mg) or 0.2 ml (1.0 mg) and growth hormone (GH) was carried in 0.1 ml. Animals were sacrificed on day 20, 4 posterior and 3 anterior mammary glands were taken for DNA determination as described previously(1).

Results. Ovariectomized mice were injected with 1 μg EB and 3 mg P daily for 19 days plus T_4 at a level of 3 μg/100 g BW. The addition of T_4 increased mammary gland growth as measured by DNA, 12.5% over the growth stimulated by EB + P alone. The addition of HCA at 100 μg/day increased the DNA of the mammary glands by 7%. A combination of T_4 and HCA resulted in a significant increase in DNA ($P<.02$) of 17%.

LGH at 0.5 or 1.0 mg/day for the last 10 days of EB + B administration did not stimulate additional growth. LGH at 0.5 mg + 50 μg HCA was also ineffective. LGH at 1.0 mg + GH at 0.5 mg/day from day 10 to 19, and 15 to 19, respectively, were not beneficial to the growth response. Thus, these 2 pituitary hormones appear not to contribute to mammary gland growth at this time.

In these studies EB and P were maintained at a ratio of 1 to 3000. When EB was increased from 1 to 2 and 4 μg during successive 6 day periods and P was reduced from 3 to 1.5 and 0.75 mg, mammary gland DNA was not increased. However, when 100 μg

* Contribution from Mo. Agr. Exp. Sta. Journal Series No. 2552. Approved by the Director.

† Supported in part by grants from USPHS and Am. Cancer Soc.

‡ Lactogenic and growth hormones kindly supplied by NIH.

TABLE I. Mammary Gland DNA Levels Induced Experimentally in Ovariectomized Mice with Daily Injections of Estradiol Benzoate, Progesterone, Thyroxine, Hydrocortisone Acetate, Prednisone, and Lactogenic Hormone over a 19-Day Injection Period.

Treatment group	No. of mice	DFFT,* mg	DNA/mg of DFFT, μg	Total DNA, mg	Final mean body wt, g	Total DNA /100 g final body wt, mg
Intact virgins	28	43.3	41.2	1.76 ± .07	29.5	5.93 ± .16[1]
33-days post-ovariectomy	12	53.8	37.5	1.95 ± .17	32.3	6.01 ± .30[2]
Ovariectomized						
1 μg EB; 3 μg P	27	78.7	40.7	3.20 ± .19	32.4	9.73 ± .43[3]
Idem + 1 μg T_4/100 g BW	16	106.1	28.4	2.98 ± .16	32.8	9.09 ± .33[4]
" + 2 μg T_4	16	99.1	33.1	3.27 ± .18	33.6	9.65 ± .35[5]
" + 3 μg T_4	19	117.4	30.3	3.39 ± .20	30.7	10.95 ± .43[6]
" + 100 μg HCA	21	101.4	28.2	2.83 ± .17	27.4	10.39 ± .56[7]
" + 100 μg HCA + 3 μg T_4	25	97.4	32.5	3.25 ± .19	28.4	11.30 ± .44[8]
" + .5 mg LGH (D-10 to D-19)	15	86.1	30.6	2.60 ± .20	27.6	9.39 ± .48[10]
" + *idem* + 50 μg HCA (D-15 to D-19)	13	66.9	35.8	2.50 ± .14	28.5	8.81 ± .37[9]
" + .5 mg growth H (D-15 to D-19) + 1.0 mg LGH (D-10 to D-19)	15	73.7	32.7	2.39 ± .15	26.2	9.10 ± .30[11]
1 μg EB; 3 mg P-7-D 2 μg EB; 1.5 mg P-6-D 4 μg EB; .75 mg P-6-D	13	87.7	34.4	3.03 ± .22	32.4	9.42 ±0.70[12]
Idem + 100 μg HCA-19D	27	116.0	34.4	4.00 ± .25	35.4	11.26 ±0.83[13]
Adrenalectomized; ovariectomized						
1 μg EB; 3 μg P + 100 μg Prednisone + 1% NaCl water	19	93.1	27.6	2.57 ± .19	25.8	9.93 ± .59[14]
1 μg EB; 3 μg P-7-D 2 μg EB; 1.5 μg P-6-D 4 μg EB; .75 μg P-6-D + 100 μg Prednisone-19-D + 1% NaCl water	18	109.1	31.1	3.44 ± .28	29.2	11.67 ± .72[15]
18-days pregnant	28	123.5	52.0	6.22 ± .37	37.4	16.90 ± .69[16]

* Dry fat-free tissue.

Student's "t"	*Probability*
1, 2 *vs* 3 to 15	.01
3 *vs* 8, 15	.02
3 *vs* 13	.05
1 to 15 *vs* 16	.01

HCA was added, DNA increased 16%.

In ovariectomized and adrenalectomized mice maintained on 1% NaCl, 100 μg Prednisone/day with changing ratios of EB + P stimulated the greatest increase in DNA 11.67 mg/100 g BW or 69% of the pregnant level.

It should be pointed out that daily injections of 0.2 to 0.3 ml of hormone in these experiments may have caused a mild stress in the mice.

Summary. When 1 μg estradiol benzoate and 3 mg progesterone were administered to ovariectomized mice for 19 days, mammary gland development as measured by DNA was only 57.6% of that observed in mice on day 18 of pregnancy. Addition of thyroxine at level of 3 μg/100 g BW increased the DNA by 12.5%. Hydrocortisone acetate at a level of 100 μg/day increased DNA by 7% while the combination of the two hormones increased DNA by 17%. Lactogenic and growth hormones were ineffective as synergists. The highest level of DNA was obtained in ovari- and adrenalectomized animals given 100 μg Prednisone and varied EB and P, an increase to 11.67 mg/100 g BW or 19.9%.

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Received March 14, 1963. P.S.E.B.M., 1963, v113.

Activity of Malic Dehydrogenase and DPN-Cytochrome-C-Reductase of Liver Mitochondria in Rats Treated with Cortisone and with an Anabolic Androgen.* (28350)

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It is apparent that hormones may modify the activity of intracellular enzyme systems. Because of known metabolic antagonism between cortisone and some anabolic steroids, it was considered possible that these two groups of hormones may differ in their action on respiratory enzymes(1). It was found previously that both C^{21} and C^{19} steroids significantly depressed Qo_2 of various tissues in rats *in vivo*(2,3). To localize the site of enzymatic impairment of respiration, further studies were made on liver mitochondria which are known to contain respiratory enzymes(4,5). It was found that the succinic dehydrogenase activity of these mitochondria was depressed in cortisone treated rats, but was not affected by anabolic steroid Methandrostenolone. Cytochrome oxidase activity was, however, not affected by the C^{21} and C^{19} steroids studied(1). It was then evident that a difference between cortisone and an anabolizer does exist as far as their effect on the activity of a respiratory enzyme of Krebs cycle is concerned. We were, therefore, interested to explore this problem further.

In the present study, rats were treated with cortisone and/or an anabolic androgen. Malic dehydrogenase and DPN-cytochrome-c-reductase of these animals were determined.

* Study supported by grants from the National Research Council of Canada and Ciba Co. of Canada. Mrs. M. E. Shargool is thanked for technical assistance. Cortisone and Methandrostenolone were supplied by Merck Co. (Canada) and Ciba Co. (Canada), respectively.

Methods. Male Sprague-Dawley rats, weighing 250-275 g, kept in individual cages and fed special grain diet(6), were used for this experiment. Animals were treated with Cortisone Acetate (Merck) and Methandrostenolone (Ciba) *per os,* in the form of crushed tablets mixed with food(7). Five groups of rats were treated for 14 days as follows: 1. Normal controls, no treatment. 2. Given cortisone, 5 mg daily/rat. 3. Given cortisone, 5 mg daily/rat and then the treatment discontinued for 14 days. 4. Given Methandrostenolone, 5 mg daily/rat. 5. Treated stimultaneously with cortisone and Methandrostenolone, each steroid 5 mg daily/rat.

At the end of the experiment the rats were lightly anaesthetized with ether. Their livers, *in situ,* were perfused with physiological saline, dissected, rinsed in ice cold saline and placed in ice cold 0.25 M sucrose solution. Mitochondria were separated immediately(8,9). Two grams of liver were homogenized with 0.25 M sucrose solution (5 × weight of sample) in a Virtis "45" homogenizer, and after decantation the flask was washed with the same volume of sucrose solution. The combined solutions were centrifuged in a Spinco Model-L ultracentrifuge at 700 × *g* for 10 minutes. The supernatant was decanted and the residue washed twice with 0.25 M sucrose solution (2½ × weight of sample) and recentrifuged as previously. Supernatant and washings combined were then centrifuged again at 10,000 × *g* for 10 minutes. The supernatant was de-