Interaction of 16 Epi-Estriol and Estradiol 17β on Uterine Growth.*† (22112)

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In a previous publication the authors reported on estrogenic activity of 16 epi-estriol, an estrogen recently extracted from urine of pregnant women by Marrian and Bauld(1). Our results showed conclusively that this estrogen was slightly less active than estriol (2). Since estriol was shown to have the ability to interfere with uterine growth-promoting action of estradiol 17β(3), it seemed of considerable interest to determine the effects of 16 epi-estriol on estradiol 17β-induced uterine growth.

Therefore, the present study takes for its central theme a series of experiments designed to elucidate the effects of 16 epi-estriol on uterine growth-promoting and vaginal cornifying ability of estradiol 17β.

Procedures. Virgin, albino rats, approximately 100 days of age, were used. They were started on treatment 7 days after bilateral ovariectomy. The 16 epi-estriol was dissolved in U.S.P. propylene glycol, and estradiol 17β was dissolved in sesame oil and/or propylene glycol. They were injected subcutaneously, each of these compounds at a separate site, daily for 3 days. Vaginal smears were taken daily, and 72 hours after first injection necropsies were performed. Uteri were removed quickly and slit longitudinally on bibulous paper, thus removing excess uterine luminal fluid. The uteri were then weighed to nearest 0.2 mg on a Roller-Smith torsion balance. After wet weights were determined, the uteri were placed in an oven at 110°C for dry weight determinations. A standard period of 24 hours at 110°C was used uniformly for drying each uterus. After this period the weight of each uterus was determined on a Voland Analytical Balance.

Results. These results clearly indicate that 5 μg of 16 epi-estriol are required to demonstrably restrict in part the uterine growth promoting action of 0.1 μg estradiol 17β on both a wet and a dry weight basis. Smaller doses of 16 epiestriol when given in combination with our standard dose of 0.1 μg estradiol 17β do not seem to appreciably modify the response of the uterus to the more potent estrogen (Table I). On the other hand it seems quite apparent that a marked reduction in uterine growth is effected when 10 μg or more of 16 epi-estriol are administered concurrently, but at different injection sites, with the estradiol 17β (Table I). Actually, a maximal re-

TABLE I. Influence of 16 Epi-estriol on Uterine Growth Promoting Effects of Estradiol 17β in Ovariectomized Rats.

Treatment	No. of animals	Uterine wet wt, mg	Uterine dry wt, mg
Ovariectomized controls, no treatment	25	115.5 ± 3.3†	22.4 ± .7
.1 μg E*	42	227.3 ± 6.1	42.3 ± 1.8
1.0 μg 16 epi + .1 μg E	21	214.2 ± 6.5	39.5 ± 4.7
2.0 μg 16 epi + .1 μg E	7	203.7 ±11.1	37.6 ± 3.2
5.0 μg 16 epi + .1 μg E	7	190.5 ± 9.5	35.1 ± 2.6
8.0 μg 16 epi + .1 μg E	13	191.4 ± 7.3	35.0 ± 2.2
10.0 μg 16 epi + .1 μg E	6	174.9 ± 6.5	34.9 ± 1.0
25.0 μg 16 epi + .1 μg E	7	173.3 ± 8.8	33.7 ± 2.1
Reference controls: 16 epi alone			
1.0 μg	8	150.0 ± 8.6	26.1 ± .8
5.0 "	8	142.7 ± 6.8	25.8 ± 1.7
10.0 "	9	171.4 ± 8.7	28.5 ± 1.6
25.0 "	9	164.3 ± 5.1	30.5 ± 1.1

* E = Estradiol 17β; epi = epi-estriol.
† Avg ± stand. error.

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striction of uterine growth is obtained on the combined treatment of 0.1 μg estradiol 17β plus 10 μg 16 epi-estriol. Of particular significance is the fact that a summation of the two estrogens does not obtain, even though the maximal growth potential of the uterus is at least 50% more than that reported for 0.1 μg estradiol 17β(3).

This substance does not seem to alter the vaginal cornifying effects of estradiol 17β. The vaginal smears of those animals on 0.1 μg estradiol 17β with and without 16 epi-estriol seem to be relatively indistinguishable, both showing a preponderance of cornified epithelial cells with degenerative nuclei. Thus, the restrictive action of 16 epi-estriol seems to be mainly on the uterine growth-promoting effects of estradiol 17β.

These results quite clearly parallel those of Hisaw, Velardo, and Goolsby(3), who reported that estriol likewise inhibited estradiol 17β on the growth of the uterus. Furthermore, Huggins and Jensen(4) showed that 16 epi-estriol was quite efficacious in restricting the effects of estrone on the uterus in hypophysectomized rats. Therefore, these results seem wholly consonant with the thought that the various estrogens and/or their metabolites act *in concert* to produce the end organ result (3). These experiments provide additional data that give credence to the concept that an endocrine response is accomplished through a precise ratio of the hormone and/or its metabolites.

Summary. The adult, ovariectomized rat was used to ascertain the effects of a new estrogen, 16 epi-estriol, on the activity of estradiol 17β. These results indicate that when 3 daily doses of 5 to 25 μg of 16 epi-estriol are subcutaneously injected into rats receiving 0.1 μg estradiol 17β daily for the 3-day period, the response of the uterus to estradiol is restricted. Normally, 3 daily doses of 0.1 μg estradiol 17β increase the uterine wet weight from the value of the ovariectomized rat from approximately 116 mg to 227 mg. The simultaneous administration of 5, 10, or 25 μg of 16 epi-estriol in addition to the estradiol treatment reduces the response to 191 down to 173 mg. The inhibitory action is likewise reflected in the dry weight data. No apparent modification of the vaginal cornifying action of estradiol could be induced by 16 epi-estriol.

1. Marrian, G. F., and Bauld, W. S., *Biochem. J.*, 1955, v59, 136.

2. Velardo, J. T., and Sturgis, S. H., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 181.

3. Hisaw, F. L., Velardo, J. T., and Goolsby, C. M., *J. Clin. Endo. and Metab.*, 1954, v14, 1134.

4. Huggins, C., and Jensen, E. V., *Abst. Nat. Acad. Sci. in Science*, 1955, v121, 622.

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Hemolysis of Sheep Erythrocytes Sensitized with Leptospiral Extracts. (22113)

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Serodiagnosis of leptospirosis presently is based on agglutination, agglutination-lysis (AL), and complement-fixation procedures. The agglutination test employing killed leptospirae has been found to parallel the AL test in its type specificity(1). Gochenour(1) found that complement-fixation tests employing sonically-disrupted leptospiral antigens(2) showed a broader spectrum than AL procedures. Various modifications of AL procedure, such as that of Stoenner(3), may alleviate some difficulties associated with AL technic, in this case the hazard of working with living leptospirae. However, several serotypes of leptospirae known to be involved in leptospirosis make it necessary to employ several antigens in the above technics used as screening tests, the choice of serotypes depending on specificity of procedure and the purpose of the investigation(4). The need