

A Comparison of 20 β -hydroxy-pregn-4-ene-3-one and Progesterone on Mammary Gland Growth of the Rat* (33466)

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Until recently it was suggested that progesterone (P) was the only naturally occurring hormone with progestational properties. However, two related compounds were isolated (1, 2) from human ripe follicles, corpora lutea, and placentae which are termed 4-pregnene-20 α -ol-3-one and 4-pregnene-20 β -ol-3-one or "20 α -ol" and "20 β -ol" in abbreviated form.

The biological activity of 20 α -ol was reported (2) to be 1/5 that of progesterone by the Hooker-Forbes assay, 1/3 to 1/2 by the Clauberg rabbit assay and less active than progesterone in women. The 20 β -ol was reported (2) to be twice as active as progesterone by the Hooker-Forbes assay, 1/5 to 1/10 by the Clauberg assay, and less active in women. Lawritzen (3) reported that it was 1/5 as active by the Clauberg assay and about 1/2 as active in women.

In a recent study (4) on the value of 20 α -ol and 20 β -ol to maintain pregnancy in ovariectomized rats, it was reported that the two epimers when injected alone were less than 1/15 as active as progesterone. When administered with estradiol, 20 β -ol produced, qualitatively, the same effects as progesterone but was only 1/15 as active.

From the data on the biological activity of these two compounds, it appears that they are much less active than progesterone. However, no one has studied the value of these compounds to substitute for progesterone (P) in the growth of the mammary glands of ovariectomized rats when injected with estradiol benzoate (EB) as measured by an increase in DNA.

Materials and Methods. Adult ovariectomized rats of the Sprague-Dawley-Rolfsmeyer strain were maintained in a room at 25.6° with 14 hr of light and 10 hr of darkness.

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Purina lab chow with an energy value of 4.41 cal/g and 23.4% total protein was fed during control and experimental periods. The EB, P, and 20 β -ol¹ were dissolved in sesame oil (USP) 1 μ g of EB/0.1 ml of oil, 3 mg of P/0.1 ml of oil, and 3 mg of 20 β -ol/0.1 ml of oil; and were injected subcutaneously daily for 19 days.

The animals were injected as follows: (i) 18 rats received 1 μ g of EB as a control group; (ii) 22 rats received 1 μ g of EB + 3 mg of P; (iii) 18 rats received 1 μ g of EB + 3 mg of 20 β -ol; (iv) 18 rats received 1 μ g of EB + 6 mg of 20 β -ol; and (v) 18 rats received 1 μ g of EB + 9 mg of 20 β -ol. The treated rats were sacrificed 1 day after the last injection; and six abdominal-inguinal glands were removed and DNA was determined by the Webb and Levy method (5). The amount of DNA/100 g of body wt. was used as an index of extent of mammary gland development.

Results. The administration of 1 μ g EB in ovariectomized rats for 19 days alone stimulated a mean DNA of 2.95 \pm 0.12 mg/100 g of body wt. (Table I). When injected with 1 μ g of EB + 3 mg of P, the mean DNA increased to 5.83 \pm 0.21. When 3, 6, and 9 mg of 20 β -ol were added to 1 μ g of EB, the mean DNA increased to 3.35 \pm 0.13, a significant increase of 14% ($p < 0.01$); to 3.86 \pm 0.17, a significant increase of 28% ($p > 0.001$) and to 3.97 \pm 0.23, a highly significant increase of 35% ($p > 0.001$), respectively, over the estradiol treated control group. However, in relation to the mean DNA stimulated by the injection of EB + P, the graded levels of 3, 6, and 9 mg of 20 β -ol stimulated 43, 34, and 32%, respectively, less DNA than did P at the 3 mg level.

Discussion. In previous studies from this laboratory, it has been shown that growth of

¹ The 20 β -ol was obtained from Mann Research Laboratory, New York.

TABLE I. Effect of 20β -ol upon Mammary Gland Growth in Ovariectomized Rats.

No. of animals	Treatment	Mean body wt. (g)	DFFT ^a mean (mg)	Mean DNA (μ g/mg of DFFT)	Total DNA (mean \pm SE; mg/100 g of body wt.)
18	Control I (1 μ g of EB)	261	364.61	21.88 \pm 0.52	2.95 \pm 0.12
22	Control II (1 μ g of EB + 3 mg of P)	262	570.38	26.90 \pm 0.57	5.83 \pm 0.21
18	1 μ g of EB + 3 mg of 20β -ol	269	387.56	23.25 \pm 9.65	3.35 \pm 0.13
18	1 μ g of EB + 6 mg of 20β -ol	275	416.33	25.58 \pm 0.60	3.86 \pm 0.17
18	1 μ g of EB + 9 mg of 20β -ol	271	451.33	23.90 \pm 0.58	3.97 \pm 0.23

^a DFFT = dry, fat-free tissue.

the lobulo-alveolar system of the rat mammary gland which occurs during normal pregnancy can be stimulated experimentally in ovariectomized rats by the combination of EB 1 μ g and P 3 mg/day for 19 days (6, 7). This growth is estimated by the mean increase in DNA of the mammary glands of such animals in comparison with control ovariectomized rats.

The discovery of 20α -ol in the ovaries and blood of rats in the last third of pregnancy (8) and of 20β -ol as well in ovarian venous blood during pregnancy (9) raises the question of their biological activity in stimulating the growth of the mammary glands comparable to that occurring during pregnancy which is stimulated by P. The present study shows that 20β -ol is far less active than P. At 3 times the level of P, 20β -ol stimulated only 68% as much mammary gland DNA as did P. It is estimated that 20β -ol is less than 1/9 as active as P.

It is interesting to note that negligible quantities of 20β -ol are present in rat ovarian blood at any time of the ovarian cycle but during pregnancy all three progestogens have been found to occur in fixed ratios of P to 20α -ol of 3.8:1 and of P to 20β -ol of 5.0:1 (9). From the present study, it appears that 20β -ol as well as P can contribute to mammary gland growth of the rat.

Summary. A comparison is presented of the biological activity of progesterone and 20β -hydroxy-pregn-4-ene-3-one with estradiol benzoate upon mammary gland growth of the ovariectomized rat. When 1 μ g of estradiol benzoate (EB) plus 3 mg of progesterone (P) was injected, the mean DNA was 5.83 mg/100 g of body wt. When 1 μ g of EB was combined with 3, 6, and 9 mg of 20β -ol, the mean DNA was 43, 34, and 32%, respectively, less. At the 9-mg level of 20β -ol only 68% as much mammary gland was stimulated as by 3 mg of P.

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