In general, the results of the experiment here reported suggest that J. F. Brown's hypothesis (*2*) may be overly restrictive, since it is demonstrated that the precision of velocity judgments is at least partially dependent upon the systematic presence of either spatial or temporal cues (*8*).

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Persistent Vaginal Cornification in Mice

Abstract. Persistent vaginal cornification induced in A/Crgl mice by brief postnatal treatment with estrogen is not prevented by later ovariectomy, adrenalectomy, or hypophysectomy. Transplantation of the persistently cornified vaginae into ovariectomized normal mice or into ovariectomized persistently estrous mice also does not eliminate cornification in the majority of transplants. Administration of "antiestrogenic" steroids temporarily alters the vaginal picture in some but not all of these mice. The vaginal epithelium of the persistently estrous mouse appears to represent an altered cell population which does not require estrogen for its constant keratinization.

Persistent cornification of the vaginal epithelium of rats and mice can be initiated by the injection of large doses of estrogen during the early postnatal period (*1*, *2*). So-called persistent estrus also occurs spontaneously in certain mice (*3*). Mice of the strains A/Crgl, C3H/Crgl, BALB/cCrgl, C57BL/Crgl, and RIII/Crgl, receiving five daily subcutaneous injections of 5γ estradiol-17β, starting on the day after birth, show persistent vaginal cornification beginning on the 15th to the 40th day of life (*2*). Ovariectomy, or transplantation of the ovary into the spleen after ovariectomy, abolishes the persistent vaginal cornification in rats (*4*) and in spontaneously constant estrous mice (*3*). However, A/Crgl mice with persistent vaginal cornification resulting from postnatal estrogen treatment continue to show a cornified vaginal epithelium despite ovariectomy, or ovariectomy and adrenalectomy, or ovariectomy and hypophysectomy (*5*). Furthermore, the ability of estrogen to produce persistent cornification is restricted to infantile mice. The administration of large amounts of estrogen to adult A/Crgl mice results in vaginal cornification only during the period of treatment (*5*).

Table 1. Effects of steroid hormones on persistent vaginal cornification in ovariectomized A/Crgl mice. There were five mice in each group, individually identified by letters.

Injection series	Daily treatment (mg)	Individual mice showing: Persistent estrus	Intermittent estrus	Constant diestrus
		Group I		
1	2.50 progesterone	A, B	C	D, E
2	2.50 progesterone	A, B	C	D, E
		Group II		
1	2.50 progesterone	F, G	H	I, J
2	1.25 cortisol	F, G	H	I, J
		Group III		
1	2.50 testosterone	K	L, M, N	O
2	2.50 progesterone	K	L, M, N	O
		Group IV		
1	1.25 deoxycorticosterone	P	Q, R, S	T
2	2.50 progesterone	Q	P, R, S, T	

These facts suggest two alternative explanations of the estrogen-induced phenomenon of persistent estrus in mice. Either the vaginal epithelium is subject to abnormal neurogenic influences caused by the early postnatal estrogen treatment, or it becomes permanently altered after this treatment. The former of these two possibilities was eliminated by observing that, when persistently cornified vaginae were transplanted to new sites, cornification was not abolished in the majority of either ovariectomized normal hosts or ovariectomized hosts showing persistent vaginal cornification (*5*). Thus, persistence of cornification must be an inherent characteristic of the vaginal epithelium.

Androgen, progesterone, and adrenal corticoids are capable of inhibiting the response of the normal vagina to estrogen (*6*). If the persistently estrous vagina was similarly inhibited from cornification, and then returned to its initial state upon withdrawal of the inhibitory steroid, one might conclude that there was a permanent alteration in such vaginal tissue.

To test this hypothesis, daily doses of 0.1 ml of an aqueous suspension of 2.5 mg of progesterone, 2.5 mg of testosterone, 1.25 mg of deoxycorticosterone acetate, or 1.25 mg of cortisol acetate were injected subcutaneously into persistently estrous A/Crgl mice, which had been ovariectomized 20 to 36 days previously. Injections were continued for 15 days. Daily vaginal smears revealed (Table 1) inhibition of persistent estrus in some but not in all of the mice. The majority showed either persistent estrus or intermittent estrus (1 to 2 days of estrus following 1 to 4 days of diestrus) despite the steroid injections. During the interim period after the first treatment with the steroid, the persistently estrous state was resumed in all cases. Thus, the capability of the vaginal tissue to show persistent cornification was not permanently altered in any mouse by these inhibitory agents.

A second course of inhibitory hormones was given (Table 1). In three of the four groups of animals, the response of the vaginal epithelium to the inhibitory hormones was similar to that in the initial treatments, although the first and second inhibitory hormones were different in two of the three groups. The fourth group was treated initially with deoxycorticosterone and subsequently with progesterone; three of the five mice showed different responses to the two steroids. A total of 17 out of 20 vaginae showed a consistent pattern of responsiveness to the first and second courses of in-

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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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