was transmissible through the milk to the offspring to cause spontaneous tumor development. The failure to induce mammary tumors in the rats receiving a cell-free filtrate prepared from the tumors induced by 3-methylcholanthrene does not seem to favor this speculation. Further investigations are now in progress.

Summary. Spontaneous mammary cancers were observed in 4 female rats among a total of 28 (7♂ and 21♀) offspring of mothers treated with 3-methylcholanthrene before pregnancy. These tumors developed at the age of 49, 85, 90, and 90 days. Histologically, 3 were mammary adenocarcinoma and 1 an undifferentiated breast cancer. One female rat among a total of 12 (3♂ and 9♀) offspring of mothers treated with 3-methylcholanthrene during pregnancy developed spontaneous mammary cancer at the age of 200 days. No mammary cancer was observed in rats whose mothers were fed 3-methylcholanthrene during lactation. Also, injection of cell-free filtrate prepared from the mammary cancer induced with 3-methylcholanthrene to the normal newborn rats of less than 24 hours old failed to induce mammary tumors in these animals. These observations are of great interest for further investigation.

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Estrogen Antagonisms: Effects of a Series of 19-Nortestosterone Derivatives on Vaginal Changes Induced by Estrone. (26072)

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Many chemical relatives of 19-nortestosterone are active as antagonists of uterine growth produced in mice by estrone and as progestational agents in Clauberg tests. There appears to be general correspondence between potencies in these tests, although no perfect correlation exists. Comparison of biological activity with chemical structure permitted conclusions regarding effects of structural alterations on activity(1). Since other studies had suggested remarkable differences in responsiveness of uterus and vagina to mixtures of steroid hormones(2,3,4,5), confirmation of these structure-activity relationships in vaginal smear tests became desirable. Comparable studies were possible only with discovery that progesterone would block estrogens administered over a 4-day period(4,6). This paper studies effects of derivatives of 19-nortestosterone as antagonists of estrone effects on the vagina.

Materials and methods. Assay methods employed here have already been described (4,6,7). Briefly, adult female Rockland mice were spayed and allowed 2 weeks to recover from effects of operation. They were then integrated into a colony and animals were chosen at random from colony for test-

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TABLE I. Comparison of Estrogen Antagonistic Effects of a Series of 19-Nortestosterone Derivatives and Other Miscellaneous Steroids in Vaginal Smear and Uterine Growth Tests. Under N, parenthetical number indicates number of tests in which a given compound was tested; number before parentheses is total number of smears upon which estimates were based. Code numbers refer to points on Fig. 1.

Compound or 17-substituent	Vaginal smear test N	Vaginal smear test Potency	Uterine growth, potency	Code No.
Progesterone	141 (4)	100	100*	1
Desoxycorticosterone acetate	101 (3)	42	10†	2
Testosterone propionate	165 (5)	200	70‡	3
19-nortestosterone	69 (2)	170	40*	4
Methyl 19-nortestosterone	61 (2)	1800	880*	5
Ethyl "	92 (3)	2100	1250*	6
Vinyl "	56 (2)	910	460*	7
Ethynyl "	52 (2)	910	800*	8
Isopropyl "	61 (2)	3100	2200*	9
n-Propyl "	71 (2)	250	100–400*	10
Allyl "	59 (2)	50	160*	11
n-Butyl "	53 (2)	20	Inactive*	12
1-Methallyl "	63 (2)	800	400*	13
2-Methallyl "	61 (2)	530	680*	14

* Data from Edgren, Calhoun, Elton and Colton(1). † Data from Edgren and Calhoun (9). ‡ Data from Edgren and Calhoun(10).

ing each week. Test materials were administered in 0.1 ml per day of corn oil solution. Combinations of steroids were mixed and given in a single 0.1 ml injection. Each animal received 4 subcutaneous injections on mornings of first 4 days of test. Vaginal contents were sampled on afternoon of day 5 and morning of day 6. Unstained smears were scored according to method of Biggers and Claringbold(8), *i.e.,* an animal was scored as positive if there were no leukocytes in smear. Mice scored positive on day 5 were not studied again on day 6; a single positive smear sufficed to give any mouse a positive evaluation. Estrone was employed at a constant dose of 2 μg, and a series of doses of other steroid were injected simultaneously. Potency evaluations were based upon dose of blocker estimated to produce 50% depression in response to estrone. In most cases the 2 μg estrone dose stimulated positive responses in about 90% of mice. ED_{50} doses for blockers were read from graphs of estimated log dose-response curves.

Results and discussion. All compounds examined in this study were active (Table I), and there was a remarkable correspondence between results in this test and those obtained in uterine growth test. Again potency increased in the straight chain, saturated compounds from a low value of 170% for 19-nortestosterone itself to a high of 2100% for the ethyl; 3 and 4 carbon chains result in subsequent decreases for n-propyl and n-butyl. The latter shows some slight activity in this test, although it was not active as estrone antagonist in uterine growth test. Branching increased potency in this test; isopropyl, considered here as a branched ethyl, was more potent than ethyl, and both methallyls were more potent than allyl or n-propyl. The high degree of correspondence between results of this test and uterine growth test is shown in Fig. 1. Most compounds fell close to a straight line when potency in one test was plotted against potency in other test, indicating that both tests largely measured the same phenomenon. In terms of potency relative to progesterone standard, the compounds, with only 2 exceptions, were more potent as antagonists in vaginal smear than in uterine growth test. The 2 exceptions to this were allyl and 2-methallyl. Since the 1-methallyl acts much as one would predict from the remainder of series, there would seem to be no obvious reason why 2-methallyl gives an abnormal response pattern. It seems reasonable to assume that deviation of 2-methallyl is a chance occurrence and that only allyl is markedly divergent; why this should be is inexplicable at this time. Desoxycorticosterone acetate and testosterone propionate also show

a close correlation between activities in this and uterine growth test.

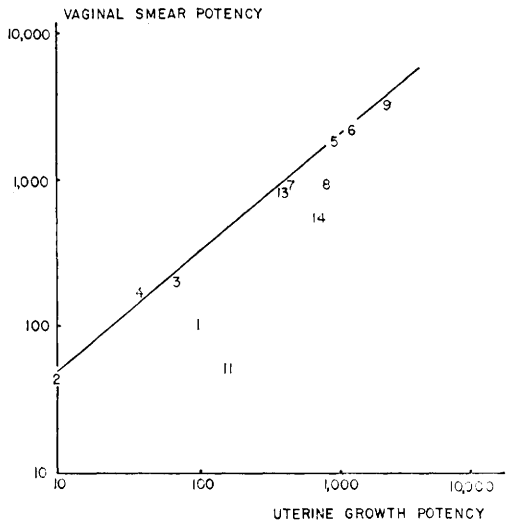

FIG. 1. Relationship of estrogen antagonistic potency in vaginal smear and uterine growth tests. Numbers refer to code numbers of Table I.

Summary. 19-Nortestosterone derivatives are antagonists of estrone in vaginal smear as well as uterine growth tests. Structure-activity relationships among these materials are largely the same in both tests.

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Suppression of LSD-25 Effects in Rats by Steroids.* (26073)

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Unpublished evidence by Resnick in our laboratories indicates that the effects of lysergic acid diethylamide (LSD-25) on smooth muscle contraction in an *in vitro* system may be altered by addition of steroid substances to the bathing medium of the muscle. This apparent antagonism is interesting to us because LSD-25 and steroids are known to affect the function of the central nervous system. Thus LSD-25 in small amounts produces a transient psychotic episode in man(1) and behavioral changes in animals(2). Likewise the balance of steroid hormones may alter mental function, *e.g.*, abnormal behavior may be seen both with adrenal insufficiency (Addison's disease) as well as excess (Cushing's disease)(3). Severe adrenal insufficiency in man is accompanied by a slowing of the electroencephalogram which is restored by cortisone(4). We have noted that adrenalectomy produces a significant decrease of approximately 10% in the dominant EEG frequency in rats and that this condition is corrected not only by most adrenal steroids but by the steroid hormone precursor Δ^5-pregnenolone as well(5). Δ^5-Pregnenolone has been shown to increase the efficiency level of a group of highly skilled motivated workers significantly above a similar group receiving placebos(6).

It occurred to us that investigation of the effects of steroids against behavioral changes produced by LSD-25 might show that steroids are effective anti-LSD agents *in vivo* as well as *in vitro*.

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