hibitory hormone treatment. The treated mice resumed persistent estrus within 18 days after completion of the second series of injections, except for those mice receiving cortisol. All of the cortisol-treated mice died within 14 days after the injections were terminated and before the resumption of persistent estrus.

These results suggest that the condition of persistent vaginal cornification reported herein is attributable to a permanent alteration in the hormone responsiveness of the vaginal epithelium. The altered cell population of the vaginal epithelium returned to its persistently estrous status after inhibition by massive doses of steroids. That the persistently cornifying vaginal epithelia may represent more than one type of cell population, was indicated by the complete nonresponsiveness of about 25 percent of the vaginae to the inhibitory steroids.

Similar cell populations from outgrowths of hyperplastic alveolar nodules of the mouse mammary gland showed altered hormone sensitivity (*7*). The occurrence and the persistence of altered cell populations in vivo suggest a selective action of hormones upon variant cells within a normal cell population. In the case of the vaginal epithelium, the early postnatal estrogen treatment results in the selection of a basal cell population which keratinized in the absence of a continued estrogenic stimulus. The resultant keratinizing epithelium is evidently the consequence of permanent alteration of the normal pattern of differentiation (*8*).

Note added in proof. Gardner (*9*) found that persistent vaginal cornification after early postnatal treatment of hybrid mice with testosterone propionate occurred in the absence of any evidence for endogenous or exogenous estrogenic influence. He also considered the change to be a permanent consequence of early exposure to androgen.

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9 July 1962

Ethynodiol Diacetate as a New, Highly Potent Oral Inhibitor of Ovulation

Abstract. Ethynodiol (17α-ethinyl-4-estrene-3,17-diol) diacetate, a potent progestin and oral inhibitor of ovulation in the rabbit, gives evidence of possessing antiovulatory activity when it is administered orally to women. When it is combined with an estrogen it provides adequate control of menstrual cyclicity, and, thus far, complete contraceptive effectiveness even in low daily doses.

Data indicating the special efficacy of certain neutral 17α-alkyl-19-norsteroids as ovulation inhibitors in animals and in women, potent progestins, regulators of menstrual cyclicity, and oral contraceptives have been published (*1–3*). In animal tests with a large number of steroids, the particular potency of 17α-alkyl-19-norsteroids as ovulation inhibitors was again brought out (*4*) and the unusual oral potency of two 17α-ethinyl-19-norsteroids was noted. In the course of our more recent studies, a third 17α-ethinyl-19-norsteroid, 17α-ethinyl-4-estrene-3,17-diol diacetate (ethynodiol diacetate, or ED), has shown properties similar to the two compounds previously described. For example, it is more active by mouth than by injection as an ovulation inhibitor in the mated estrus rabbit, and it has a high oral progestational activity by carbonic anhydrase assay in the Clauberg rabbit (*5*). Elton and Nutting were the first investigators to report that, depending upon dosage, this steroid, when given by injection, both promotes and inhibits progestational activity in the Clauberg assay (*6*); moreover, they found that it is a weak "impeded" estrogen which acts also as an estrone inhibitor in rats and mice. But since they also found it active as an oral progestin, they described it as "an agent that exerts unique hormone effects." The chemistry of this compound has been described by Colton and Klimstra (*7*).

The oral administration of ethynodiol diacetate to normally cyclic, regularly ovulating women by our previously described regimen, namely, one tablet a day from the 5th to the 25th day of the menstrual cycle, apparently inhibited ovulation. Table 1 summarizes data obtained with 22 volunteer subjects. Each woman was observed during a control cycle with no medication, then ten of these women received in various regimens 2 mg of ethynodiol diacetate per day alone, or in combination with the estrogen, the 3-methyl ether of 17α-ethinylestradiol (EEME), for one or more cycles. Notable was the rather high frequency of breakthrough bleeding when either no estrogen was used or when the dose was low. One should also note the data for the 27 medication cycles of the 12 women who took 1 mg of ED plus 0.1 mg of EEME. These data suggest inhibition of ovulation in over 90 percent of the cycles by basal body temperature tests, and in all but one by endometrial biopsy. The reduced pregnanediol excretion may also be interpreted as a suppression of ovulation. The three cases of "ovulation" deduced from basal body temperature probably are reflections of the slow development of a thermogenic effect with this dosage.

A group of about 50 volunteers in

Table 1. Effects in normally cyclic women of ethynodiol diacetate (ED) alone, and in combination with the 3-methyl ether of 17α-ethinylestradiol (EEME). BBT, basal body temperature; EB, endometrial biopsy on days 19, 20, 21, or 22; BTB, breakthrough bleeding. Doses in milligrams per day.

No. ovulation cycles by		No. cycles with BTB	Excretion (mg/day) means	
BBT	EB		Pregnanediol	17-Ketosteroid
Control, 10 cycles mean 26.9 ± 2.4 days				
9	8	2	3.7 ± 1.6	7.1 ± 2.6
2 mg ED, 6 cycles mean 23.5 ± 1.1 days				
1?	1?	5	1.2 ± 0.7	6.7 ± 1.0
2 mg ED + 0.5 mg EEME, 5 cycles, mean 26.8 ± 0.4 days				
0	0	5	1.6 ± 0.6	5.5 ± 1.8
2 mg ED + 0.1 mg EEME, 7 cycles, mean 27.0 ± 1.2 days				
0	0	3	0.40 ± 0.2	4.2 ± 1.2
Control, 12 cycles, mean 28.0 + 3 days				
12	12	0	2.9 ± 1.9	7.7 ± 2.5
1 mg ED + 0.1 mg EEME, 27 cycles, mean 28.0 ± 2.9 days				
3	1	5	0.63 ± 0.3	4.9 ± 1.4

Table 2. Contraceptive trial with ethynodiol diacetate.

Cycle No.	No. of women	Percent reporting "reactions"	Percent with breakthrough bleeding
1	124	16.9	6.5
2	119	9.3	4.2
3	103	10.7	1.9
4	75	1.0	5.0
5–6	99	1.0	5.0
7–8	88	6.8	0.0
9–10	64	6.2	0.0
Total	662	8.6	3.2

San Juan initiated contraceptive trials with the 5th- to 25th-day oral medication regime by taking a tablet containing 2 mg of ED plus 0.1 mg of EEME. At the beginning of the experiment (July and August 1961), each subject was given a thorough physical examination. Four to six months later, most of the women of this first group were given a second examination. Approximately 70 additional volunteers were added to the study, and the record of the first ten cycles of use is analyzed in Table 2. During this period, no detectable conception occurred. As was the case when other 19-norsteroids were tested, the volunteers reported "reactions" in turns of comments or complaints which were noted during the medicated cycles. More objective was the occurrence of bleeding or spotting on one or more days while the medication was being taken. This we call breakthrough bleeding. As previously noted for other 19-norsteroids (*8, 9*), the first medication cycle was the period of maximum occurrence of these phenomena. The data on breakthrough percentage resemble those found in trials with the daily administration of 10 mg of norethynodrel plus 0.15 mg of EEME, whereas the "reaction" frequencies are similar to those reported for dosages of 5 mg of norethynodrel plus 0.075 mg of EEME. Thus this combination of ED and EEME would appear to yield a lower reaction incidence and a better control of menstrual bleeding than the combination of norethynodrel and EEME.

Table 3. Responses to questioning of ED users after 4 to 6 months of test.

Concerning	Percent claiming		
	Increase	No change	Decrease
Weight	23	40	37
Breast size	7	88	5
Menstrual pain	10	76	14
Menstrual flow	7	61	32
Libido	2	95	2

Amenorrhea, which here means no menstruation upon withdrawal of the medication, occurred in 1.2 percent of the 662 cycles. This is about the incidence which was observed with Enovid (*8*). Analysis of menstrual cycle lengths discloses a mean of 27.4 days per cycle, with 3.4 percent of the cycles less than 24 days. Practically all of these "short" cycles were reported by individuals who omitted the dose for several days. These data emphasize the relative regularity of the imposed cyclicity.

The physical examinations of 45 of the original volunteers have disclosed no notable effect other than menstrual regulation and contraception already noted. Endometrial biopsies disclosed a similar, typical sequence of early progestational change in the endometrial glands, with continuing stromal stimulation so characteristic of 19-norsteroid administration (*8, 10*). Papanicolaou smears were of classes I and II only; before medication 67 percent were class I, during medication 77 percent were class I. The uterine size as measured by palpable fundal area was unchanged in 53 percent of the women after medication. It was decreased in 40 percent and increased in 7 percent.

Table 3 summarizes the replies to questioning of the volunteers at the time of the second examination. No very remarkable change was recorded, although 32 percent of the women who used ED reported some reduction in the amount of menstrual discharge; this reduction had also been observed with other 19-norsteroids. Otherwise, the increases reported by some subjects are balanced by the decreases reported by others (*11*).

In conclusion, we find the ED plus EEME combination a potent antifertility agent, when it is administered orally in low daily dosage. By analogy with our experience with Enovid, contraceptive effectiveness may be expected at a low daily dose (1 mg/day) and presumably with minimal "side effects." Menstrual cyclicity with no untoward effects is adequately controlled. Because of the low dosage regimen, this drug may be not only physiologically safer than others but also more economical.

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4 September 1962

Localizing Tritiated Norepinephrine in Sympathetic Axons by Electron Microscopic Autoradiography

Abstract. Following intravenous infusion of tritiated norepinephrine, rat pineals were prepared for combined autoradiography and electron microscopy. Concentrations of photographic grains were observed only over regions of preterminal autonomic axons containing granulated vesicles, thereby directly demonstrating uptake of norepinephrine into these axons and strongly suggesting that their granulated vesicles contain norepinephrine.

Electron-microscope studies (*1–5*) have established the presence of characteristic "granulated vesicles" in many autonomic axons. These granulated vesicles are 40 to 50 mμ wide, contain a 20 to 30 mμ electron-dense core, and seem to be concentrated in preterminal axoplasm (Fig. 1). It has been suggested that granulated vesicles contain serotonin (*1*), norepinephrine (*2*), or one of several "reducing amines" (*3*). These suggestions rest upon such circumstantial evidence as the morphological analogy between granulated vesicles and chromaffin cell granules possessing a limiting membrane and a dense core, the known concentration of norepinephrine in sympathetic nerves (*6*), and the evidence from centrifugation studies of splenic nerve homogenates that at least 20 percent of the total norepinephrine is associated with "particles" somewhat similar to catecholamine-containing granules obtained from adrenal homogenates (*7*). From one brief