post-ganglionic fibers as well as liberation of acetylcholine(5). Acetylcholine or epinephrine injections also induced ejaculation in mice but pretreatment with atropine and Priscoline prevented, respectively, the ejaculations induced by these drugs but not those induced by KCl. Ejaculations induced by acetylcholine were qualitatively and quantitatively better than those induced by epinephrine, suggesting predominance of parasympathetic control of the efferent ejaculatory pathway.

It is assumed that KCl produces only ejaculation of the fluid present in the seminal vesicles because no sperm were ever found in the ejaculates. Repeated daily application of KCl apparently does not injure the animals so it is believed that it is a safe means of inducing ejaculation of seminal fluid without sacrificing the animals for collecting fluid for bio-assay studies.

Summary. Intraperitoneal injection of KCl has been demonstrated to induce intravital ejaculation in mice, rats, guinea pigs, hamsters and ground squirrels, but not in pigeons or cats. The response is an all-or-nothing response regardless of the size of the dose. A dose of 400 mg/kg of 3.5% KCl was found to be the most effective in mice. Only potassium-containing salts were effective in producing ejaculation. It was not possible to standardize the ejaculatory response by measuring ejaculatory time or by weighing the ejaculate. Extirpation of the seminal vesicles, bilateral castration, and analysis of the fructose content of the semen indicated the seminal vesicles as the source of the fluid. No sperm were found in the fluid at any time. Repeated daily injections of KCl cause ejaculation without apparent harm to the animals.

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Uterine Growth Stimulating and Testicular Growth Suppressing Activities of 17α-Ethinylandrostane-3β,17β-diol, Its Δ^5-Analog and Derivatives. (22493)

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Comparative studies of biological activity of 17α-ethinylandrostane-3β,17β-diol and its Δ^5-analog(1) have been concerned largely with androgenic and luteoid potency as related to other androstane and pregnane derivatives (2-5). Our observations have indicated that these steroids are characterized by predominantly estrogenic activity (growth promotion of infantile rat uterus and inducement of vaginal cornification in ovariectomized rats). The present report is a structure-activity relationship survey of ethinylandrostanediol, ethinyl-Δ^5-androstenediol, and esters of each, with respect to relative activity in stimulation of uterine growth (UGSt) and suppression of testicular growth (TGSp) in immature intact rats. Observations on the UGSt and TGSp activities of estradiol-17β, ethinylestradiol and Δ^5-androstenediol are included for reference purposes. Definitive endocrinological studies on certain compounds of the series will be reported separately.

Materials and methods. Immature male or female Sprague-Dawley rats weighing 35-40 g were used. Unless noted otherwise all test substances were dissolved in cottonseed oil containing 10% ethanol v/v and administered subcutaneously in 0.2 ml of vehicle. The uterine growth tests were performed essentially in accordance with procedures outlined by

TABLE I. Uterine Growth Stimulating and Testicular Growth Suppressing Activities of Estradiol-17β.

A. Uterine growth stimulating (UGSt) activity

Treatment	Dose, mg/kg/day	No. of rats	Uterine wt, mean ± S.E., mg	% stimulation	ED_{50}, mg/kg/day
None	—	8	38.4 ± 3.4	—	
Estradiol-17β	.00037	8	49.5 ± 3.5	15	
	.00075	8	77.8 ± 4.9	55	.0008
	.0015	8	89.8 ± 7.1	72	
	.003	8	96.8 ± 4.2	82	

B. Testicular growth suppression (TGSp) activity

Treatment	Dose, mg/kg/day	No. of rats	Testicular wt, mean ± S.E., mg	% suppression	ED_{50}, mg/kg/day
Controls					
Initial	—	10	220 ± 10	—	
Final	—	9	556 ± 17	—	
Estradiol-17β	.05	5	406 ± 36	45	.055
	.10	5	301 ± 17	76	
	.20	5	251 ± 12	91	

Lauson *et al.*(6). The test material was injected once a day for 3 days. On the 4th day, 24 hours after the last injection, the uteri were removed, blotted and weighed on a micro-torsion balance. For testicular growth suppressing activity the test agents were injected once a day for 5 days. On the 3rd day after the last injection the testes were removed, cleaned and weighed. At least 3 dose levels of each compound were employed within the active range. The dose of each compound which effected 50% of maximum change (ED_{50}) was calculated from log-dose probit graph paper according to the method of Miller and Tainter (7). A uterine weight of 110 mg was used as the maximum uterine stimulation. This value was obtained from standard dose-response curves established for estradiol-17β. The per cent suppression of testicular growth was calculated on the basis of the difference in degree of growth of testes from treated *vs* untreated controls. Esterification of the parent ethinyl-steroid(1) was effected by reaction with the appropriate acid chloride or anhydride in pyridine solution. The crystalline compounds gave analysis in good agreement with theory and possessed optical rotations within the expected range.

Results. Dose-response relationships for estradiol-17β in UGSt and TGSp assays are shown in Table I. The degree of response in the two tests was proportional to dose. Con-

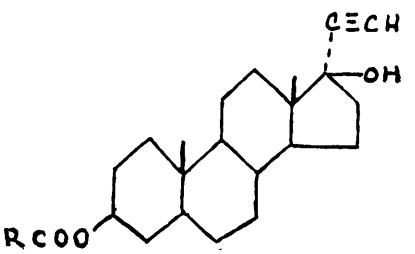
TABLE II. Comparison of Uterine Growth Stimulating (UGSt) and Testicular Growth Suppressing (TGSp) Activities of Various Diol Steroids.

Compound	A UGSt activity ED_{50},* mg/kg/day	B TGSp activity ED_{50},† mg/kg/day	Quotient B/A
Δ^5-Androstene-3β,17β-diol	8.2	47.0	5.7
17α-Ethinyl-Δ^5-androstene-3β,17β-diol	4.75	5.8	1.2
17α-Ethinylandrostane-3β,17β-diol	5.25	6.5	1.2
Estradiol-17β	.0008	.055	68.
17α-Ethinylestradiol-17β	.00045	.0037	9.

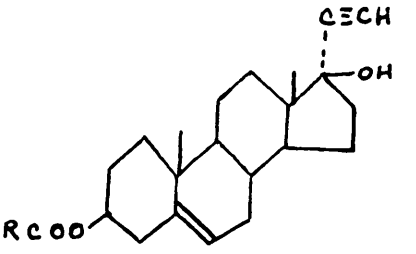
* Dose required to effect 50% of maximum growth of uterus of infantile rats following daily subcut. inj. for 3 days. Estradiol-17β was employed as standard reference.

† Dose required to effect a 50% suppression in normal growth of the testes of immature rats following daily subcut. inj. for 5 days.

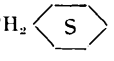
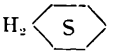
TABLE III. Comparison of Uterine Growth Stimulating (UGSt) and Testicular Growth Suppressing (TGSp) Activities of Ethinyl-diol Esters.



Series I



Series II

R	Series	M.P., °C, cor.	A UGSt activity ED_{50}, mg/kg/day	B TGSp activity ED_{50}, mg/kg/day	Quotient B/A
I $-CH_3$	I*	201.2–204.1	.96	3.8	3.9
II $-CH_2CH_2CH_3$	II	115.4–117.0	2.2	4.75	2.2
III $-(CH_2)_5CH_3$	I	89.8– 91.4	8.0	15.0	1.9
IV $-CH_2CH_2COOH$	II	203.0–204.4	16.0	11.8	.74
V $-CH_2$ 	II	191.8–194.8	13.0	9.0	.69
VI $-CH_2CH_2$ 	I	91.8– 94.2	31.0	17.5	.56
VII $-C(CH_3)_2-CH_3$	I	216.2–218.0	2.7	4.0	1.5
VIII $-(CH_2)_2CH(CH_3)_2$	II	135.6–138.4	5.2	6.1	1.2
IX $-CH(CH_2CH_3)_2$	II	171.4–173.8	21.2	10.5	.49
X $-CH_3$	II*†	171.3–172.9	1.25	6.25	5.1
XI $-CH_2CH_3$	II†	149.7–152.2	3.75	3.75	1.0
XII $-CH_2CH_2$ 	II†	115.2–116.6	9.0	7.25	.8

* *Cf* Ruzicka *et al.*(1). † Aqueous suspensions.

siderably more estradiol was required to suppress testicular growth than to stimulate uterine growth. The TGSp ED_{50} was found to be 68 times larger than the UGSt ED_{50}. Although Δ^5-androstenediol was greatly less active mg per mg than estradiol (Table II), the UGSt TGSp dose ratio was only about 1/12th that determined for the natural estrogen. 17-Ethinylation of estradiol and Δ^5-androstenediol resulted in increased UGSt and TGSp activities. The increment in TGSp activity effected by this chemical modification was much greater than was the increase in UGSt activity, resulting in a considerably lower UGSt/TGSp dose ratio. The extent to which UGSt and TGSp activities as well as dose ratios are altered by esterification of the ethinyl-diol steroids together with the melting points for each compound is shown in Table III.

Within the series of ethinyl-diol esters, the 3-acetate (I) possessed 5 times more UGSt activity than its corresponding alcohol. In-

creasing the size of the ester group resulted in proportionate decrease of this activity. Thus, the 3-heptanoate (III) exhibited approximately 1/8 and the 3-cyclohexylpropionate (VI) approximately 1/32nd the uterine growth stimulating capacity of the 3-acetate (I). A similar structure-activity relationship was obtained for these esters in regard to testicular growth suppressing activity. The 3-acetate of ethinylandrostanediol (I) and the aqueous suspension of the 3-propionate of ethinyl-Δ^5-androstenediol (XI) were the most active in the series. The TGSp activity diminished as the size of the ester group was increased above 3 carbon atoms, but this loss of inhibition was relatively less than was the decrease in UGSt activity. Several of the high molecular weight esters possessed lower UGSt/TGSp ED_{50} ratios than numerous esters lower in the series. The extent to which this dose ratio was affected by enlarging the ester group is shown in the last column of Table III. The α-ethylbutyrate of ethinylandrostenediol (IX) exhibited the lowest UGSt/TGSp dose ratio. This compound possessed 1/22 the UGSt activity of the 3-acetate (I) but was more than 1/3 as active in suppressing testicular growth.

A comparison of activity ratios for the acetate (X), propionate (XI) and cyclohexylpropionate (XII) of ethinylandrostenediol, when administered as aqueous suspensions (Table III), shows that here also the UGSt activity falls off much more abruptly with increasing size of the ester group than does the TGSp activity. Whereas the 3-propionate (XI) was 1/3 as estrogenic as the 3-acetate (X), it was nearly twice as active as the latter in suppressing testicular growth. It should be noted that the aqueous suspension of ethinylandrostenediol 3-cyclohexylpropionate (XII) was considerably more active in the two tests than was the oil solution of the corresponding ester in the ethinylandrostanediol series (X).

It has been found that aqueous suspensions of 3-cyclohexylpropionate (XII) are slowly absorbed from sites of injection and provide extended duration of activity. This compound is well tolerated by tissues and is being examined clinically under the name "ethandrostate" as a possible inhibitor of pituitary gonadotrophin. When administered subcutaneously to mature male rats daily for a period of 2 weeks this ester produced 50% of castration atrophy of the seminal vesicles at dose levels which were 0.3 and 0.6 the estrogenic ED_{50} (UGSt) unit for the oil solution and aqueous suspension, respectively.

Discussion. These observations, in addition to pointing out structure-activity relationships, serve to indicate that a particular chemical change of a biologically active compound does not necessarily modify all activities manifested by that compound to the same degree. The finding that 17-ethinylation increased the TGSp activity of estradiol and Δ^5-androstenediol considerably more than it did UGSt activity, and that certain large molecular weight esters of ethinylandrostanediol and its Δ^5-analog brought about a greater loss of UGSt than TGSp, illustrates altered UGSt/TGSp dose ratios due to different increments of activity in one case and to different degrees of loss of activity in the other case. Several factors suggest themselves as contributory to such effects. A particular chemical change may modify the manifestations of an active agent by altering its rate of absorption from the site of injection, by changing its rate of degradation by tissues, by modifying its rate of entry into the responsive organ or tissue or by any combination of the three. Which of these factors is operative in regard to the compounds studied and reported here has not been established.

The observation that considerably more estradiol-17β was required to suppress testicular growth than to stimulate estrogen sensitive tissues of female immature rats is in accord with earlier reports(8,9). Byrnes and Meyer (10), however, have reported that significant suppression of ovarian growth is effected by subestrogenic doses of estradiol administered daily to 70 g intact female rats for 10 days. Greep and Jones(9), on the other hand, did not observe ovarian regression with less than fully estrogenic doses of estradiol benzoate administered daily for 45 days to rats of similar age and weight. Although the literature

is not definitive in respect to the relative sensitivity of the male and female pituitary to estrogens, there is suggestion that the pituitary of the adolescent male rat is somewhat less sensitive to estradiol than is the female rat pituitary. It should be pointed out that 35-40 g male rats were used in the studies reported here because of the satisfactory dose response of the testes to estrogen, in contrast to the inadequate ovarian changes induced by estrogens in intact rats of this age. Pincus and Werthessen(11) noted that the ovarian response in comparable rats was a function of duration of dosage rather than size of dose, hence we deemed immature female rats unsuitable for assay of suppressive activity.

The much lower UGSt/TGSp dose ratios obtained for several high molecular weight esters of ethinylandrostanediol and its Δ^5-analog than obtained for estradiol or ethinylestradiol suggests that these esters may be relatively more specific than the natural estrogens as inhibitors of pituitary gonadotrophic function. This possibility is being explored in a variety of appropriate follow-up experiments.

Summary and conclusions. (1) The biological activity of ethinylandrostanediol and its Δ^5-analog has been found to be predominantly estrogenic rather than androgenic. The relative testicular growth suppressing (TGSp) activity effected by these ethinyl-diols was considerably greater than observed for equivalent estrogenic doses of estradiol-17β or ethinylestradiol. (2) Low molecular weight esters (less than 6 carbon atoms) of these non-phenolic steroids were more active than their parent alcohols, but the degree of estrogenicity (UGSt) diminished more abruptly than did TGSp activity as the size of the ester group was increased. Of the compounds tested, the 3α-ethylbutyrate of ethinyl-Δ^5-androstenediol effected the greatest degree of testicular growth suppression (TGSp) per unit of estrogenicity (UGSt-ED_{50}), when 35-40 gram immature intacts rats were used.

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Relation of Gonad Hormones to X-Irradiation Sensitivity in Mice.* (22494)

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It has been reported(1-5) that a single injection of estradiol benzoate 10 days before an LD 50/30 day exposure to whole body x-rays will increase the resistance of the mouse to the exposure. The present study was designed to determine what effect estradiol benzoate would have in the intact as compared with the castrated male mouse when each is exposed to the LD 50/30 day level of whole body x-rays.

Materials and methods. Ninety-one mature CF_1 male mice were castrated by surgical excision. Aureomycin was sponged over the

* Based on work performed under contract for the Atomic Energy Commission.