an inhibitory response. Preliminary studies with I^{131} labeled DDIH indicate however that the major portion of injected DDIH is excreted via the urine in an unchanged form. If the compound is inhibitory *per se* this would indicate that an inhibitor of the deiodinase responsible for thyroxine degradation need not possess a free hydroxyl group. This possibility has already been suggested by earlier authors(1).

Sheahan *et al.*(6) have generalized as to the structures of compounds which exhibit BHDB characteristics. They suggest that one of structures A or B of Fig. 4 is required for BHDB-like action. Our data would indicate that structure C must now be added to this group of antithyroid substances.

Summary. The diacetyl derivative of 2,6-diiodohydroquinone has been shown to behave as an antithyroid substance. The available evidence would suggest that the compound inhibits the deiodinase responsible for conversion of thyroxine to triiodothyronine.

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Received June 26, 1961. P.S.E.B.M., 1961, v107.

Effect of Pregnenolone, Progesterone, and Derived Metabolites on Mammary Gland Growth in Rats.* (26821)

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A number of steroid compounds have been shown to have varying mammary gland growth promoting (mammogenic) capacities when injected into ovariectomized mice(1). Differences in extent of lobule-alveolar growth were evaluated by visual examination of whole mounts of glands, and criterion of response was based on percentage of mice showing a minimum amount of lobule-alveolar growth after 10 daily subcutaneous injections of material to be assayed. More recently, improvements have been made in the method of measurement of mammary gland growth of mouse (2) and rat(3), using desoxyribosenucleic acid (DNA) as an index of normal and experimental mammary gland growth. With these methods and employing a 19-day injection period (normal gestation) it is now possible objectively to quantitate amount of mammary gland growth resulting from injection of a given amount of steroid.

Recent studies(4) have established a steroidogenetic pathway involving the following transformations: pregnenolone → progesterone → 17α-hydroxyprogesterone → androstenedione → testosterone. Progesterone in synergism with estradiol has been shown to stimulate mammary gland growth in rats equal to that stimulated during pregnancy as measured by total DNA(3). It seemed of interest to determine whether pregnenolone would be converted to progesterone efficiently in ovariectomized rat as measured by mammary gland growth and further to determine whether steroids involved in the biosynthetic transformation of progesterone to androgens showed mammary gland stimulating properties which might result from these further biogenetic changes.

Materials and methods. Sprague-Dawley-Rolfsmeyer rats with an initial weight of 220-250 g were ovariectomized. Animals killed 35

* Contribution from the Mo. Agri. Exp. Sta. Journal Series No. 2334. Approved by the Director. Supported in part by grants from Am. Cancer Soc. and U. S. Public Health Service.

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TABLE I. Effect of Pregnenolone, Progesterone, and Derived Metabolites on Rat Mammary Gland DNA.

Treatment	No. of animals	Body wt (g) mean	DFFT* (mg) mean	DNA (μg/mg DFFT)	Total DNA (mg/100 g BW)
Castrate controls	8	282	682	11.6	2.807 ± .22
EB, 1 μg	12	304	823	13.0	3.523 ± .18
EB, 1 μg + P, 3 mg	10	318	1499	15.4	7.009 ± .34
Idem	17	278	618	30.2	6.66 ± .46†
"	19	269	589	35.7	7.72 ± .25‡
EB, 1 μg + pregnenolone, 3 mg	9	267	824	14.7	4.535 ± .36
EB, 1 μg + 17α-OH-progesterone, 3 mg	10	316	1089	12.9	4.448 ± .17
EB, 1 μg + androstenedione, 3 mg	10	289	998	13.8	4.772 ± .42
EB, 1 μg + testosterone propionate, 3 mg	9	282	748	17.6	4.673 ± .27

* DFFT = dry, fat-free tissue. † Data from(5). ‡ Data from(3).

days post-castration served as controls. Remaining animals were divided into groups receiving 1 μg estradiol benzoate (EB), 1 μg EB plus 3 mg progesterone (P), 1 μg EB plus 3 mg pregnenolone, 1 μg EB plus 3 mg 17α-hydroxyprogesterone, 1 μg EB plus 3 mg Δ^4-androstene-3,17-dione (androstenedione), and 1 μg EB plus 3 mg testosterone propionate daily for 19 days commencing 14 days after castration. Steroids were dissolved in propylene glycol such that 0.1 ml solution was injected daily in each rat. Groups were killed one day following last injection, skinned rapidly and 6 abdomino-inguinal glands removed and frozen. DNA was determined as described previously(3). Visual observations were made for presence of lobule-alveolar development but no attempt was made to subjectively quantitate extent of growth by this method.

Results. Total DNA produced by injection of 1 μg EB daily for 19 days differed significantly from DNA observed in castrate controls ($P < .001$) (Table I). P injected in synergism with EB, produced total DNA of approximately same order as observed in previous studies, and differed significantly from DNA observed in rats receiving EB alone ($P < .001$). All groups receiving pregnenolone or metabolites of progesterone plus EB had total DNA which was significantly higher than DNA in rats receiving EB alone ($P < .05$) but lower than in rats receiving EB plus P ($P < .01$). Groups receiving EB plus pregnenolone, 17α-hydroxyprogesterone, androstenedione and testosterone propionate produced 29.0%, 26.5%, 35.8% and 33.0% of growth produced by EB plus P over that produced by injection of EB alone. Variable but definite lobule-alveolar development was observed in groups receiving EB plus progesterone, pregnenolone, 17α-hydroxyprogesterone, androstenedione, and testosterone.

Discussion. Previous study(1) has shown that a number of steroids are effective to variable degrees in production of lobule-alveolar mammary gland growth in mice. The present study confirms these findings in rats, and indicates that, of compounds tested, progesterone is most effective. One objective of this study was to elucidate configurations among naturally occurring neutral steroids necessary for stimulation of maximum mammary gland lobule-alveolar growth. Since progesterone is most active, it is evident that most potent biological activity is related to following steroid configurations: (a) C_{21} nucleus, (b) Δ^4-3-ketone, and (c) C-20 ketone. From other compounds tested, it would appear that replacement of any of these groupings with following configurations will decrease mammogenic activity: (a) Δ^5-3β hydroxyl grouping, (b) 17α-hydroxyl, (c) 17-ketone group and (d) C_{19}, C-17 propionate.

From steroidogenetic point of view, it would appear that pregnenolone could promote mammogenesis by one of two mechanisms. Either its action is attributable to partial conversion to progesterone, or it is active *per se*. In either case, it would appear that the Δ^5-3β

hydroxy grouping of pregnenolone is detrimental to the molecule's mammogenic action, for replacement of this group by the Δ^4-3 ketone group (as in progesterone) greatly increases its mammogenic potential.

There is no evidence indicating conversion of 17α-hydroxyprogesterone, androstenedione, or testosterone propionate to progesterone. Results indicated that these compounds promoted considerably reduced, although definite mammary gland lobule-alveolar development compared to EB and P. It would seem entirely possible that these metabolites of P produced in normal biogenetic pathway, may be responsible in part for mammary gland lobule-alveolar growth observed in rats injected with P.

Summary. Compounds in a steroidogenetic pathway (pregnenolone → progesterone (P) → 17α-hydroxyprogesterone → androstenedione → testosterone propionate) were tested to determine their mammogenic effectiveness (as determined by desoxyribosenucleic (DNA) measurement) in ovariectomized rat when injected with estradiol benzoate (EB). Results indicated that EB plus P produced greatest total mammary gland DNA. EB plus pregnenolone, 17α-hydroxyprogesterone, androstenedione or testosterone propionate produced 29.0%, 26.5%, 35.8%, and 33.0% of DNA produced by EB plus P over that produced by injection of EB alone.

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Received June 26, 1961. P.S.E.B.M., 1961, v107.

17-Ethynyl-4-estrene-3,17-diol Diacetate: A Unique Steroidal "Progestin".* (26822)

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In recent years the organic chemist has provided the biologist and clinician with a wide variety of synthetic steroidal substances. These have taken the form of potent estrogens, progestins and adrenal corticoids. Separation of hormonal effects has become a reality: the masculinizing properties of androgens have been decreased without reducing their anabolic effects(1,2) and separation of lipid-shifting from "estrogenic" properties of the estrogens has been achieved(3). In addition, the progesterone-like activity of the nortestosterone agents has been increased while their androgenic activities have been largely eliminated(4). This report describes a new steroid, 17α-ethynyl-4-estrene-3,17-diol diacetate, which is progesterone-like yet antiprogestational and also estrogenic as well as estrogen-antagonistic.

Methods. Progestational and progesterone-antagonistic activities of 17α-ethynyl-4-estrene-3,17-diol diacetate (EED) were studied using immature female rabbits primed with estradiol-17β for 6 days (Clauberg Assay). On the day following last injection the animals received the test compound alone (for progesterone-like activity) or in combination with progesterone (progesterone-antagonistic activity). The daily dose, in each instance, was contained in 0.1 ml corn oil and was administered subcutaneously or buccally for 5 days.

Animals were sacrificed on the day following last injection and a segment of each uterus was removed, fixed and prepared for histological examination. Degree of glandular arborization was determined and graded

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