Induction of Deciduoma in Rabbits Without Uterine Trauma by Treatment with Ethynodiol Diacetate: A Synthetic Progestogen. (31002)

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In other studies we demonstrated that decidual cell formation could be produced in immature female rabbits treated with combinations of estrone and progesterone(1). An independence of action for these 2 steroids in producing deciduoma in the endometrium was shown. At the same time it was shown that deciduomata could not be produced by progesterone treatment alone.

With the recent emphasis on synthetic progestins, steroids exhibiting mixed endocrine properties were made available for study. One of these, 17α-ethynyl-17β-hydroxy-5(10)-estren-3-one (norethynodrel), has both progestational and estrogenic properties(2). Norethindrone has been shown to have, in addition to progestational activity, some androgenic properties(3). Recently, we described the endocrine properties of still another steroid, 17α-ethynyl-4-estrene-3β,17β-diol diacetate (ethynodiol diacetate). This substance displays both progestational and estrogenic effects. However, under certain conditions, it is able to antagonize the effects of progesterone and estrogen(4,5). Thus, it was of interest to determine if this compound could cause deciduogenesis in rabbits in a manner similar to that observed in animals treated with combinations of estrogen and progesterone.

Materials and methods. Immature female rabbits weighing about 1 kg were primed with 5 μg 17β-estradiol for 6 days and, starting on the day after the last priming injection, ethynodiol diacetate was administered subcutaneously in doses ranging from 0.5 to 10 mg per day for 5 or 6 days. In some groups of animals 17α-ethynyl-17β-estradiol 3-methyl ether (mestranol) was combined with the progestin. Compounds were given either as a solution or suspension in corn oil so that the daily dose was contained in 0.1 ml vehicle.

In the first group of experiments the animals were killed on the day after the last treatment with ethynodiol diacetate and uterine segments were removed and prepared for histological evaluation. The presence of decidual tissue was determined using methods described previously(6).

A second series of experiments was designed in an attempt to determine whether decidual cell responses (DCR), once produced, could be maintained. Groups of 12 animals were treated for 14 days with 1 or 2 mg ethynodiol diacetate per day. Uterine segments were removed surgically after 5, 8, 12, and 15 days of treatment so that each animal could be studied over a prolonged period. To minimize any effect attributable to operative trauma, the uterine tissues examined on the 8th, 12th, and 15th days were taken from sites not affected by previous surgery. The presence of decidual tissue was determined as above.

Results and discussion. Ethynodiol diacetate, administered in doses of 1, 2, 4, and 10 mg per day to immature estrone-primed rabbits produced decidual cell responses (DCR) in 58, 74, 58, and 25% of the animals (Table I. No responses were observed at the 0.5 mg dose level. The ability of this material, known to exert both progesterone-like and estrogenic effects, to stimulate DCR in rabbits in the

TABLE I. Stimulation of Decidual Responses in Uteri of Immature Rabbits Treated with Ethynodiol Diacetate Alone or Combined with Mestranol.* No exogenous trauma were applied in these experiments.

Daily dose (mg)		No. of rabbits	Length of treatment (days)	No. DCR‡ (%)
E.D.†	M*			
.5	0	8	6	0
1.0	0	19	5	11 (58)
2.0	0	20	5	14 (74)
4.0	0	20	5	11 (58)
10.0	0	4	6	1 (25)
.1	.05	4	6	0
.1	.1	4	6	1 (25)
.5	.05	4	6	3 (75)
.5	.1	4	6	3 (75)
.5	.3	4	6	4 (100)

* 17α-ethynyl-17β-estradiol 3-methyl ether.
† Ethynodiol diacetate.
‡ Decidual cell responses.

absence of uterine trauma is in keeping with results obtained when combinations of progesterone and estrogen are administered(1). The absence of DCR in rabbits treated with 0.5 mg agrees with data previously published showing that at this dose level ethynodiol diacetate exerts predominately progesterone-like effects (4,5). At doses above 0.5 mg the estrogenic effects become dominant. Thus, the importance of the estrogenic component for producing DCR is demonstrated in this test system.

Combination of mestranol and ethynodiol diacetate resulted in augmentation of the response to ethynodiol diacetate administered alone. Whereas no responses were obtained when 0.5 mg of ethynodiol diacetate was given alone, 25% of the animals treated with 0.1 mg combined with 0.1 mg of mestranol responded (Table I). Furthermore, when 0.5 mg of ethynodiol diacetate per day was administered along with 0.05, 0.1, and 0.3 mg of mestranol 75 to 100% of the animals had DCR in their uteri. Thus, while the progestational component of ethynodiol diacetate appears sufficient to support deciduogenesis at the 0.5 mg dose level, it is postulated that the intrinsic estrogenic property has been reduced or antagonized by the progestational component to such an extent that no responses are obtained unless estrogen is added.

In the second group of experiments optimal doses of ethynodiol diacetate capable of producing and maintaining DCR in rabbits were determined. Under this protocol, 1 mg of ethynodiol diacetate produced a response in 4 of 12 animals by the 5th day of treatment (Table II). Of these, 3 maintained the response through the 8th day of treatment while only one animal had decidual tissue at termination of the study. Following 8 days of treatment, another animal (I-7) had decidual tissue which then was maintained throughout the study. Two mg of ethynodiol diacetate produced DCR in 5 of 12 animals after 5 days, and 8 of 11 animals after 8 days of treatment. Once produced, this dose maintained the response throughout the rest of the experiment. Thus, 1 mg represents the threshold dose for this compound when administered alone. As the dose was increased the number of DCR increased and, equally important, the responses produced were maintained. In addition, these studies show that the time required to produce optimal responses appears to be on or near the 8th day of treatment. This corresponds closely to the time of deciduogensis during normal pregnancy in this species(7). It is of interest to note that the formation of decidual cells at an earlier time, both in these experiments as well as others reported by us (6), is in agreement with the effects of these synthetic steroids on endometrium of humans. These steroids have been reported to accelerate the endometrial changes in uterine morphology observed during the menstrual cycle(8). Indeed, not only do these steroids bring about secretory changes in the endometrium of humans at an earlier time, they also are able to produce decidual (predecidual or pseudodecidual) changes earlier(8). These "pre- or pseudodecidual" cells, observed in endometrium during the days immediately preceding catamenia(9), have also been induced by

TABLE II. Effect of Ethynodiol Diacetate in Maintaining Decidual Responses in Rabbits.

Presence or absence of decidual response indicated by (+) or (—).

Animal No.	Dose (mg/day)	Days on which uterine segments removed 5	8	12	15
I- 1	1.0	—	—	—	+
I- 2	1.0	—	—	—	—
I- 3	1.0	+	+	—	—
I- 4	1.0	—	+	—	—
I- 5	1.0	+	+	—	—
I- 6	1.0	—	—	—	—
I- 7	1.0	—	+	+	+
I- 8	1.0	+	—	+	—
I- 9	1.0	—	—	—	—
I-10	1.0	—	—	—	—
I-11	1.0	+	+	+	+
I-12	1.0	—	—	—	—
	Total	4/12	5/12	3/12	3/12
II- 1	2.0	—	—	+	+
II- 2	2.0	+	*		
II- 3	2.0	+	+	+	+
II- 4	2.0	—	+	+	+
II- 5	2.0	—	+	*	
II- 6	2.0	+	+	+	+
II- 7	2.0	—	+	+	†
II- 8	2.0	+	+	+	+
II- 9	2.0	—	+	+	+
II-10	2.0	—	—	—	+
II-11	2.0	+	+	+	+
II-12	2.0	—	—	—	—
	Total	5/12	8/11	8/10	8/9

* Died from anesthesia.
† Not enough tissue to evaluate.

treatment with progesterone and estrogen combinations(10,11,12). Thus, the deciduogenic effects observed in the uteri of rabbits treated with ethynodiol diacetate correspond closely to the pre- or pseudodecidual changes observed in the endometrium of women. The effect of other synthetic steroids known to be effective in the treatment of menstrual disorders and control of ovulation is being evaluated by this procedure.

Summary. Ethynodiol diacetate (E.D.) was administered to immature estrogen-primed rabbits in an attempt to stimulate formation of decidual tissue in the uterus. No uterine trauma was employed in these experiments. DCR were obtained after treatment with 1, 2, 4, or 10 mg of E.D. per day for 5 or 6 days. Combination of mestranol, in doses of 0.05 to 0.3 mg per day, with as little as 0.1 mg E.D. resulted in the formation of DCR. When E.D. was administered by itself, doses of 2 mg per day were required to stimulate deciduogenesis and to maintain the response over a 15-day period. The similarity of the responses in humans and rabbits is discussed.

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1. Elton, R. L., Calhoun, D. W., Nutting, E. F., 1965, submitted for publication.
2. Saunders, F. J., Edgren, R. A., Drill, V. A., Endocrinology, 1957, v60, 804.
3. Drill, V. A., Fed. Proc., 1959, v18, 1040.
4. Elton, R. L., Nutting, E. F., Proc. Soc. Exp. Biol. and Med., 1961, v107, 991.
5. Elton, R. L., Nutting, E. F., Saunders, F. J., Acta Endocrinol., 1962, v41, 381.
6. Elton, R. L., ibid., 1965, submitted for publication.
7. Mossman, H. W., Contr. Embryol. Carneg. Inst., 1937, v26, 129.
8. Pincus, G., Rock, J., Garcia, C.-R., Ann. N. Y. Acad. Sci., 1958, v71, 677.
9. Noyes, R. W., Hertig, A. T., Rock, J., Fertil. and Steril., 1950, v1, 3.
10. Eichner, E., Golar, G. G., Reed, J., Gordon, M. B., Am. J. Obstet. & Gynecol., 1951, v61, 253.
11. Long, R. C., Bradbury, J. T., J. Clin. Endocrin. & Metab., 1951, v11, 134.
12. Durham, W. C., Fertil. & Steril., 1961, v12, 45.

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Hydrocephalus in Mice Infected with Polyoma Virus. (31003)

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Previously this laboratory reported(1) that a strain of polyoma virus isolated from Swiss mice bearing sarcoma 180 produced tumors in hamsters by intracerebral inoculation. However, when the virus was inoculated intracerebrally (IC) into 1-day-old Swiss white mice, 30% developed hydrocephalus and the virus was recovered from the brain tissue of the hydrocephalic mice. No tumors were observed in the Swiss white mice after IC or subcutaneous inoculation of the virus.

Hydrocephalus in mice and small rodents caused by various viruses has been reported earlier. Levaditi *et al*(2) and Jones *et al*(3) produced hydrocephalus in mice and Findlay (4) demonstrated that pleuropneumonia-like organisms in association with a neurotropic virus caused hydrocephalus in the same animal. Shortly after our previous report(1), Vandeputte(5) produced hydrocephalus in rats with SE polyoma virus while Huebner *et al*(6) and Yabe *et al*(7) observed that the intracerebral inoculation of type 12 adenovirus produced hydrocephalus in hamsters.

In an attempt to clarify the nature of the hydrocephalus observed in this laboratory when mice were inoculated with our strain of polyoma virus, histologic sections prepared from the brains of 115 Swiss mice inoculated IC were examined. A number of (C_3 H + A K R) F_1 mice were also inoculated with the

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