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Effect of Various Hormones on Mammary Gland Growth of Ovariectomized Rats. (32144)

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Study of the hormones which stimulate mammary gland growth has been aided greatly by estimation of the deoxyribonucleic acid (DNA) content of the glands. It has been shown that pregnant rats at the end of pregnancy and ovariectomized rats stimulated with estradiol benzoate (EB) plus progesterone (P) show great variation in the total DNA/100 g bw. The theory has been advanced that part of the variation in the total DNA of the glands of individual rats is due to variation in the secretion rates of various hormones from the pituitary and the placentae of pregnant rats.

In a previous study(1) the mean total DNA of the mammary glands of rats at the end of pregnancy was 7.41 mg/100 g bw. In the present study (Table I) the mean total DNA of a group of ovariectomized rats administered 2 μg EB + 6 mg P for 19 days was 5.57 mg; thus, the pregnant animals showed 33% greater DNA than the rats administered the 2 ovarian hormones. While it is believed the EB + P are of primary importance in stimulating growth of the mammary gland during pregnancy, these data suggest that these hormones may not be able to stimulate increased secretion of other hormones in the absence of the placenta. These other hormones may synergize with EB + P in pregnant animals to increase the DNA of the mammary glands. If the administration of an exogenous hormone has a greater synergistic effect upon DNA in the ovariectomized rats than upon the pregnant animal, it would suggest that the pregnant condition is stimulating the secretion of more endogenous hormone.

In a previous report the effect of various hormones on mammary gland growth of pregnant rats was presented(1). The object of the present report is to present data on the effect of these same hormones in synergism with EB + P on the DNA of the mammary glands of ovariectomized rats.

Materials and methods. Groups of adult ovariectomized rats of the Sprague-Dawley-Rolfsmeyer strain with a mean body weight of 245 g were maintained in a room at 78 $\pm$ 1°F. Purina Lab Chow with an energy value of 4.41 calories per gram and 23.4% total protein was fed during control and experimental periods. Ovariectomized rats were allowed 7 days for recovery and were sacrificed on day 27 after castration.

EB and P were dissolved in sesame oil (USP) and injected daily subcutaneously in 0.2 ml of oil for 19 days. The other hormones were dissolved in alkaline physiological saline solution and were injected subcutaneously daily at approximately the same time: protamine zinc insulin‡ 1 unit/0.1 ml, L-T_4 3 μg/0.1 ml/100 g bw, and GH 1 mg/0.1 ml. Bovine growth hormone (GH)§ was injected in increasing doses of 1 mg from day 1 to 6, 2 mg from day 7 to 12, and 3 mg from

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‡ Kindly supplied by Eli Lilly Co., Indianapolis, Ind.

§ Kindly supplied by NIH, Bethesda, Md. It was reported to contain 1.17 μ/mg GH and contaminated with 0.4 μ/mg of lactogenic hormone.

TABLE I. DNA Content of Mammary Glands of Ovariectomized Rats Injected with Various Hormones.

	Treatment	No. of rats	Mean body wt (g) Initial	Mean body wt (g) Final	DFFT (mg) mean	Total DNA (μg/mg DFFT) mean ± S.E.	Total DNA (mg /100 g body wt) mean ± S.E.	% Increase over control DNA/100 g body wt based on initial mean body wt
1.	2 μg EB + 6 mg P (control)	28	242	248	459	13.47 ± .64	5.50 ± .24[1]	
2.	*Idem* + L-T_4 3 μg/100 g body wt	21	240	248	434	14.32 ± .63	5.97 ± .29[2]	7
3.	" + insulin 3 units	18	228	243	493	15.72 ± 1.34	6.89 ± .57[3]	24
4.	" + L-T_4 + insulin	18	241	254	487	14.83 ± .57	6.15 ± .27[4]	10
5.	" + GH + insulin	12	253	299	586	19.27 ± .75	7.62 ± .27[5]	37
6.	" + L-T_4 + GH + insulin	11	255	297	600	21.46 ± 1.06	8.41 ± .37[6]	51

DFFT = Dry, fat-free tissue. S.E. = Standard error of mean. EB = Estradiol benzoate; P = Progesterone; L-T_4 = L-thyroxine; GH = Bovine growth hormone.

"Student's" t probability comparisons of treatment means with the mean of the control group. N.S. = Not significant.

[1] *vs* [2]	N.S.	[1] *vs* [6]	<.001	[4] *vs* [5]	N.S.
[1] *vs* [3]	<.05	[2] *vs* [3]	N.S.	[4] *vs* [6]	>.001
[1] *vs* [4]	N.S.	[2] *vs* [4]	N.S.	[5] *vs* [6]	<.02
[1] *vs* [5]	<.001	[2] *vs* [5]	<.001	[3] *vs* [6]	>.001

day 13 to 19 in combination with other hormones.

The animals were injected as follows: (1) 28 rats received 2 μg EB + 6 mg P + 0.5 ml of alkaline saline; (2) 21 rats received EB + P + 3 μg/100 g bw of L-T_4; (3) 18 rats received EB + P + 3 units of insulin; (4) 18 rats were given EB + P + 3 μg L-T_4/100 g bw + 3 μ of insulin; (5) 12 rats received EB + P + bovine GH + 3 μ of insulin; (6) 11 rats received EB + P + 3 μg L-T_4/100 g bw + GH + 3 μ of insulin. The rats were sacrificed one day after the last injection and 6 abdominal-inguinal glands removed and DNA determined by the Webb-Levy method(2) previously described using highly polymerized DNA as a standard.‖ The initial body weight was used in determination of total DNA/100 g bw.

Results. The mean mammary DNA of the control rats injected with 2 μg EB plus 6 mg P based on the initial body weight was 5.57 ± 0.24 mg/100 g bw (Table I). Injection of EB + P + 3 μg L-T_4/100 g bw increased mean DNA to 5.97 ± 0.29 mg/100 g bw, a non-significant increase of 7% over the controls. Three units of protamine zinc insulin plus EB + P increased the mean DNA to 6.89 ± 0.57 mg/100 g bw, a significant increase of 24%. The combination of EB + P + L-T_4 and insulin increased the mean DNA to 6.15 ± 0.27 mg/100 g bw, a non-significant increase of 10%. EB + P plus graded levels of bovine GH plus insulin increased the mean DNA to 7.62 ± 0.27 mg/100 g bw, a significant increase of 37%. The combination of EB + P + GH + insulin + L-T_4 increased the mean DNA to 8.40 ± 0.37 mg/100 g bw, a highly significant increase of 51%.

Discussion. The injection of 2 μg EB + 6 mg P daily for 19 days stimulated the greatest growth of the lobulo-alveolar system (as measured by DNA) in ovariectomized rats(4).

In pregnant rats, the injection of GH had a non-significant effect upon mammary gland DNA, whereas in the ovariectomized rats GH + EB + P stimulated gland growth 53%(3). This suggests that increased GH may be secreted during pregnancy but not by

‖ From Nutritional Biochem. Corp.

the injection of EB + P. Similarly, insulin had little effect on gland growth in pregnant rats(1) but in ovariectomized rats, a significant 24% increase in DNA was observed in synergism with EB + P. In these studies L-T_4 had little effect in either type of animals. While GH stimulated a significant 13% increase, insulin had little effect in pregnant animals when administered separately. The combination of the two hormones had a significant mammary growth stimulating effect of 62% in pregnant(1) and 37% in ovariectomized rats. Similarly, while GH and L-T_4 had little value separately in pregnant animals, the combination stimulated an increase of 29% (3) when the latter were stimulated also with EB + P.

The combination of insulin and L-T_4 stimulated slight growth in both types of animals. Finally, the combination of GH, L-T_4 and insulin stimulated a significant 41% increase in DNA in pregnant animals(1) and a significant increase of 51% in ovariectomized rats in synergism with EB + P.

With the exception of GH alone and with L-T_4, the mean DNA of the pregnant rats considerably exceeded the mean DNA of the ovariectomized rats. This observation suggests that the pregnant state stimulates the increased secretion of endogenous hormones which are not stimulated by EB + P.

Summary. The synergistic effect of various hormones upon mammary gland growth has been studied in adult ovariectomized rats stimulated with estradiol benzoate (EB) and progesterone (P) for 19 days. The mean deoxyribonucleic acid (DNA) of 28 control rats was 5.57 mg/100 g bw. When treated with EB and P plus 3 units of insulin, a mean DNA of 6.89 mg/100 g bw, a significant increase of 24% ($P<0.05$) was observed. EB and P plus growth hormone (GH) plus protamine zinc insulin increased the mean DNA to 7.62 mg/100 g bw, a highly significant increase of 37% ($P<0.001$). When EB + P + GH + insulin + L-thyroxine (L-T_4) were added, a mean DNA of 8.40 mg/100 g bw was observed, a highly significant increase of 51% ($P<0.001$). Non-significant increases in DNA were observed with L-T_4 and with L-T_4 plus protamine zinc insulin.

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Erythropoietic Recovery of Chronically Radiated Rats. II. Response to Phenylhydrazine. (32145)

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Sustained depression of erythropoiesis and development of progressive anemia during continuous exposure to irradiation has been well documented by Lamerton(1). Resumption of erythropoiesis during continued irradiation was, however, observed by Brambel *et al* who exposed rats to 70 R per day(2). After 5 to 6 weeks of exposure, there was a rapid increase of normoblasts in the spleen, followed shortly thereafter by a sustained reticulocytosis and stabilization of the hemoglobin level in the peripheral blood. Brambel *et al* suggested that the observed resumption of erythropoiesis during continued irradiation might represent an adaptation phenomenon. The present paper will adduce evidence for the alternative hypothesis that erythropoiesis develops in the spleen in response to a severe degree of anemia which develops at about 5 weeks of chronic radiation. To test this hypothesis, severe anemia was produced early in the course of chronic radiation by the in-