oxidase and cytochrome oxidase of the total homogenate or the fractions after 16-140 hours of starvation.

Discussion. These experiments indicate that much less of the total succinoxidase and cytochrome oxidase activities were recovered with the renal cortex mitochondria isolated in sucrose than in whole kidney mitochondria isolated in alkaline water(1). This may be due to differences between cortical and medullary mitochondria or to differences induced by the suspending medium. In addition, less succinoxidase activity can be demonstrated in the renal cortex mitochondria than in mitochondria from other tissues. Thus, although there is qualitatively a tissue and species biochemical similarity of mitochondria, quantitatively there may exist tissue and possibly cellular differences.

Summary. 1. The results with 0.88 M sucrose solution as the dispersion medium agree essentially with those obtained with alkaline water medium. 2. Succinoxidase activity of the mitochondria accounts for 43% of the total homogenate activity and cytochrome oxidase 47% on a wet weight basis. 3. On nitrogen basis the activities are 2-3 times greater than that of the homogenate. 4. After 2 washings the activity of both enzymes on a wet weight basis is reduced; on a nitrogen basis succinoxidase activity is reduced to 33% but the cytochrome oxidase activity is the same as in the original mitochondria. 5. Small amounts of the final supernatant fluid added to mitochondria increase the succinoxidase activity to 85% of the original homogenate. This effect does not represent an addition of the activities of the 2 fractions. 6. There is no change in intracellular succinoxidase and cytochrome oxidase activities after a period of starvation of 16 to 140 hours.

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Anti-Gonadal Hormone Activity of 11α-Hydroxyprogesterone. (20080)

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The antagonism which one gonadal hormone may show for another is frequently brought about by the hormones' decreasing the secretion of gonadotrophins by the pituitary, and, therefore, secondarily diminishing the production of the endogenous sex hormones. On the other hand, there is evidence that a gonadal hormone may inhibit the effects of another sex hormone directly. Early investigations by de Jongh(1-3) revealed that androgens are capable of preventing certain responses in estrogen-treated animals. These observations were confirmed and extended by Zuckerman and Parkes(4), Robson(5), Gardner and Pfeiffer(6) and many others. The anti-androgen effects of estrogens were reported about the same time by Gley and Delor(7) and Mühlbock(8). Early experiments by Macht and Stickels(9), Hisaw and Lendrum(10) and Dessau(11) brought out the inhibitory effect which progesterone may exert on the biological activity of estrogen. Conversely, estrogen has been shown to be antagonistic to the development of certain progestational effects. The inhibition of the placentoma reaction by estrogen has been demonstrated by Courrier(12), Brouha(13), Votquenne(14) and others. Although both estrogen and progesterone are

needed for the production of the progestational type of endometrium, an excess of estrogen prevents this uterine response(15-17). There are numerous other reports of antagonism between gonadal hormones. The antagonism which one gonadal hormone has for another under certain conditions has led to their use in clinical medicine in attempts to counteract sex hormone-linked pathology. Although this therapy has proven successful in some instances, the effectiveness of a steroid in antagonizing the endogenous hormone is frequently vitiated by its own sex hormone activity. It is reasonable to assume that the efficacy of this technic would be enhanced if a steroid were available which exhibited antigonadal hormone activity without having other pharmacological activity.

A number of 11-oxygenated compounds related to the steroidal hormones have recently become available through new processes developed in the laboratories of The Upjohn Company(18-20). As part of a broad program of biological evaluation of these steroids, we have investigated the endocrinological behavior of 11α-hydroxyprogesterone (U-0384), a compound differing from progesterone in that it has an α-hydroxyl group at position 11 of the steroid nucleus. This new steroid has been found to antagonize estrogen and androgen while having little or no other hormone-like effects.

Procedures. U-0384 was tested for antigonadal hormone activity in estrogen-treated gonadectomized male and female rats and in androgen-treated castrated male rats as well as in intact untreated male and female rats. The compound was also assayed for estrogenic, androgenic, progestational, and adrenal cortical hormone activity. The animals used in these procedures were rats of the Upjohn Strain which are of Sprague-Dawley ancestry. At the beginning of the experiments the rats were divided into groups of equal body weight. The various steroids which were used were homogenized and suspended in a diluent prepared in this laboratory (0.4% Tween 80, 1.5% benzyl alcohol, 0.9% NaCl, and 0.5% low viscosity carboxy-methyl cellulose in distilled water). The experimental rats were injected subcutaneously once a day with the steroid suspended in 0.4 cc of the diluent; control animals were given the diluent alone. In procedures where U-0384 and a gonadal hormone were administered simultaneously to the animals, the two steroids were injected at different sites.

Anti-estrogen effect in ovariectomized female rats. Fifteen immature female rats with an average body weight of 63 g were ovariectomized and divided into 3 groups. The rats were injected daily with diluent, 0.05 μg estradiol (17β-estradiol), or 0.05 μg estradiol and 10 mg of U-0384. The animals were treated for 4 days and killed 24 hours after the last injection. The uterine weight of the rats injected with estrogen plus U-0384 was 76 mg as compared with 111 mg in the estrogen-treated rats and 53 mg in the controls. This experiment has been repeated several times with substantially the same results. It is concluded that U-0384 partially counteracted the stimulation of the uterus in ovariectomized rats injected with estradiol. *Anti-estrogen effect in castrated male rats.* Fifteen immature male rats averaging 78 g were castrated and divided into 3 groups. The rats in the first group were injected with 0.4 cc of the diluent. The second group was given 20 μg of estradiol and 0.4 cc of the diluent, and the rats in the third group were injected with 20 μg of estradiol and 10 mg of U-0384. The animals were treated for 5 days and killed 24 hours after the last injection. The seminal vesicles of the estrogen-treated rats weighed 26.4 mg as compared with 7.0 in the uninjected controls. Since the administration of U-0384 to estrogen-treated rats reduced the seminal vesicle weight to 18.6 mg, it appears that this steroid partially counteracted the exogenous estrogen. This experiment has been repeated under varying conditions and essentially the same results obtained.

Anti-estrogen effect in intact immature female rats. Twenty-five immature female rats of the Upjohn strain were divided into 5 groups. The rats in the first group were injected with the diluent. The rats in the remaining groups were given 5 or 10 mg of progesterone or U-0384. The animals were treated for 10 days and autopsied 24 hours after the last injection. The data presented

TABLE I. Anti-Estrogen Activity of U-0384 in Intact Immature Female Rats.

Treatment (mg/day)	Body wt, g	Absolute wt (mg) Ovaries	Uterus	Thymus	Adrenals
Diluent	120	25.3	156	423	34.6
P* 5	127	16.1	123	383	27.8
P 10	122	13.2	117	366	25.1
U 5	121	20.5	88	390	28.4
U 10	121	19.6	96	404	31.4

* P = Progesterone; U = U-0384.

TABLE II. Anti-Estrogen Effect of U-0384 Compared with Testosterone in Intact Female Rats.

Treatment	Uterus (mg)	Ovaries (mg)	Body wt (g)	No. rats
Diluent	154	27.3	123	15
TP,* 500 μg/day	254	19.7	131	10
U-0384, 5 mg/day	81	18.4	119	15

* TP = Testosterone propionate.

in Table I demonstrate that U-0384 caused considerable atrophy of the uterus. Although progesterone was more effective in inhibiting the pituitary secretion of gonadotrophic hormones by ovarian weight criteria, the uteri of the animals were much heavier than in the rats treated with U-0384. This indicates that U-0384 is much more effective than progesterone in antagonizing endogenous estrogen. *Anti-estrogen effect compared with an androgen in intact female rats.* To compare the anti-estrogen effect of U-0384 with androgen, immature female rats were divided into 3 groups. The animals were injected for 10 days with diluent, testosterone propionate, or U-0384. On the eleventh day the rats were killed and the ovarian and uterine weights taken. The data from this experiment appear in Table II. The secretion of gonadotrophic hormones was suppressed by each of the steroids injected as evidenced by the decreased ovarian weights. U-0384 was effective in causing uterine atrophy whereas the androgen caused further stimulation of uterine growth. It was considered unreasonable to give the testosterone at the same dosage as the U-0384. The dose given is very large and probably comparable to any amount used clinically in women. The increase in body weight of the rats getting the androgen confirms that this amount of testosterone is more than adequate to produce the anabolic effect. Although androgens under some conditions counteract estrogens, in this experiment the testosterone brought about an enlarged uterus whereas U-0384 effectively antagonized the endogenous gonadal hormones.

Anti-androgen effect in castrated male rats. Fifteen young male rats were castrated and divided into 3 groups. The rats in the first group were injected with the diluent, and the second group was given 200 μg testosterone propionate. The animals in the third group were injected with 200 μg testosterone propionate and 10 mg of U-0384. The animals were treated for 3 weeks and autopsied 24 hours after the last injection. The results of the experiment are recorded in Table III. The purpose of this experiment was not only to test the activity of U-0384 in decreasing the androgen-induced growth of the sex accessories, but also upon the nitrogen-retention effect of androgen as measured by the weight of the body, kidney, and the levator ani muscle. From the data in Table III it is seen that U-0384 partially counteracted the androgen-induced hypertrophy of the seminal vesicles, prostate and levator ani muscle. This experiment has been repeated several times with essentially the same results. *Anti-androgen effect in intact male rats as compared with estrogens.* To compare the anti-androgen activity of U-0384 with estrogen, adult male rats were divided into 4 groups, 5 rats to the group. The rats were injected for 10 days with diluent, estradiol, stilbestrol, or U-0384. On the eleventh day the animals were killed and the weights of the seminal vesicles, prostates, and testes were taken. The data from this experiment are in Table IV. Contrary to expectation, the dose of estradiol was not enough to cause great atrophy of the secondary sex organs. The stilbestrol, however, was injected in amounts which were sufficient to elicit marked involution. It should be emphasized that the doses of estrogen used were sufficient to produce great uterine stimulation in females. The decrease in body weights obtained with the estrogens indicates that the doses were excessive. Based on the weight of the seminal

TABLE III. Anti-Androgen Activity of U-0384 in Castrated Male Rats.

Treatment	Body wt (g)	Absolute wt in mg			
		Sem. ves.	Prost.	Kidney	Lev. Ani.
Diluent	188	11.5	17.4	1870	39.8
TP,* 200 μg/day and diluent	204	214.8	276.2	1943	158.6
TP, 200 μg/day, and U-0384, 10 mg/day	179	163.4	211.6	1990	109.6

* TP = Testosterone propionate.

TABLE IV.
Anti-Androgen Activity of U-0384 as Compared with Estrogens in Intact Male Rats.

Treatment	Body wt (g)	Sem. ves. (mg)	Prostate (mg)	Testes (g)
Diluent	247	199	196	2.634
Estradiol, 5 μg/day	231	125	110	2.469
Stilbestrol, 50 μg/day	199	46	44	2.110
U-0384, 5 mg/day	251	118	147	2.430

vesicles (which is better criterion of androgen activity than prostate weight) U-0384 was about as effective as 5 μg of estradiol in antagonizing the androgen. All of the compounds produced some testicular atrophy. While U-0384 caused atrophy of the seminal vesicles, it is not estrogenic and caused no decrease in body weight.

Estrogenic activity. Twenty immature female rats were ovariectomized and divided into 4 groups. The animals were injected for 8 days with diluent, or U-0384, in a daily dose of 1, 5, or 10 mg. U-0384 had no estrogen-like activity as determined by uterine stimulation: on the contrary, there was some decrease in uterine weight at the larger doses. The uterine weight of rats given 10 mg of U-0384/day was 43 mg as compared with a weight of 57 mg in the untreated controls. It is postulated that this was due to the anti-estrogen effect of this compound. Since the rats were ovariectomized, it is presumed that there was a small amount of estrogen being produced by the adrenals(21,22).

Androgen-like activity. Twenty male rats were castrated and divided into 4 groups. The rats in the first group were injected with the diluent, and the rats in the remaining groups were given 1, 5, or 10 mg of U-0384. The animals were treated for 10 days and autopsied 24 hours after the last injection. The weight of the seminal vesicles and prostates of the untreated rats averaged 12.0 and 16.4 mg respectively. Since the corresponding weights in rats treated with U-0384 in doses as high as 10 mg/day were 11.7 and 18.7, this steroid has little or no androgenic activity.

Progestational activity. The method chosen for testing U-0384 for progestational activity was that described by Astwood(23), based on the fact that a progestin is required for the formation and maintenance of the deciduoma in the traumatized uterus of the pseudopregnant rat. Adult female rats of the Holtzman-Rolfsmayer strain, 200-220 g body weight, were mated with vasectomized males to induce pseudopregnancy. On the fourth day after mating, the rats were ovariectomized, and the left horn of the uterus was traumatized by scarification of the endometrium with a needle. Beginning on the day of operation and continuing for 3 days, the rats were injected once daily with A) progesterone, 0.25 or 0.50 mg, or B) U-0384, 1.0 or 3.0 mg. On the following day following the last injection the rats were sacrificed, the uteri were dissected out, and mean diameters of control (right) and traumatized (left) horns of the uteri were estimated with a millimeter rule. It was concluded that since a positive response was obtained with 0.25 mg of progesterone, and since negative responses were obtained with 3.0 mg of U-0384, this compound has less than 1/12 the progestational activity of progesterone.

Effect on gonadotrophic hormone secretion.

Although U-0384 did not have estrogenic, androgenic, or progestational activity in the doses tested, it is of interest that this steroid caused a moderate suppression of gonadotrophic hormone secretion based on ovarian atrophy (Tables I, II). In the preceding experiments in which U-0384 had an anti-gonadal hormone effect in intact animals, it is probable that the action was both by direct antagonism to the sex hormone, and via an inhibitory action on the secretion of gonadotrophic hormone.

Effect on the secretion of ACTH. In Table I data are presented from intact female rats given U-0384 in doses of 5 and 10 mg/day. Using relatively large amounts there was no evidence of thymolysis, although there was some suppression of the secretion of ACTH as evidenced by slight adrenal atrophy. U-0384, however, was less active in this regard than comparable amounts of progesterone. U-0384 was also administered to gonadectomized male and female rats in doses of 5 and 10 mg/day without producing a decrease in body or thymic weight, although again there was slight adrenal involution. Since this compound had very little effect on the adrenal weight and no effect on the thymic and body weights of rats given very large amounts, it is probable that the steroid has little or no adrenal cortical hormone activity.

Summary. 1. U-0384 (11α-hydroxyprogesterone) partially counteracted the effects of exogenous estradiol in stimulating the uteri of ovariectomized rats and the seminal vesicles of castrated male rats. This compound also decreased the hypertrophy of the seminal vesicles, prostate, and levator ani muscle in castrated males injected with testosterone propionate. In intact rats U-0384 decreased the weight of the sex accessories by its anti-gonadal hormone activity, and through its inhibitory effect on gonadotrophic hormone secretion. 2. This steroid has no estrogenic, androgenic, or progestational activity in the amounts assayed. This compound, when injected in large amounts, produced only slight adrenal atrophy and no thymic involution, and can, therefore, be presumed to have very little or no adrenal cortical hormone activity. 3. Although the anti-estrogen and anti-androgen activity of this new steroid is limited in animals, inasmuch as it lacks other gonadal hormone effects, it may be effective in therapy of some pathological conditions in which a reduction of endogenous sex hormones is considered desirable.

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