range as the effect on calcifiability. This is in contrast to the effects on β-metachromatic granules of the non-calcifiable proliferative zone which were practically complete after lower doses of irradiation that only partly inactivated the calcifiability. The appearance of the γ-metachromasia in the tissue when it becomes calcifiable, and the similar sensitivity of the γ-metachromatic material and calcifiability to irradiation, suggest that a relationship may exist between them.

Summary. 1. X-irradiation of rachitic epiphyseal cartilage with 250,000 R resulted in small decrease in calcifiability, loss of β-metachromatic granules from some cells of the proliferative zone, and a slight general decrease in staining of the hypertrophic zone. X-irradiation with 500,000 R resulted in marked decrease in calcifiability, loss of most of the β-metachromatic granules from the proliferative zone, and loss of γ-metachromatic granules from some cells of the hypertrophic zone. X-irradiation with 1,000,000 R resulted in almost complete loss of both calcifiability and stainability with toluidine blue. 2. Toluidine blue staining of fresh sections of epiphyseal cartilage was employed for demonstration of metachromatic substances. 3. Once the process of calcification had been initiated, the inactivating effect of irradiation on calcifiability was considerably reduced. 4. A change in mucopolysaccharide resulting in increase of free electronegative groups is a factor associated with calcifiability of epiphyseal cartilage.

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Estrogen Antagonisms: Inhibition of Estrone-Induced Uterine Growth and Vaginal Smear Effects with Testosterone Derivatives. (25943)

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19-Nortestosterone derivatives are highly potent antagonists of uterine growth and vaginal smear effects of estrogens(1). Lengthening of the chain at carbon 17 increased potency in straight-chained forms to ethyl, beyond which potency decreased, and unsaturation affected potency which seemed increased. Data are now available on natural forms of certain of these materials, allowing comparison of structure-activity relationships between 19-nor and natural derivatives of testosterone.

Materials and methods have been described (1). Briefly, in uterine weight studies, various doses of test compound were administered mixed with 0.3 μg of estrone in 0.3 ml of corn oil. 0.1 ml of the mixture was injected daily for 3 days and mice were sacrificed 24 hr after final injection. At autopsy the cleaned, blotted uteri were weighed on a torsion balance. Dose of test compound that was esti-

mated to produce 50% depression in uterine response to the estrone was employed for comparative purposes. Estrone at 0.3 μg generally produces a uterus weighing 35 mg; oil treated mice average 10 mg uterine weight. After 4 days of treatment, vaginal smears were taken from spayed mice on days 5 p.m. and 6 a.m.(2). The presence of leukocytes in fresh smears was the criterion of activity(3). All mice received 2 μg of estrone which generally produces about 90% smears lacking leukocytes, and dose of antagonist estimated to produce 50% depression in this response was employed for comparative purposes.

Results. For both tests progesterone is standard estrogen antagonist and has been assigned a potency of 100%. All 17-substituted testosterones studied were active antagonists (Table I), although their potencies were extremely low. In both tests methyl testosterone was most potent, free testosterone and ethyl being considerably less so. In uterine growth test ethyl testosterone seems somewhat more potent than vinyl or ethynyl, but this is not supported by vaginal smear studies in which ethyl, vinyl and ethynyl are essentially indistinguishable. Results in the two tests show close correspondence of potencies, such as has been found for entire 19-nortestosterone series.

Comparison of results from this test and those from studies with 19-nortestosterones is instructive (Table II). In each case and in both tests 19-nor configuration is considerably more potent than natural. In natural series, methyl testosterone is most potent compound, whereas activities continue to rise to ethyl in 19-nor series. These data indicate that biological effects of modifications of testosterone molecule at carbon 17 are intimately related to presence or absence of angular methyl grouping at carbon 10. This would suggest that both positions are involved in attachment of molecule to whatever receptor site is involved in estrogen antagonisms. This is not a unique finding here, since earlier studies with compounds related to progesterone showed that although neither 6α-methyl progesterone nor 17-acetoxyprogesterone was markedly different in potency from progesterone itself, 6α-methyl-17α-acetoxyprogesterone was almost 3 times more potent than progesterone (3). In the present series free testosterone and ethyl, vinyl and ethynyl testosterone are all of roughly similar potencies, whereas cleavage of the angular methyl grouping at carbon 10 (19-nor configuration) results in major differences in potency among these 4 materials.

TABLE I. Potencies of Various Testosterone Derivatives as Antagonists of Uterine Growth Effects of 0.3 μg of Estrone and Vaginal Smear Effects of 2 μg of Estrone.

	Potency as estrogen antagonist			
	Uterine growth test		Vaginal smear test	
Compound	N*	Potency	N†	Potency
Progesterone	24 (7)	100	141 (4)	100
Testosterone	14 (3)	7	137 (4)	80
Methyl	17 (4)	23	103 (3)	130
Ethyl	11 (2)	14	56 (2)	22
Vinyl	12 (3)	7	57 (2)	27
Ethynyl	11 (3)	8	103 (3)	27

* No. before parentheses indicates No. of groups of 8-10 mice employed in estimation of potency of compound; No. in parentheses indicates No. of tests in which compound was studied.

† No. in front of parentheses indicates total No. of vaginal smears employed in estimation of potency; parenthetical No. is No. of tests in which material was evaluated.

TABLE II. Comparison of Estrogen Antagonistic Potencies of 19-nor and Natural Analogues of Testosterone Derivatives in Mouse Uterine Growth and Vaginal Smear Studies. Progesterone = 100%.

	Potency			
	Uterine growth		Vaginal smear	
Compound	Natural	19-nor*	Natural	19-nor†
Testosterone	7	40	80	170
Methyl	23	880	130	1800
Ethyl	14	1250	22	2100
Vinyl	7	460	27	910
Ethynyl	8	800	27	910

* Data from(1). † In preparation.

Summary. Free testosterone, and its methyl, ethyl, vinyl and ethynyl derivatives were tested as antagonists of estrone-induced uterine growth and vaginal smear changes. Methyl testosterone was most potent of the compounds; there were no major potency differences among remainder. These findings are in contrast to 19-nor derivatives, which are considerably more potent and among which the ethyl is most potent.

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Estrogen Antagonisms: Effects of Several Steroidal Spirolactones on Estrogen-Induced Uterine Growth in Mice. (25944)

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There has been continuous growth of interest in compounds that antagonize renal action of aldosterone. Pharmacology of the first of these agents to be discovered was summarized by Kagawa, Sturtevant and Van Arman(1), who noted briefly that SC-5233, 3-(3-oxo-17β - hydroxy - 4 - androsten - 17α - yl)propionic acid γ-lactone was inactive as estrone antagonist. Recently, interest has shifted to 19-nor (SC-8109) and 7α-acetylthio (SC-9420) derivatives of SC-5233(2,3). Since Hertz and Tullner(4) were able to demonstrate progestational activity for SC-5233 and SC-8109, it seemed worthwhile to study these materials as estrone antagonists. In addition to these 3 compounds, several other steroidal spirolactones have been studied. Chemistry of these materials has been discussed by Cella and his colleagues(5,6,7).

Materials and methods. Structural formulae for the compounds tested are presented in Fig. 1. Each substance was tested initially at a dose of 1000 μg, against 0.3 μg of estrone, following technic described earlier(8). Briefly, immature mice received test compound mixed with the standard dose of estrone in 3 daily injections, each containing $\frac{1}{3}$ of the total doses indicated in this paper. Twenty-four hrs after final injection mice were sacrificed and cleaned uteri weighed on a torsion balance. Compounds were compared at doses estimated to produce a 50% depression in response expected from estrone alone. Earlier experiments, including certain studies dealing with SC-8109, employed Rockland mice and method of evaluation has been discussed(8). Most studies to be described here employed Carworth mice obtained from Small Animals Farms, Des Plaines, Ill., which responded with greater increment of uterine growth. Three groups of 10 animals each that received only estrone averaged 46.3 mg uterine weight; oil-treated controls averaged 9.4 mg. Thus, dose of material estimated to produce uterine weight response of 28.4 mg, which corresponds to a 50% depression, was employed for comparison, and any material that failed to produce this level was considered inactive. In contrast to earlier studies no material considered as inactive here produced any suggestion of inhibition. Progesterone, standard in this test, produces 50% depression at a dose of about 40 μg. Secondary testing of each compound progressed by decreasing dose at increments of about $\frac{1}{3}$ until activity was lost. Progestational potencies were determined from subcutaneous Clauberg test(8). For purposes of comparison, potencies were based upon doses that produced a +2 response.

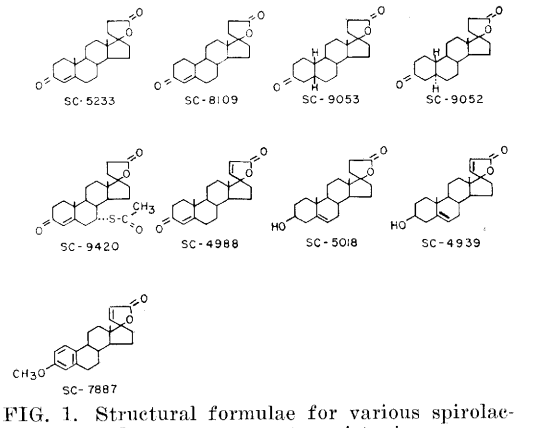

FIG. 1. Structural formulae for various spirolactones tested as estrone antagonists in a mouse uterine growth assay.