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Ability of Some Progestational Steroids to Stimulate Male Accessory Glands of Reproduction in the Rat. (24690)

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The principle of multiplicity of actions of steroidal hormones has long been recognized. Steroids thought of as "estrogens" because of ability to stimulate female reproductive tract may also be "androgens" because of ability to stimulate the male tract. Similarly, hormones thought of primarily as adrenal corticoids have ability to produce progestational changes in the uterine endometrium. Progesterone resembles "androgens" because of its property of stimulating the prostate of castrated animals(1). This fact has recently assumed practical significance because of the finding of Wilkins *et al.* that occasionally women treated with progestational substances during pregnancy deliver mildly masculinized female infants(2). Some steroids recently introduced into therapeutic use are remarkably potent in causing glandular proliferation of endometrium, inhibiting ovulation, and to greater or lesser degree maintaining pregnancy in ovariectomized animals(3,4.5). Because of the possible importance of "androgenic" properties of progestins in treatment of pregnant women, a comparison has been made of ability of several of these compounds to stimulate the reproductive tract of castrate male rat. It is not known whether a substance promoting sex gland growth in the male rat will promote differentiation of the human female tract in a male direction, but the data are offered with the belief that a parallelism between the actions on these different end-points may exist.

Methods. Steroids. The drugs, together with some of their synonyms and abbreviations used in this communication, are listed below: 1. 17-α-methyl testosterone, methyltestosterone, (MT), 2. 6α-methyl-17α-acetoxy progesterone, medroxyprogesterone acetate, (MAP)*, 3. 17α-ethinyltestosterone, ethisterone, anhydrohydroxy progesterone, pregneninolone, (ET)†, 4. 17α-ethinyl-19-nortestosterone, norethindrone, (ENT)‡, 5. A mixture of norethynodrel (17α-ethinyl-5, 10-estrene-17β-ol-3-one) and 17α-ethinylestradiol, 3-methyl ether, (EN)§, 6. 17α-acetoxy progesterone, (AP)‖, 7. Progesterone (P), 8. 17α-(2-methallyl) - 19-nortestosterone, SC-9022, (MNT), 9. Testosterone propionate (TP). For administration all compounds were suspended or dissolved in cottonseed oil. *Animals.* Male rats of the Upjohn colony (Sprague-Dawley ancestry) were castrated between 26 and 28 days old. On day following castration steroid treatment began. The rats were divided into groups of 10, each group being treated with single daily oral (0.2 cc) or subcutaneous (0.1 cc) doses of cottonseed

* *Provera,* Registered trademark, The Upjohn Co., Kalamazoo, Mich.

† *Pranone,* Registered trademark, Schering Corp., Bloomfield, N. J.

‡ *Norlutin,* Registered trademark, Parke, Davis & Co., Detroit, Mich.

§ *Enovid,* Registered trademark, G. D. Searle & Co., Chicago, Ill.

‖ *Prodox,* Registered trademark, The Upjohn Co., Kalamazoo, Mich.

TABLE I. Stimulation of Male Sex Accessory Organs by Progestins and Androgens Administered Orally.

Compound	Dose (mg/day)	No. rats	Seminal vesicle wt (mg)	Prostate wt (mg)
Vehicle only		20	6	8
Ethinyl testosterone	1	10	9	23
	2	"	10	29
	4	"	14	40
	8	"	22	50
Ethinyl-19-nortestosterone	1	"	10	13
	2	"	14	18
	4	9	18	26
Enovid	1	10	15	12
	2	"	17	17
	4	"	18	21
Progesterone	5	"	8	29
	10	"	7	28
17-acetoxy progesterone	2	"	7	15
	4	"	6	11
	8	"	6	9
	16	"	6	9
6-methyl-17-acetoxy progesterone	1	"	7	14
	2	"	7	"
	4	"	8	"
Methyl testosterone	1	20	16	48
	2	"	30	67
	4	"	87	116

TABLE II. Stimulation of Male Sex Accessory Organs by Progestins and Androgens Given Subcutaneously.

Compound	Dose (mg/day)	No. rats	Seminal vesicle wt (mg)	Prostate wt (mg)
Vehicle only		25	7	9
Ethinyl testosterone	.25	10	14	40
	.5	"	33	58
	1.0	"	44	75
	2.0	"	57	84
Ethinyl-19-nortestosterone	.25	"	13	17
	.5	"	18	24
	1.0	"	30	36
Enovid	.25	"	17	13
	.5	"	20	15
	1.0	"	19	"
Methallyl-19-nortestosterone	.25	5	6	10
	.5	5	7	13
	1.0	5	8	14
6-methyl acetoxy progesterone	.25	10	9	16
	.5	"	12	22
	1.0	"	16	25
Testosterone propionate	.01	5	13	40
	.02	5	34	62
	.025	20	48	78
	.04	5	67	90
	.05	20	84	108
	.10	"	126	146

oil alone or with steroid. Animals receiving subcutaneous treatment were sacrificed the day following tenth injection and orally-treated rats the day following ninth dose. Weights of seminal vesicles and ventral prostates were recorded at autopsy.

Results. Tables I and II present organ weights of rats treated orally with MT and 6 of the progestins and subcutaneously with TP and 5 of the progestins.

Table III ranks estimated potencies of the 7 compounds studied with respect to the 2 routes of administration and the 2 end-points. When "activity" is estimated to be $<1\%$ as effective as standard androgen, "v. sl." is entered.

TABLE III. Progestins Ranked by Order of Potency in Stimulating Androgen Target Organs.

Compound	Oral efficacy		Subcut. efficacy	
	Seminal vesicle	Prostate	Seminal vesicle	Prostate
Ethinyl testosterone	3	1	1	1
Ethinyl nortestosterone	2	2	2	v.sl.
Progesterone	v.sl.	3		
Enovid	1	4	3	"
Acetoxy progesterone	0	5		
Methyl acetoxy progesterone	v.sl.	6	v.sl.	"
Methallyl nortestosterone			0	"

Figures indicate potency in descending order, *i.e.*, most potent steroid in a series is designated "1." Thus, of the compounds showing measurable oral prostate-stimulating ability, methyl acetoxy progesterone is least potent. When potency is so slight as to be judged less than 1% of standard androgen, it is indicated as "v.sl."

Although some androgenicity is seen with most of these progestins, none of them has "androgenic" activity approaching the potency of standard androgens. In most instances androgenic actions of the progestins are so weak that their potencies cannot be compared with those of standard androgens. In general, however, they can be compared with one another. For example, the greatest prostate response to ENT is a 26-mg gland at dose of 4 mg. This is much less than that with the lowest dose of MT, so an estimate

comparing its activity with MT cannot be made. But since the response falls between 23-mg gland produced by 1 mg of ET and the 29-mg gland of 2 mg of ET, with respect to prostate stimulation, ENT is greater than 25% and less than 50% as active as ET.

MAP administered *orally* showed no activity on seminal vesicle and very slight effect on the prostate. The prostate response did not increase as the dose increased, so a potency estimate would have little meaning. By *subcutaneous* route this compound showed measurable activity by both end-points, being approximately one-fourth and one-tenth ET on seminal vesicle and prostate respectively.

AP by *oral* route showed no androgenic activity except for slight elevation of prostate weight at 2-mg dose, an effect which decreased at 4 and disappeared at 8 and 16 mg.

P *orally* produced no stimulation of seminal vesicle and slight (though not dose-related) stimulation of the prostate. At 5 mg. the prostate response was equal to that with 2 mg of ET.

ET *orally* was the most potent prostate stimulator and was exceeded only by ENT and EN in ability to stimulate the seminal vesicle. By *subcutaneous* route it was the most effective stimulator of both seminal vesicle and prostate.

ENT *orally* produced substantial stimulation of seminal vesicle, being about twice as potent as ET; in stimulating the prostate it was one-fourth to one-half as active as ET and approximately equal to P. *Subcutaneously,* it was the second most potent of the compounds tested, being somewhat less than half as potent as ET as a seminal vesicle stimulator and less than one-fourth ET on the prostate.

EN *orally* was the most potent seminal vesicle stimulator of progestins studied, although its dose-response curve was sufficiently different from those of other compounds to prohibit potency estimate. Its action on the prostate was less than that of P or ENT, being only about one-fourth as active as the latter compound. By the *subcutaneous* route it also had a rapidly-plateauing curve in its effect on the seminal vesicle, and had virtually no effect on the prostate at doses used.

MNT was only tested *subcutaneously,* and by this route of administration was inert as seminal vesicle stimulator and produced only minimal stimulation of the prostate.

These results are interesting from the standpoint of differential sensitivity of the 2 glands, supporting previously established concept(6) that seminal vesicle and prostate grow differentially under the influence of compounds of varying chemical structure. ET, for example, when given orally, is more potent than ENT as a prostate-stimulator, but less potent as a stimulus to the seminal vesicle. This shift in relative activity also occurs when one compares EN with ET.

The tendency of EN and of ENT to be more potent in causing weight increase of the seminal vesicle than of the prostate may be associated with "estrogenic" activity of these 2 agents. EN contains a potent estrogen(4), and ENT has been shown(3) to be estrogenic to a significant degree. Ability of estrogen to augment seminal vesicle response to androgen while having little effect on prostate response to androgen has been known for many years (7).

The shifts in activity which accompany varying routes of administration are becoming so commonplace that those observed here scarcely require comment. EN, for example, is more potent in stimulating the seminal vesicle than is ET orally, but is less potent than ET subcutaneously. This is reminiscent of the relative lack of progestational activity of the major component of the EN mixture (norethynodrel) by subcutaneous route compared with its marked activity by oral route in the rabbit(4).

One important fact must be kept in mind in analyzing these results: if the capacity to stimulate male sex accessories is considered indicative of an "undesirable side effect" in clinical situations, the relative androgenic potency of the series of compounds must be considered in relation to the potency of therapeutic activity. The progestational potencies of these compounds vary widely(3,4,5,8,9). Thus, although MAP may be one-eighth as potent a seminal vesicle stimulator as ET by

the oral route, it is hundreds of times as potent a progestin(8). Thus for an effective progestational dose of MAP one gives an infinitesimal amount of androgenic potential compared with that of an effective progestational dose of ET.

Summary. When tested for ability to stimulate seminal vesicles and prostates of castrate, immature rats, a series of progestational steroids produced results as follows: 1) None of the compounds has androgenic activity approaching that of standard androgens, methyltestosterone or testosterone propionate. 2) All compounds tested *orally* produced some stimulation of the prostate, although it was extremely slight in the case of 17α-acetoxy progesterone and 6α-methyl-17α-acetoxy progesterone. Significant stimulation of seminal vesicles was seen only with 17α-ethinyltestosterone, 17α-ethinyl-19-nortestosterone, and EN. 3) Of compounds tested *subcutaneously,* only 17α-(2-methallyl)-19-nortestosterone failed to stimulate the seminal vesicle. All compounds produced some stimulation of the prostate, although EN and 17α-(2-methallyl)-19-nortestosterone produced minimal responses.

Samples of Enovid and 17α-(2-methallyl)-19-nortestosterone were generously supplied by Dr. Lee Hershberger and Dr. V. A. Drill respectively, G. D. Searle and Co. All other steroids used were provided by Dept. of Chemistry, The Upjohn Co.

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Particle Counts of Meningopneumonitis Virus by Phase Microscopy.* (24691)

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A rapid and simple technic for counting psittacosis virus particles would be of obvious value. Gogolak(1) described a modification of the method of Backus and Williams(2) for counting murine pneumonitis virus particles in which virus was mixed with a suspension of known concentration of *E. coli,* sprayed onto a glass slide and stained with Gram stain. The ratio of virus particles to bacteria was determined by counting each by light microscopy. This method was criticized by Crocker(3) on the basis that proof was lacking that the proportions of virus particles and bacteria are unchanged by the staining procedure, and that it does not allow differentiation between virus and non-infectious, non-elementary body forms. The need for a simple and rapid method for counting unpurified and partially purified virus preparations prompted the present study. The technic of a method and confirmation of its validity by egg infectivity tests, electron microscopy counts, and nitrogen determination is reported here.

Materials and methods. (1) *Preparation of virus suspensions.* Meningopneumonitis virus from the American Type Culture Collection was passed serially approximately 20 times in

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