THE DEPRESSION OF GROWTH OF THE UTERUS, ADRENALS, AND OVARIES BY FLUORINATED STEROIDS IN THE PREGNANE SERIES*

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This paper is concerned with the depression of growth of specific tissues of the rat by steroids related to progesterone. As growth antagonists, certain members of the pregnane series were found to have the following unique properties: The depression of growth is selective. Without interfering with body growth, certain pregnanes cause a decrease in the size of the adrenals and ovaries of intact young rats compared with the size of those of uninjected controls. In hypophysectomized rats, these steroids inhibit the growth of the uterus when administered concurrently with estrone or testosterone, hormones which are powerful stimulators of uterine growth.

Steroidal inhibition has pertinence in problems of growth, notably cancer. It is known that several neoplasms including those of the prostate of dog (1) and man (2) are maintained and propagated in their growth by testosterone and that they undergo regression following the administration of phenolic estrogens. These estrogenic phenols while suppressing cancers promote undesirable growth in the breast and other organs. The pregnane derivatives which depress steroid-induced growth have special interest since, administered separately, they excite little or no cellular proliferation.

It will be shown that a fluorine atom in steroids related to progesterone can augment significantly their power to inhibit steroid-induced growth and to depress as well glands which synthesize growth-promoting steroids.

It has been known for some time that certain steroids in the pregnane series can inhibit the growth-stimulatory effects of phenolic estrogens upon the tissues of the female genital tract under special conditions. Conversely the characteristic progesta-

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tional effect induced in the uterus of the rabbit by progesterone can be completely inhibited by large doses of estrone (3, 4).

The scarlet edematous perineum induced by estradiol in the castrate baboon undergoes complete deturgescence and becomes pale when this estrogen is supplemented with progesterone (5). Hertz *et al.* (6) found that the simultaneous administration of progesterone and phenolic estrogens resulted in a marked inhibition of the estrogen-induced growth of the oviduct of the chick. After a high degree of inhibition was obtained, additional increments of progesterone did not cause further decrements of estrogen-induced tissue growth. Also it was observed (6) that a sixteenfold increase in estrogen dosage did not reverse the inhibitory action of a maximally effective quantity of progesterone upon the oviduct.

The studies of the school of Lipschütz on the inhibition of estradiol-induced growth in the castrate female guinea pig have revealed that other pregnane derivatives are also effective inhibitors. The most powerful inhibitor of estrogen-induced uterine growth described by these workers is 19-*nor*progesterone (7); other inhibitors in decreasing order of effectiveness are progesterone (8, 9), desoxycorticosterone (10), and testosterone (9); weak inhibitors which they reported were 11-ketoprogesterone (11) and 11β-hydroxyprogesterone (11). Cortisone (12) was found not to be an inhibitor of uterine growth.

Progesterone (13) and desoxycorticosterone (10) are such potent inhibitors of estradiol in the guinea pig that supplementary administration of either of them stops uterine bleeding and permits closure of the vaginal membrane in the presence of the estrogen.

11α-Hydroxyprogesterone is the only compound which has been reported to have the capacity to inhibit growth induced by steroids in both the estrane and androstane series. Byrnes, Stafford, and Olson (14) stated that 11α-hydroxyprogesterone, when administered to gonadectomized rats in large doses (5 to 10 mg. daily), partially suppressed uterine growth in females treated with estradiol and depressed as well the growth of the seminal vesicles in males injected with testosterone. Large amounts of this compound produced a slight decrease in weight of the ovaries and the adrenals in intact rats.

Recently it was shown by Fried and Sabo (15, 16) that the introduction of a chlorine, or especially of a fluorine, atom at position 9 in cortisone, hydrocortisone, and 11β-hydroxyprogesterone (17) profoundly increases the capacity of these steroids to deposit glycogen in the liver as well as to cause retention of sodium in the body. The ability of these fluorinated steroids to inhibit steroid-induced growth of tissues has not been described.

The present studies consisted in the administration of derivatives of steroids in the pregnane series alone or in addition to estrone or testosterone to hypophysectomized rats maintained under highly standardized conditions. Compounds which were found to be the most effective steroid inhibitors in hypophysectomized animals were then administered to intact young rats. The principal indicator of suppressive effect was growth of the uterus, and this is defined as an increase of weight and of nitrogen content of this organ. Histo-

logical studies of the vagina provided useful supplementary evidence of the activity of steroids in combination upon the female genital tract.

Methods

The experimental procedure has been described (18, 19) in detail. In brief, steroids were administered on a strict schedule first to hypophysectomized rats kept in a controlled climate and fed a steroid-free synthetic ration. The steroids were dissolved or suspended in ethyl alcohol and these preparations were diluted with 9 volumes of sesame oil; such freshly prepared solutions (0.2 ml. daily) were injected from age 38 to 44 days inclusive. When combinations of steroids were administered they were dissolved in the same solution. Tests of inhibition of growth were carried out against constant daily doses of growth-promoting steroids; the standard dose of estrone was 0.5 μg., of testosterone, 1 mg. *Throughout this paper dosage refers to the amount administered each day.*

Six rats were tested at each dose level of every compound. Each compound was tested in triplicate at least and on fresh batches of rats and at different times. In studies of inhibition the statistical probability, P, of a significant effect was derived from Fisher's table (20) of t values. The results were considered significant when P values less than 0.02 were obtained for both nitrogen content and uterine weight.

Necropsy was performed at age 45 days. The uterus was excised, blotted lightly, and weighed. Its nitrogen content was determined by a micro-Kjeldahl technique; the results are stated as percentage of nitrogen (gm./100 gm.) of the moist weight of the uterus. Paraffin sections of the vagina were stained with hematoxylin and eosin, and with celestin blue for mucoprotein.

Intact female rats were injected with steroids daily from age 23 to 50 days with necropsy at age 51 days. The preputial glands, thymus, adrenals, and ovaries were weighed in addition to the uterus and vagina.

Chemical.—Many of the steroids were obtained from other laboratories.[1] 4-Pregnen-20β-ol-3-one, 4-pregnen-20-one, and 4-androsten-3-one were synthesized in this laboratory by methods to be described elsewhere.

RESULTS

Characteristics of Uterine Growth Induced by Estrone or Testosterone.—Increasing amounts of estrone or testosterone promote brisk growth of the vagina and uterus of the hypophysectomized rat until a plateau of growth is reached; estrone, 0.5 μg. (19) or testosterone, 1 mg. (21), the standard doses employed in these experiments, are amounts considerably larger than are required for the weight of the uterus to reach the growth plateau. The type of growth which these steroids evoke differs considerably.

The uterus of uninjected hypophysectomized rats is delicate, thread-like and opaque; the average concentration of nitrogen in 34 uteri was 2.9 ± 0.1 per cent. The vagina is atrophic.

The uterus whose growth has been excited by estrone is tough, pale, translucent and is distended with fluid; the nitrogen concentration in 16 uteri was

[1] We acknowledge with gratitude generous gifts of compounds used in these experiments from Dr. Josef Fried, the Squibb Institute for Medical Research; Dr. D. H. Peterson, The Upjohn Co.; Dr. Paul Talalay, The Ben May Laboratory for Cancer Research.

2.52 ± 0.1 per cent. The vagina of rats with such uteri is opaque and many long keratin fibres are visible by microscopy; mucoprotein is not present.

The uterus whose growth has been provoked by testosterone is soft, jelly-like, red in color due to dilated capillaries and contains little or no secretion; the nitrogen concentration in 16 uteri was 2.09 ± 0.05 per cent. The vagina is pale, translucent and contains much mucoprotein.

The administration of estrone 0.5 μg. and testosterone 1 mg. together did not result in decrease in size of the uterus. Some of the physiologic character-

TABLE I

Inhibitors of Estrone-Induced Growth of Uterus

In all cases estrone 0.5 μg. injected daily with or without added steroids. Δ indicates the change in uterine weight induced by added steroids. The rats were hypophysectomized.

No.	Added steroid	Daily dose	Change in body weight	Weight of uterus		Δ
				Estrone alone	Estrone + added steroid	
		μg.	gm.	mg.	mg.	per cent
	(a) Strong inhibitors					
I	Progesterone	250	+1	126 ± 12	80 ± 9	−36
II	5-Pregnene-3,20-dione	1000	+2	128 ± 15	81 ± 10	−37
III	Desoxycorticosterone	250	+1	131 ± 13	84 ± 11	−36
IV	9α-Fluoro-11β-hydroxyprogesterone	250	−1	126 ± 12	62 ± 10	−51
V	9α-Fluorohydrocortisone	250	−3	120 ± 12	59 ± 12	−51
	(b) Weak inhibitors					
VI	4-Pregnen-20β-ol-3-one	1000	+1	128 ± 21	92 ± 14	−28
VII	5-Pregnen-3β-ol-20-one	1000	+5	128 ± 15	98 ± 11	−23
VIII	11β-Hydroxyprogesterone	1000	−2	137 ± 21	107 ± 23	−22
IX	Cortisone	1000	−8	128 ± 21	108 ± 17	−16
X	Hydrocortisone	1000	−8	128 ± 21	109 ± 12	−15

± indicates standard deviation.

istics of each compound are present. The uterus is pink, soft, and distended with secretion. The nitrogen concentration was 2.31 ± 0.1 per cent. The vagina is pale, soft, and gelatinous; keratin fibres are absent and an outstanding characteristic is the large amount of mucoprotein which is present.

Pregnane Inhibitors of Estrone-Induced Uterine Growth.—Members of the pregnane series which inhibited the growth of the uterus when administered with estrone were classified as strong or weak inhibitors (Table I).

Our criteria for strong inhibitors are fourfold: (*a*) All values of uterine growth (weight and nitrogen content) must be lower than the lowest determination found in the absence of the inhibitor. (*b*) The weight and nitrogen content of the uterus in the presence of estrone and the inhibitor must be

diminished more than 30 per cent of average values obtained when estrone alone was administered. (*c*) The *P* value of Fisher (20) must be less than 0.01. (*d*) Repeated assays at different times must yield inhibition of a consistent order of magnitude.

In the case of weak inhibitors, the order of inhibition was 15 to 28 per cent of the growth obtained when estrone alone was injected. The *P* values were less than 0.02. The results were in agreement in serial experiments at various times.

The nitrogen concentration of all the uteri of rats treated with estrone with or without added pregnane compounds, fell in a closely grouped series, the range being 2.47 to 2.6 per cent, so that a simple determination of increment of uterine weight was a significant criterion of growth.

Progesterone (I)[2] is a strong inhibitor of estrone-induced growth of the uterus. A considerable inhibition was obtained when 0.1 mg. of progesterone was administered and an increase of dose of this compound to 3 mg. did not enhance the effect (Text-fig. 2). 9α-Fluoro-11β-hydroxyprogesterone (IV) and 9α-fluorohydrocortisone (V) were more effective inhibitors than progesterone (Table I).

A significant loss of body weight (Table I) resulted when 9α-fluorohydrocortisone, cortisone (IX), or hydrocortisone (X) was injected at dosage levels required to exert inhibitory effects against estrone. The loss of weight with 9α-fluoro-11β-hydroxyprogesterone (FHP)[3] in dosage up to 0.5 mg. was inconstant or slight.

None of the pregnane inhibitors, in the absence of estrone, induced growth of the vagina or uterus in dosage up to 1 mg. The preputial glands of hypophysectomized rats weighed 8 to 9 mg. FHP, 0.25 to 1 mg. daily caused an increment of weight of these structures to 10 to 13 mg.

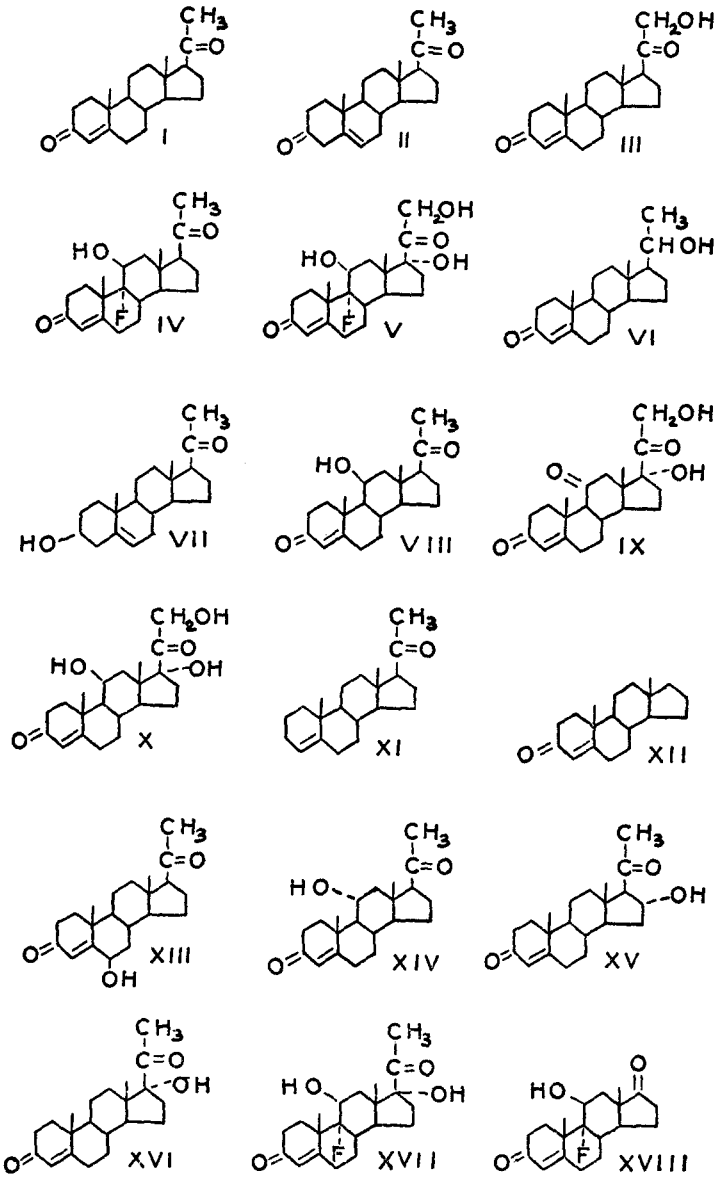
Many members of the pregnane series (Table II) failed to modify significantly the effectiveness of estrone in promoting growth.

Effect of Pregnane-Inhibitors of Estrone on the Vaginal Epithelium.—When injected with estrone, only 2 steroids in the pregnane series caused transformation of the vaginal epithelium to mucus cells. The effective compounds were progesterone and FHP.

Progesterone, 0.25 mg., permitted keratin sheets, characteristic of estrone activity, to form in the vagina (Fig. 1) although this amount was sufficient to cause maximal inhibition of uterine growth. When the dosage of progesterone was increased to 0.5 mg. the cells desquamated before keratinizing and leucocytes appeared amidst them (Fig. 2). Progesterone, 1 mg., caused mucification and many leucocytes appeared amidst the vaginal epithelium

[2] The chemical formulae of the compounds used in these experiments are given in Text fig. 1.

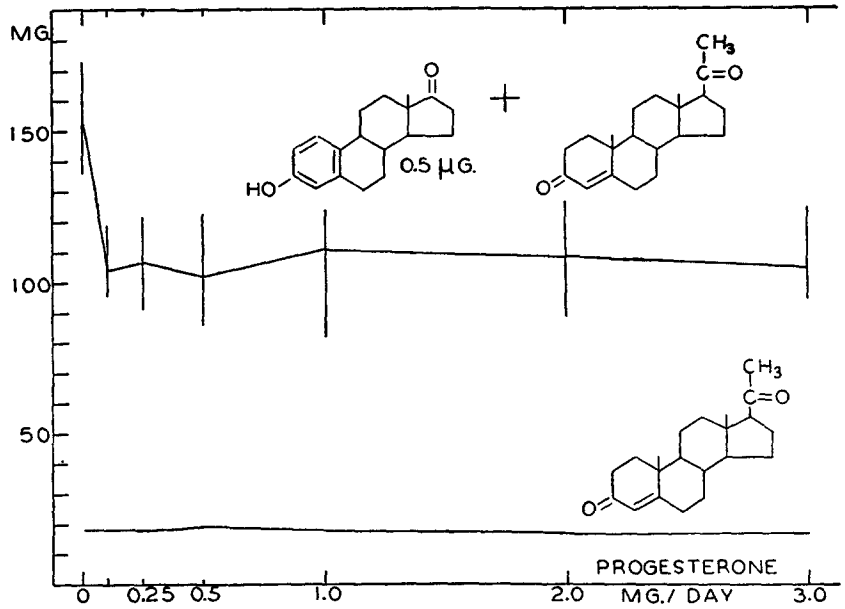
[3] 9α-Fluoro-11β-hydroxyprogesterone.



TEXT-FIG. 1. Chemical formulae of the compounds.

(Fig. 3); the formation of mucus cells was much less than in pregnancy in the rat.

FHP was a more powerful inhibitor than progesterone of estrone-induced growth of the vagina. Mucoprotein appeared in the vaginal epithelium at a



TEXT-FIG. 2. The upper curve shows decrease of weight of the uterus induced by supplementing estrone (0.5 μg. daily) with increasing amounts of progesterone (abscissae). The ordinates indicate weight of the uterus in milligrams. The lower curve is weight of the uterus in the presence of progesterone alone.

TABLE II

Non-Inhibitors of Estrone-Induced Growth of Uterus

In all cases estrone 0.5 μg. injected daily with or without added steroids. Δ indicates the change in uterine weight induced by added steroids. The rats were hypophysectomized.

No.	Added steroid	Daily dose	Weight of uterus		Δ
			Estrone alone	Estrone + added steroid	
		mg.	*mg.*	*mg.*	*per cent*
XI	4-Pregnen-20-one	1.0	109 ± 14	119 ± 15	+9
XII	4-Androsten-3-one	1.0	119 ± 16	108 ± 14	−9
XIII	6β-Hydroxyprogesterone	0.5	119 ± 14	122 ± 18	+2
XIV	11α-Hydroxyprogesterone	1.0	109 ± 14	105 ± 11	−4
XV	16α-Hydroxyprogesterone	1.0	109 ± 14	115 ± 11	+6
XVI	17α-Hydroxyprogesterone	1.0	109 ± 14	124 ± 24	+14
XVII	9α-Fluoro-21-desoxyhydrocortisone	0.25	128 ± 15	123 ± 17	−4
XVIII	9α-Fluoro-11β-hydroxy-4-androstene-3,17-dione	0.5	119 ± 16	105 ± 4	−12

lower dose level (Fig. 4) than when progesterone was injected, it was more extensive (Fig. 5), and leucocytes were absent from the vaginal secretion

(Fig. 6). 5-Pregnene-3,20-dione (II), 1 mg., injected with estrone caused disappearance of keratin and many leucocytes were present in the vaginal wall and fluid but mucoprotein was not present.

In large dosage, desoxycorticosterone (III), 9α-fluorohydrocortisone, and 11β-hydroxyprogesterone (VIII) caused a decrease in the amount of keratin fibres but there was pronounced growth of the basal cells of the vagina which is characteristic of estrogenic stimulation, and mucification did not occur.

Pregnane Inhibitors of Uterine Growth Induced by Testosterone.—11β-Hydroxyprogesterone and FHP were strong inhibitors of uterine growth elicited by testosterone, 1 mg. (Table III). This inhibitory property was not shared by progesterone. None of these compounds caused loss of weight. FHP was an

TABLE III

Effect of Pregnanes on Testosterone-Induced Growth of Uterus

In all cases, testosterone, 1 mg, injected daily with or without added steroids. Δ indicates the change in uterine weight induced by the added steroids. The rats were hypophysectomized.

Added steroid	Daily dose	Change in body weight	Weight of uterus		Δ
			Testosterone alone	Testosterone + added steroid	
	μg.	*gm.*	*mg.*	*mg.*	*per cent*
Progesterone	1000	+2	105 ± 9	100 ± 13	−5
11β-Hydroxyprogesterone	500	+2	96 ± 6	89 ± 6	−7
11β-Hydroxyprogesterone	1000	0	104 ± 6	71 ± 14	−32
9α-Fluoro-11β-hydroxyprogesterone	100	+1	104 ± 6	68 ± 13	−35
9α-Fluoro-11β-hydroxyprogesterone	250	−1	104 ± 6	65 ± 10	−38
9α-Fluorohydrocortisone	250	−5	104 ± 6	67 ± 8	−36

effective inhibitor at a dosage level of 0.1 mg. The first inhibition of growth of the uterus by 11β-hydroxyprogesterone was induced at a dosage level above 0.5 mg. (Table III).

9α-Fluorohydrocortisone was a strong inhibitor of testosterone-induced uterine growth but it induced a significant loss of body weight (Table III).

The concentration of nitrogen in the uterus of animals treated with testosterone, with or without supplementary pregnane compounds, was 2.03 to 2.14 per cent. In each case keratin sheets were absent and there were considerable amounts of mucoprotein present, although far less than in pregnancy.

Pregnane Compounds Administered to Intact Young Rats.—Progesterone and steroids with a closely related molecular structure were administered to intact female rats, beginning at the onset of puberty; the compounds differed in the number of hydroxyl groups (0 to 3) and fluorine atoms (0 or 1) which were present (Table IV).

FHP was found to have unusual inhibitory properties. It did not cause loss of body weight at a dose level (0.1 mg.) such that selective inhibition of growth was evident. Whereas all the pregnane compounds induced some depression of uterine weight relative to that of uninjected controls, only FHP was a strong inhibitor of growth of the uterus. FHP caused a significant de-

TABLE IV

Pregnane Derivatives Administered to Intact Rats

Compounds were injected daily from age 23 to 50 days with autopsy at age 51 days. There were 8 female rats in each group. The weight at age 23 days was 43 to 46 gm.

Compound	Daily dose	Final body weight	Thymus	Preputial glands	Adrenals	Ovaries	Uterus	Δ Uterus
	mg.	*gm.*	*mg.*	*mg.*	*mg.**	*mg.**	*mg.**	*per cent*
None; uninjected	—	168	503	88	56 (48–64)	50 (44–56)	341 (298–409)	—
Progesterone *OH, 0; F, 0*	0.2	169	523	117	53 (46–64)	51 (44–70)	250 (180–329)	−27
11β-Hydroxyprogesterone *OH, 1; F, 0*	0.2	166	471	96	54 (43–68)	50 (45–60)	268 (232–327)	−21
9α-Fluoro-11β-hydroxyprogseterone *OH, 1; F, 1*	0.1	176	444	88	39 (28–47)	43 (34–55)	192 (118–330)	−44
9α-Fluoro-11β-hydroxyprogesterone *OH, 1; F, 1*	0.2	159	327	74	33 (23–45)	31 (23–34)	167 (111–275)	−51
9α-Fluoro-21-desoxyhydrocortisone *OH, 2; F, 1*	0.1	156	387	90	41 (32–46)	48 (36–59)	259 (164–314)	−24
9α-Fluorohydrocortisone *OH, 3; F, 1*	0.1	139	186	96	33 (18–40)	57 (52–70)	278 (188–416)	−18

* Average values and the range of weight. Δ, the change in weight of uterus with respect to uninjected controls.

crease (30 to 41 per cent) of the weight of the adrenals, but inhibition of this order of magnitude was induced by all of the fluorinated steroids. FHP alone caused a decrease (38 per cent) in the weight of the ovaries.

The nitrogen concentration of the uteri of the intact rats injected with FHP was 2.49 to 3.10 per cent. The vaginal epithelium of 8 rats injected with FHP 0.2 mg. was classified as follows: keratin sheets, 2; atrophy, 2; mucification, 4 rats. The mucification was of extremely high grade—of an extent only seen previously by the authors in pregnancy. Mucification of so high a grade oc-

curred with no other compound in this series. The vaginal mucosa of 8 uninjected control rats fell in the following categories: keratin sheets, 5; stratified epithelium, 1; mucification (slight), 2.

DISCUSSION

Although none of the members of the pregnane series completely suppressed growth of the uterus in hypophysectomized rats injected simultaneously with large amounts of estrone or testosterone, some of the compounds were found to be effective to a significant extent.

Progesterone is a strong inhibitor of estrone-induced growth of the uterus. This compound loses its effectiveness by removal of the ketone group at C_3 as in 4-pregnen-20-one (XI) or the 2-carbon side chain as in 4-androsten-3-one (XII).

No great modification of the inhibitory characteristics of progesterone followed the reduction of the ketone group in the side chain at C_{20} to form 4-pregnen-20β-ol-3-one (VI) or the introduction of a hydroxyl at position 21 as in desoxycorticosterone. Each change abolished the power of induce vaginal mucification. Progesterone lost its inhibitory characteristics when hydroxylated at position 6 (β) (XIII), 16 (α) (XV) or 17 (α) (XVI).

The efficacy of progesterone in the inhibition of estrone-induced growth was weakened by the introduction of a hydroxyl group at C_{11} to form 11β-hydroxyprogesterone; however, this compound was found to be a significant inhibitor of testosterone-induced growth of the uterus when large doses were administered. At the dosage level (1 mg.) employed in the present experiments, 11α-hydroxyprogesterone (XIV) did not inhibit steroid-induced growth although in the experiments of Byrnes *et al.* (14) this compound exerted weak inhibitory effects when given in large doses (5 to 10 mg).

The introduction of a fluorine atom in the 9 (α) position can exert a striking influence on the growth-inhibitory properties of a steroid. Both FHP and 9α-fluorohydrocortisone are stronger inhibitors of estrone-induced growth than 11β-hydroxyprogesterone or hydrocortisone.

The fluorine atom alone does not bring about the growth-inhibitory activity since 9α-fluoro-21-desoxyhydrocortisone (XVII) and 9α-fluoro-11β-hydroxy-4-androstene-3,17-dione (XVIII) did not inhibit estrone-induced uterine growth.

Three compounds, cortisone, hydrocortisone, and 9α-fluorohydrocortisone (to a smaller extent) induced a significant loss of body weight at a dosage of 0.25 mg. and inhibition of growth of the uterus following treatment with these compounds can be explained at least in part on the basis of a general inhibition of protein synthesis. FHP in similar amount caused slight or no decrease in weight of the body. It is known that hydroxyl groups at positions 11, 17, and 21 increase the catabolic activity of steroids in the pregnane series.

Determination of the concentration of nitrogen in the uterus was helpful in the interpretation of the inhibitory effects of FHP. Estrone and testosterone induce uterine growth with differing and characteristic values[4] for nitrogen concentration. With estrone the nitrogen concentration (about 2.5 per cent) of the uterus is considerably higher than in testosterone-induced growth (about 2.1 per cent). Although testosterone does not inhibit the growth of the uterus when it is administered with estrone, it modifies the composition of the uterus (nitrogen concentration about 2.3 per cent). FHP does not alter the nitrogen concentration of the uterus; as an inhibitor of estrone, the nitrogen concentration of the uterus remains characteristic for estrone-stimulated growth and as an inhibitor of testosterone, the nitrogen content remains low. The fluorine atom in FHP intensifies the inhibitory characteristics of 11β-hydroxyprogesterone *vis à vis* testosterone or estrone but it does not change qualitatively the characteristic uterine growth elicited by either of these growth-promoting steroids.

Vaginal mucification is not a prerequisite to the inhibition of growth by steroids. In common with testosterone (22), progesterone (23) and FHP induce the formation of mucus cells when administered with estrone. In contrast to testosterone, these pregnane derivatives are inhibitors of estrone-induced growth of the uterus. 11β-Hydroxyprogesterone did not cause vaginal mucification in rats treated with estrone while FHP was more effective than progesterone in this regard. The high grade of mucification of the vagina of many of the intact rats to which FHP was administered is similar to that of advanced pregnancy; it is evidence that FHP has some of the characteristics of a highly active progestational compound.

In addition to its highly developed progestational activity, there is evidence that FHP has some of the physiological properties of an adrenal cortical steroid. Adrenal atrophy was induced by FHP, 9α-fluoro-21-desoxyhydrocortisone and 9α-fluorohydrocortisone; of these fluorinated steroids only FHP induced a decrease of ovarian weight.

CONCLUSIONS

Progesterone, 5-pregnene-3,20-dione, and desoxycorticosterone were found to be partial but significant inhibitors of estrone-induced growth of the uterus

[4] Among members of the androstane series which are androgenic in that they elicit growth of the prostate and preputial glands of the rat, the site of unsaturation in the steroid molecule can determine the ability of the compound to elicit keratin fibres (an estrogenic action) or mucification in the vaginal epithelium (16). As in the case of estrone and testosterone these compounds cause characteristic changes in the nitrogen concentration of the uterus. 5-Androstene-3β,17β-diol, 1 mg., induces the formation of keratin fibres and the average concentration of nitrogen was 2.54 per cent. 4-Androstene-3β-17β-diol, 1 mg., causes vaginal mucification and the concentration of nitrogen in the uterus was 2.15 per cent.

of hypophysectomized rats but they did not depress the amount of growth elicited by testosterone.

The presence of both the 2-carbon side chain at position 17 and an oxygenated function at position 3 is necessary for the inhibitory activity of progesterone. The introduction of a hydroxyl group in the progesterone molecule at positions 6 (β), 11 (α), 16 (α) or 17 (α) destroyed its growth-inhibitory activity.

11β-Hydroxyprogesterone is weaker than progesterone in the inhibition of estrone-induced uterine growth; unlike progesterone it has the capacity to depress growth elicited by testosterone

The introduction of a fluorine atom in the 9 (α) position resulted in a striking increase of the growth-inhibitory activity of 11β-hydroxyprogesterone and hydrocortisone.

9α-Fluoro-11β-hydroxyprogesterone is a partial but significant inhibitor of growth of the uterus in hypophysectomized rats injected simultaneously with large doses of estrone or testosterone. It is more powerful than progesterone in transforming the vaginal epithelium to mucus cells in the presence of estrone. Administered alone, the only detectable growth was a slight increase in size of the preputial glands.

9α-Fluoro-11β-hydroxyprogesterone suppresses in part the growth of the adrenals, ovaries, and uterus in intact rats at a dose level which does not cause loss of body weight or inhibit body growth. Many rats treated with this compound in small amount had extensive mucification of the vagina resembling that of late pregnancy.

BIBLIOGRAPHY

1. Huggins, C., and Clark, P. J., *J. Exp. Med.*, 1940, **72,** 747.
2. Huggins, C., and Hodges, C. V., *Cancer Research*, 1941, **1,** 293.
3. Courrier, R., *Compt. rend. Soc. biol.*, 1936, **122,** 661.
4. Robson, J. M., *J. Physiol.*, 1936, **88,** 100.
5. Gillman, J., and Stein, H. B., *Endocrinology*, 1941, **28,** 274.
6. Hertz, R., Larsen, C. D., and Tullner, W., *J. Nat. Cancer Inst.*, 1947, **8,** 123.
7. Mardones, E., Iglesias, R., and Lipschütz, A., *Proc. Soc. Exp. Biol. and Med.*, 1954, **86,** 451.
8. Lipschütz, A., Murillo, R., and Vargas, L., Jr., *Lancet*, 1939, **2,** 420.
9. Lipschütz, A., Vera, O., and Gonzales, S., *Cancer Research*, 1942, **2,** 204.
10. Lupschütz, A. Luco, J. V., and Zanartu, J., *Cancer Research*, 1942, **2,** 200.
11. Mardones, E., Iglesias, R., and Lipschütz, A., *Nature*, 1954, **174,** 839.
12. Lipschütz, A., Mardones, E., Iglesias, R., Fuenzalida, F., and Bruzzone, S., *Science*, 1952, **116,** 448.
13. Lipschütz, A., and Maas, M., *Cancer Research*, 1944, **4,** 18.
14. Byrnes, W. W., Stafford, R. O., and Olson, K. J., *Proc. Soc. Exp. Biol. and Med.*, 1953, **82,** 243.

15. Fried, J., and Sabo, E. F., *J. Am. Chem. Soc.*, 1953, **75,** 2273.
16. Fried, J., and Sabo, E. F., *J. Am. Chem. Soc.*, 1954, **76,** 1455.
17. Fried, J., Herz, J. E., Sabo, E. F., Borman, A., Singer, F. M., and Numerof, P., *J. Am. Chem. Soc.*, 1955, **77,** 1068.
18. Huggins, C., Jensen, E. V., and Cleveland, A. S., *J. Exp. Med.*, 1954, **100,** 225.
19. Huggins, C., and Jensen, E. V., *J. Exp. Med.*, 1955, **102,** 335.
20. Fisher, R. A., Statistical Methods for Research Workers, Edinburgh, Oliver and Boyd Ltd., 9th edition, 1944.
21. Huggins, C., Parsons, F. M., and Jensen, E. V., *Endocrinology*, 1955, **57,** 25.
22. Korenchevsky, V., and Dennison, M., *J. Path. and Bact.*, 1936, **43,** 345.
23. Desclin, L., *Compt. rend. Soc. biol.*, 1934, **115,** 439.

EXPLANATION OF PLATE 40

The photomicrographs are of paraffin sections of the vaginal mucosa stained with hematoxylin and eosin. The magnification of each is × 350.

In each case a standard amount of estrone, 0.5 μg. daily, had been injected with various steroids in the daily dosage stated below each photomicrograph, from age 38 to 44 days with necropsy at 45 days.

The photomicrographs show that 9α-fluor-11β-hydroxyprogesterone (administered with estrone) induces more mucification of the vaginal epithelium and at a lower dosage than progesterone does.

FIG. 1. Long keratin sheets are present; this is characteristic of unmodified estrone activity.

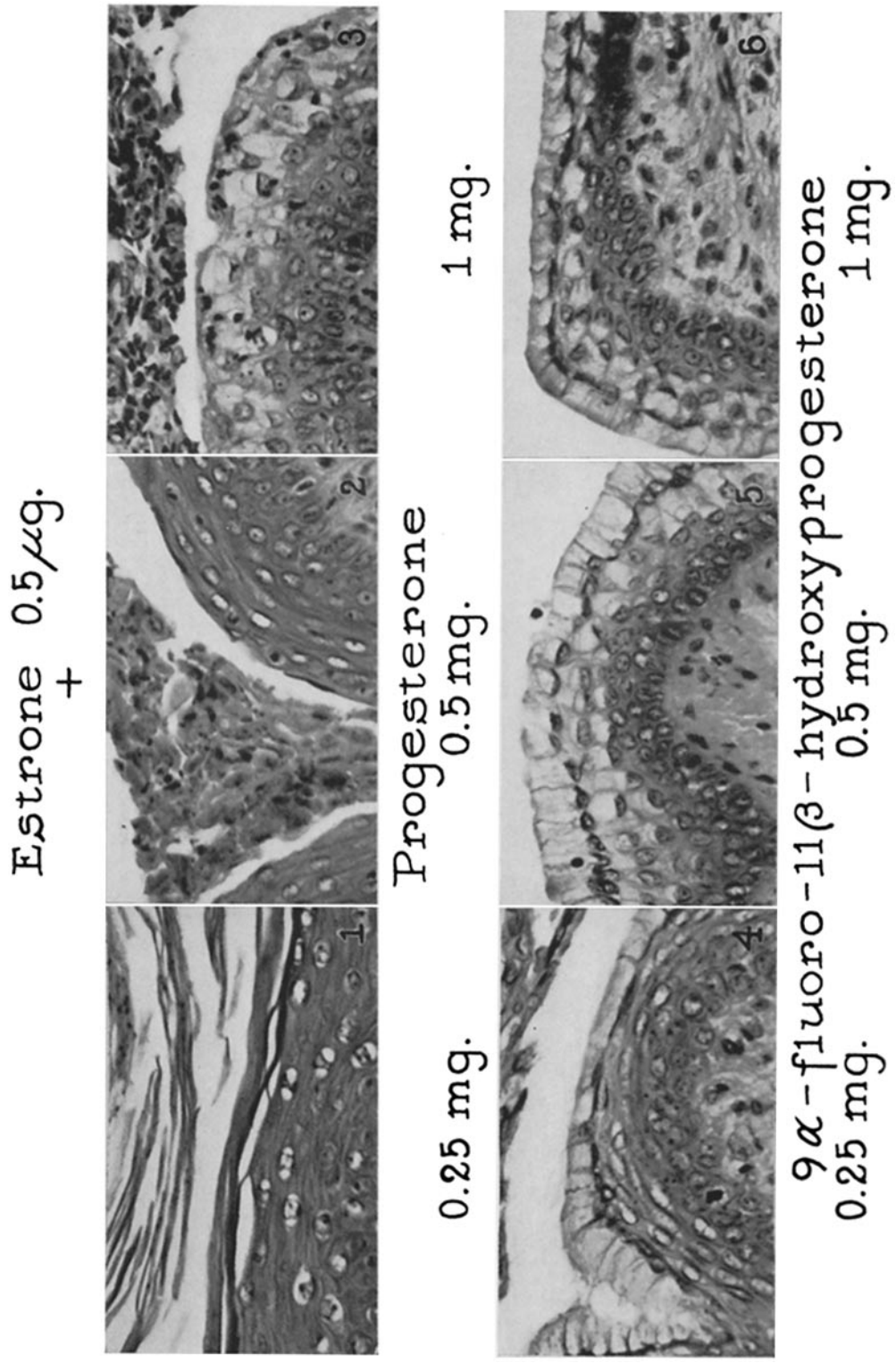
FIG. 2. The keratin sheets are short and leucocytes are present in the vaginal secretion.

FIG. 3. Many mucous cells are seen in the vaginal epithelium and leucocytes are visible between these cells and in the vaginal secretion.

FIG. 4. Mucous cells are seen on the surface of the vaginal mucosa.

FIG. 5. The mucous cells are more extensive than in Figs. 3 and 4.

FIG. 6. While mucification of the vaginal epithelium was extensive when 9α-fluoro-11β-hydroxyprogesterone was administered at a dosage level of 1 mg., leucocytes were not present. The vaginal epithelium is thinner at this dosage level than occurred when 0.5 mg. was the daily amount of the inhibitor.



(Huggins and Jensen: Steroid inhibitors of growth)