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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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

* Synthesized by Drs. Frank B. Colton and Paul Klimstra, Division of Chemical Research, G. D. Searle & Co.

after the method of McPhail(5). Estimates of progestational potency were obtained from dose-response curves of the compound and standard, subcutaneously or buccally administered progesterone. Anti-progestational activity of the compound was dependent upon depression of the uterine arborization produced by a daily dose of 0.1 mg progesterone. In addition, progesterone-like activity was assessed using the technique of McGinty(6) in which the compound was administered to ligated segments of uteri in estrogen primed mature-spayed rabbits. Arborization of the glandular epithelium again served as the index of activity.

Estrogen and estrogen-antagonistic properties were obtained from studies involving *uterine growth* in intact immature female mice treated with EED alone (for estrogenic activity) or in combination with 0.3 μg estrone (for estrone-antagonistic activity)(7). The total dose was given in 3 equal subcutaneous injections over 3 successive days. The daily dose of the compound or compound plus estrone was given in a single injection of 0.1 ml corn oil. At autopsy, 24 hours after final injection, uteri were removed, cleaned, scored and blotted to remove fluids and weighed on a torsion balance. A second method for determination of estrogenic activity was the rat *vaginal smear assay*. The rats were spayed at least 30 days before being placed in the test colony. Colony rats responding positively to estrone priming on 2 consecutive weeks were chosen and placed on treatment during the third week(8). The test substance was given in 2 injections 24 hours apart and vaginal smears were taken 56 and 72 hours after the first treatment. The smears were read according to the method of Biggers and Claringbold (9) where the absence of leukocytes was considered as a positive response.

Results and discussion. 17α-Ethynyl-4-estrene-3,17-diol diacetate (EED) proved to be a potent progestin as well as a progesterone-antagonist as judged from its effects on the uterine epithelium in rabbits (Fig. 1 & 2). Comparison of the dose-response curves obtained with EED and progesterone, when both were administered buccally, revealed EED to be about 40 times more effective than progesterone (Fig. 1). Following subcutaneous administration the potency of EED was estimated to be 1 to 2 times that of progesterone (Fig. 2). Maximal arborization following subcutaneous administration was obtained at a dose of 0.1 mg per day. However, as the dose was increased above this level the response of the glandular epithelium in the uterus was reversed and was absent at the 2 and 4 mg dose levels. No such reversal was obtained with progesterone at high dose levels. Thus, EED differs from progesterone in this regard. When EED was administered buccally, no depression of the epithelial response was observed. This suggests differences in absorption and/or metabolism of EED by the 2 routes.

When administered in combination with 0.1 mg of progesterone per day, 1 and 2 mg of EED antagonized the progesterone-induced arborization of the uterine epithelium (Fig. 2). Doses of EED below 0.5 mg failed to antagonize the progesterone response but it will be noted that at these levels the compound itself was acting as a progestin. Thus, EED not only antagonizes its own progestational effects, as shown earlier, but also inhibits the response to added progesterone.

Administered locally (McGinty Assay), EED was not effective in stimulating proliferation of the glandular epithelium in rabbits (Table I). Since the hormonal effects of EED have been shown to change at different dose levels the lack of activity in this single study may be due to the dose at which it was tested. Differences in absorption at the site of action can not be discounted.

Estrogenic activity of EED was demonstrated in both bioassays to which it was subjected. It produced vaginal cornification in spayed rats; ED_{50} values for EED and estrone were 54.0 and 2.4 μg per rat respectively. Thus, the relative potency of EED at this response level was estimated to be 4% (Fig. 3). EED was found to have uterine growth-stimulating properties in immature mice (Fig. 4). However, EED differed from estrone in this study since it had a shallow (impeded) dose-response curve and did not produce maximal uterine growth. In contrast to this uterine stimulating property, EED antagonized the effects of 0.3 μg of estrone on uterine growth (Fig. 3). Maximal inhibition of the response

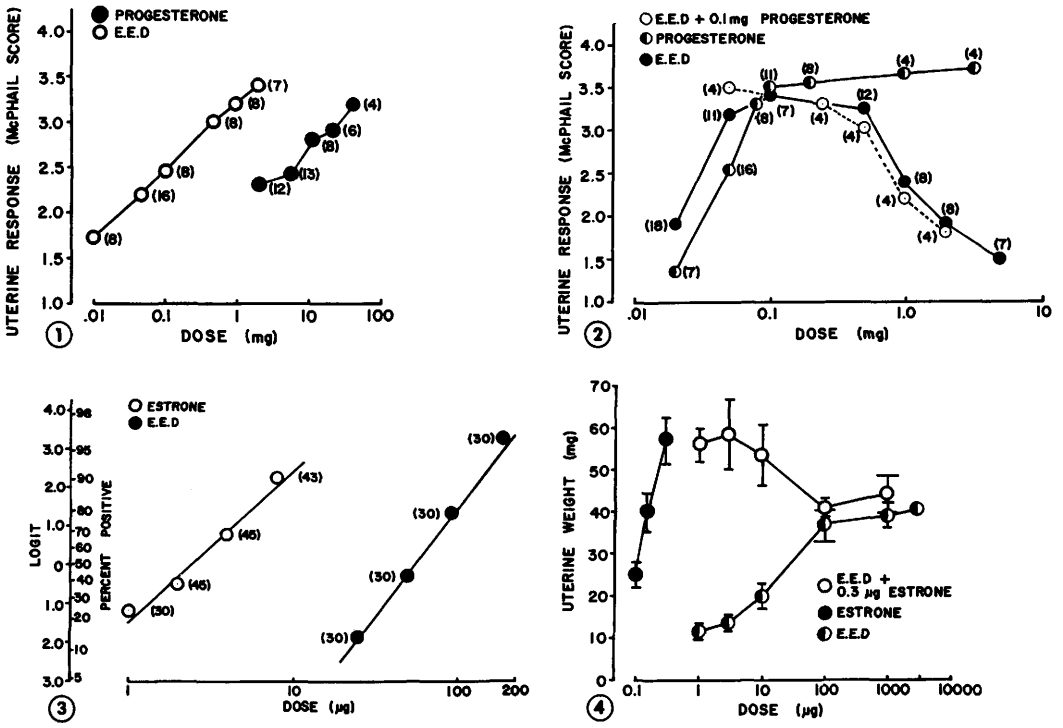

FIG. 1. Response to buccally administered EED and progesterone in uterine epithelium of rabbits. Numbers in parentheses refer to number of animals tested.

FIG. 2. Progesterone-like and progesterone-antagonistic effects of subcut. administered EED as measured by proliferative response in uterine epithelium in immature rabbits. Numbers in parentheses refer to number of animals tested.

FIG. 3. Comparison of EED and estrone on vaginal cornification in spayed rats. Dose-response lines and ED_{50} values were obtained by the minimum logit X^2 method(10). The observed responses were transformed from per cent to logit values and plotted as a function of dose. Parenthetical numbers indicate number of animals in each observation.

FIG. 4. Estrogen-like and estrogen-antagonistic effects of subcut. administered EED as measured by uterine weights in immature mice. Each point represents mean uterine weight for 8-10 mice; vertical bars represent ± 2 stand. errors of mean.

to estrone (approximately 35%) was obtained with 100 μg EED. However, doses above 100 μg failed to antagonize estrone-induced uterine growth since the uterine weights produced with these doses approach the asymptote obtained with the compound alone. It is of interest to note that in these studies both uterine growth (estrogenic effect) and estrone-antagonism (progestational effect) are obtained over the same dosage range. However, in the rabbit studies progestational and estrogenic effects were patently separated. The progesterone-antagonistic property of EED in rabbits can best be explained on the basis of its estrogenic component at the 1 to 4 mg dose levels since it was estimated to have about 4% of the potency of estrone. At lower doses the progestational component is dominant.

TABLE I. Endometrial Response to 17α-ethynyl-4-estrene-3,17-diol diacetate (EED) Administered to Ligated Segments of Rabbit Uteri.

Compound	Dose (μg)	N	McPhail score*
Oil	—	5	1.1
Progesterone	.5	7	2.3
EED	100	4	1.2
	10	4	1.6

* In our studies only values of 2 or over on the activity scale are considered positive (active); 4 represents maximal activity.

Summary. 17α-Ethynyl-4-estrene-3,17-diol diacetate is an agent that exerts unique hormonal effects. By the subcutaneous route EED is 1 to 2 times more potent than progesterone in producing proliferation of the uterine epithelium in rabbits, yet it is also capable of inhibiting this progestational response.

Buccally, EED is approximately 40 times more effective than progesterone, administered by the same route. EED is estrogenic as measured in both the mouse uterine growth assay and the rat vaginal smear assay yet when administered in conjunction with estrone EED acts as an estrogen-antagonist. These opposing activities can best be explained by the differences in hormonal dominance at low and high dose levels.

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Enhancement of Resistance in Mice to Staphylococcal Infection by Preliminary Treatment with a Staphylococcal Extract.* (26823)

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Protection against staphylococcal infection has been the subject of extensive clinical and experimental investigations. Among the most provocative experiments are those in which resistance is enhanced by methods which are independent to those involved in specific acquired immunity(1,2).

While investigating the properties of a staphylococcal extract which we were using as an hemagglutinating antigen, it was found that this extract had a definite resistance enhancing effect in mice against experimental staphylococcal disease. This resistance could be demonstrated after 20 hours and held for 2 heterologous strains of staphylococci as well as for the homologous strain and for one strain of *E. coli.* The extract did not visibly disturb mice and in rabbits did not behave like an endotoxin. In this study the details of these experiments are reported.

Materials and methods. Mice—Swiss mice (CFW) weighing 15-20 g were used in all experiments, except where indicated. *Staphylococci*–"Bartlett", an epidemic, 80-81 strain, resistant to penicillin, obtained from Dr. David Rogers, then at the New York Hospital. was used for preparation of the extract and except as indicated in the text for challenging the mice. For the latter purpose the organisms from an overnight culture in trypticase soy broth were obtained by centrifugation and suspended in sterile saline. The concentration of organisms per inoculum was determined by plating out serial dilutions on blood agar plates. Two other strains were used: "H"—a non typable strain sensitive to penicillin which was obtained from Dr. Stanley Schneierson, Mt. Sinai Hospital, and "Giorgio"—a coagulase positive strain which was sent by Dr. Rene Dubos, Rockefeller Institute for Medical Research.

Staphylococcal extract. 40 ml of trypticase soy broth were inoculated with a few drops of a 4 to 6 hour culture of the "Bartlett" strain of staphylococcus. After 18 hours of incubation at 37°C the culture was centrifuged and

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