TABLE II. Degree of Hemorrhage Seen Grossly in Treated Dogs with Malignant Hypertension.

Type of pretreatment	No. of animals	No. of animals with each degree of hemorrhage 0	+	++	+++
Control (procaine only)	5	1	3	1	0
Catechin and procaine	5	1	3	1	0
Rutin and procaine	6	2	1	2	1

TABLE III. Arteriolar Necrosis and Myocardial Necrosis in Treated Dogs with Malignant Hypertension.

Type of pretreatment	No. of animals	No. of animals with each degree of arteriolar necrosis 0	+	++	+++
Control (procaine only)	5	0	3	2	0
Catechin and procaine	5	0	2	3	0
Rutin and procaine	6	2	0	4	0

sidered, the apparent difference in the rutin-treated group loses its significance.

Discussion. Two reviews of the literature pertaining to the flavonol compounds indicate wide variations in the effectiveness of these substances in hemorrhagic states and other vascular disease(4,5). Contrary to the previous observations of one of us (J.L.O.), the results obtained in this experiment fail to indicate any effectiveness of rutin in malignant hypertension in dogs. The only differences between this experiment and the previous one using rutin are the use of different samples of rutin and a different injection schedule. The injection schedule used previously was three times daily, whereas in this experiment, the injections were given once daily. It is possible that either or both of these factors may have caused the difference in results.

Rutin was also found to be ineffective in the prevention of arterial lesions in hypersensitivity. Catechin was ineffective in both types of vascular lesions. It would appear, therefore, that if either flavonol has any effect, it must be too slight to be demonstrated by the procedures used.

Summary. Neither rutin nor catechin altered the vascular lesions of hypersensitivity arteritis in rabbits or of experimental malignant hypertension in dogs.

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Myotrophic Activity of 19-Nortestosterone and Other Steroids Determined by Modified Levator Ani Muscle Method.* (20301)

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Many steroids produce both androgenic and protein anabolic effects(1). Protein anabolic effects have been measured by body weight gain(2), by nitrogen retention(3-6), and by renotropic activity(7,8). Eisenberg and Gordon(9) have proposed a myotrophic test for the assay of protein anabolic activities of androgens. Steroids which had the greatest protein anabolic activity as measured by the classical nitrogen retention methods, also had the greatest myotrophic activity as measured by a levator ani muscle assay(9,10).

In this study a modification of the Eisenberg and Gordon myotrophic assay is proposed which eliminates the 23-day post-castration

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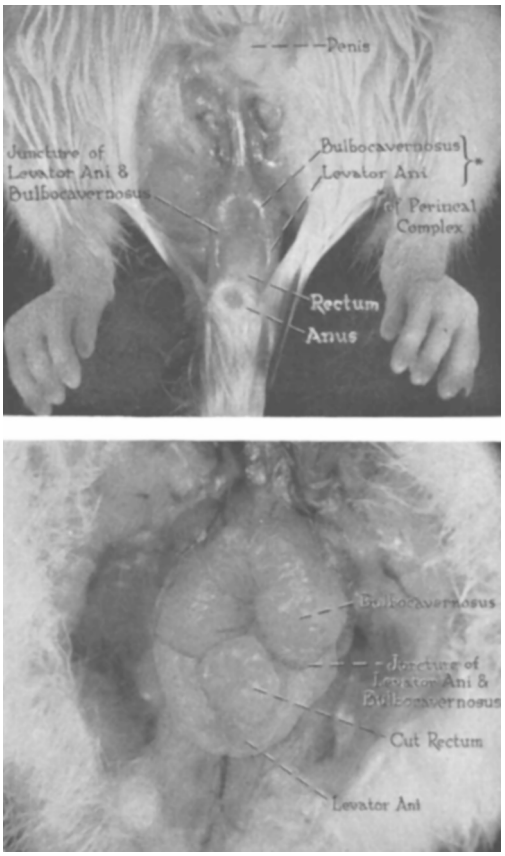

FIG. 1 and 2. Levator anti muscle and related structures in the rat.

rest period for the animals and reduces total assay time from 31 days to 8 days. The advantages are: 1) the rapid turnover of animals reduces the required size of the assay colony, and 2) it becomes possible to start an assay at any time, without the long post-castration delay. The assay has been used in this laboratory to study a number of steroids not previously tested in order to establish a ratio between their myotrophic and their androgenic activities. A series of compounds possessing high myotrophic activity with little or no androgenic activity might be of practical value, as well as of theoretical interest.

Materials and methods. Male rats of the Holtzman-Rolfsmeyer strain, 21 days of age, were castrated and maintained on Rockland Farm rat diet and tap water throughout the experiment. Each animal was given daily subcutaneous oil injections of the test substances for 7 days, beginning with the day of castration. On the 8th day, 22-26 hours after the last injection, the animals were sacrificed. Dissection of the levator ani muscle was effected after removing the skin in the scrotal area between the base of the penis and the anus. The posterior aspect of the perineal complex (Fig. 1) was cleared of fat and connective tissue with forceps, particular care being taken to disclose the constrictions at either end of the levator ani where it joins the bulbocavernosus muscle. The rectum was transected just caudad of the point where the levator ani loops around it dorsally (Fig. 2). The body of the levator ani was then freed of the rectum and removed by incisions at the points of attachment to the bulbocavernosus muscle. The levator ani was cleared of any connective tissue and weighed to the nearest 0.1 mg on a torsion balance. The ventral prostate and seminal vesicle weights (free of coagulating glands) were also recorded, for comparison of androgenic and myotrophic

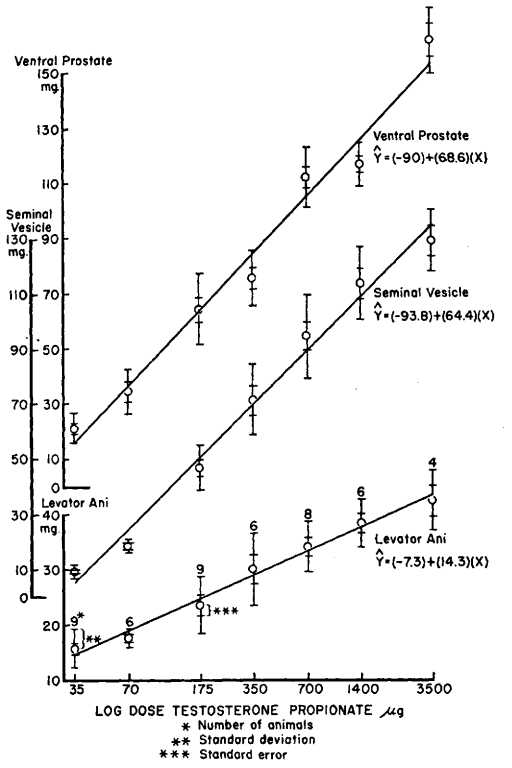

FIG. 3. Responses of the levator ani, ventral prostate, and seminal vesicles to increasing doses of testosterone propionate.

TABLE I. Myotrophic and Androgenic Activities of Standard Steroids and Some of Their Analogues.

Treatment		Total* dose, mg	Body wt, mg	Ventral prostate wt, mg	Seminal vesicle wt, mg	Levator ani wt, mg	Lev. ani /v. pros. ratio†
Castrated control	(25)‡	—	65	9.7± .4	7.0± .3§	12.2± .5	
Testosterone propionate	(9)	.035	68	21.5±1.9	9.8± .4	15.7±1.1	.30
	(6)	.070	67	34.8±3.5	18.7±1.0	17.6± .7	.22
	(9)	.175	68	64.7±4.3	47.0±2.8	23.7±1.7	.21
	(6)	.350	68	75.8±4.0	71.7±5.1	30.1±2.7	.27
	(8)	.700	67	112.1±3.9	94.5±5.4	34.2±1.6	.21
	(6)	1.400	69	114.7±3.1	113.7±5.3	38.4±1.7	.25
	(4)	3.500	71	161.7±6.0	128.8±5.6	42.3±2.7	.20
Testosterone	(6)	.350	62	35.3±1.8	14.7± .9	20.4±2.3	.32
	(8)	.700	68	46.5±4.6	17.8±1.3	21.9±2.0	.26
	(11)	1.400	66	48.4±2.3	27.4±2.3	23.7±1.0	.30
	(8)	2.100	70	63.5±4.5	38.2±5.5	28.8± .9	.31
	(9)	3.500	68	71.3±4.9	51.6±4.4	28.7±2.2	.27
[a]19-Nortestosterone	(5)	.700	66	19.7±2.5	12.1±1.7	21.6± .5	.94
	(11)	1.400	73	20.7±1.8	12.5± .6	24.5±2.0	1.10
	(5)	2.100	70	27.5±5.1	14.4±1.1	28.1±2.2	.89
	(6)	3.500	67	21.0±2.2	15.4±1.1	26.0± .6	1.20
[a]17-β Hydroxy-5(10) estren-3-one	(6)	2.800	66	12.7± .7	15.1±1.1	20.0±2.2	2.60
	(6)	3.500	63	14.1± .5	15.9±1.2	18.7±1.0	1.50
[a]19-Norandrostenedione	(6)	3.500	68	22.4±1.4	9.7± .7	18.3± .7	.48
Methylandrostenediol	(5)	.140	64	16.2±1.1	7.3± .3	11.4±1.7	
	(6)	.350	66	24.0±1.8	9.0±1.0	14.4±1.9	
	(11)	.700	67	29.5±1.7	11.6± .6	15.4± .9	.16
	(5)	1.400	69	37.1±2.3	16.5±1.3	17.0±1.0	.18
	(5)	1.750	65	55.3±2.9	33.6±2.7	22.9±1.1	.23
	(3)	3.500	65	66.3±5.3	58.5±6.2	25.8±1.1	.24
[b]Androsterone	(3)	.672	69	69.9±8.9	7.6± .5	15.5± .5	
	(6)	1.750	68	93.0±3.7	11.2± .5	16.6±1.3	.05
	(6)	3.500	64	109.4±4.1	13.8± .7	15.4±2.0	
Estrone	(4)	21.000	51	10.1±1.0	21.0± .8	8.8±1.0	
Estradiol dipropionate	(4)	7.000	54	9.3± .6	18.8± .5	10.0± .5	
[c]Cortisone acetate	(4)	21.000	55	9.4± .8	6.5± .7	6.6± .3	
Desoxycorticosterone-acetate	(6)	7.000	71	9.6± .8	7.7± .4	11.5± .7	
Progesterone	(6)	3.500	68	29.5±2.8	7.4± .8	13.4±1.2	
[d]19-Norprogesterone	(5)	3.500	75	14.1± .8	7.1± .9	14.7±1.2	
Pregnenolone acetate	(6)	3.500	66	17.3±3.0	7.1± .2	14.3± .9	

* Administered subcut. once/day for 7 days.

† Ratio of increase in levator ani to increase in ventral prostate. (Included only if levator ani response showed a highly significant increase, $P < .01$, over controls.)

‡ Figures in parentheses represent No. of animals used.

§ Stand. error.

Furnished by: [a] Drs. A. L. Wilds and N. A. Nelson, Univ. of Wis.; [b] Ciba Pharmaceutical Products; [c] Merck and Co.; [d] Chemical Specialties, Inc.

activity. Dry weights were taken on the levator ani muscles following the method of Astwood(11), except that the muscles were desiccated at 72°C.

Results and discussion. Testosterone propionate was selected for a dose-response curve (Fig. 3), since it has been shown to be highly active in producing the classical androgenic stimulation of the accessory organs of reproduction, in nitrogen retention(12), and, more recently, it has been shown to possess high myotrophic activity(9). In this study responses to testosterone propionate by the levator ani muscle and by the accessory glands of reproduction are termed the myotrophic and the androgenic activities, respectively.

When the weights obtained in these experiments for the levator ani, ventral prostate and seminal vesicles are plotted against the log of the dose of testosterone propionate, a straight line is obtained for each end organ. The slope of the levator ani dose-response curve does not parallel those for the accessory glands; the curves for ventral prostate and seminal vesi-

cles are approximately parallel (Fig. 3). The organ weight responses obtained in these experiments (Table I) are in agreement with previous observations of Eisenberg and Gordon(9) that testosterone propionate is more active, both androgenically and myotrophically, per unit weight than other commonly used androgens. However, in evaluating compounds for possible myotrophic use where androgenic activity is undesirable, the ratio of the response of the levator ani to the response of the ventral prostate may be an important consideration. In order to compare the myotrophic activity of test substances to their classical androgenic activity, the ratios of the levator ani response to the ventral prostate response (myotrophic/androgenic ratios) were devised. The levator ani/ventral prostate ratios (l.a./v.p. ratios) in Tables I and II were calculated as follows:

$$\frac{\text{l.a.}}{\text{v.p.}}\ \text{ratio} = \frac{\text{experimental levator ani wt} - \text{control l. ani wt}}{\text{experimental v. prostate wt} - \text{control v. prost. wt}} = \frac{\text{increase in levator ani wt}}{\text{increase in v. prostate wt}}$$

Using the responses to a dose of 35 μg testosterone propionate (Table I) as an example,

TABLE II. Steroids with Weak Myotrophic and Androgenic Activity.

		Total* dose, mg	Body wt, g	Ventral prostate wt, mg	Seminal vesicle wt, mg	Levator ani wt, mg	Lev. ani /v. pros. ratio†
Castrated control	(25)‡	—	65	9.7	7.0	12.2	
[a]Allopregnane-3(β),17(α)-diol-20-one	(3)	1.40	60	11.9	9.5	13.8	
[b]10-Methyl-6-(trans-4'-hydroxycyclohexyl)-$\Delta^{1:9}$-octalone-2	(6)	7.00	66	8.9	6.8	14.2	
[b]6-(4'-Ketocyclohexyl)-Δ^{1-9}-octalone-2	(3)	7.00	60	9.4	7.3	14.8	
[b]6-(Cis-4'-hydroxycyclohexyl)-Δ^{1-9}-octalone-2	(3)	7.00	74	14.6	7.7	15.9	
[a]$\Delta^{1,4}$-Androstadiene 3,17-dione	(5)	1.40	62	27.3	7.1	11.7	
[a]$\Delta^{4,6}$-Androstadiene-dione 3,17	(3)	.70	64	21.4	9.2	13.0	
[a]2-Acetoxy-testosterone-acetate	(3)	.17	62	39.4	9.4	14.1	
[c]1,8-Diketoperhydro-10a-methyl chrysene	(3)	2.55	71	10.7	7.9	15.0	
[a]$\Delta^{1,4,6}$-Androstatriene-17-ol-3-one-acetate	(2)	1.05	68	16.5	10.5	15.5	
[d]17-Vinyl-Δ^{4}-androsten-3-one-17(β)ol	(3)	1.40	64	42.6	22.7	18.5	.19
[a]16-Methyl-17-iso-Δ^{4}-pregnene-3,20-dione	(3)	3.50	66	15.1	6.5	12.8	
[d]Dehydroisoandrosterone	(4)	4.20	74	35.5	8.4	13.6	
[a]Δ^{1}-Androstenedione-3,17	(3)	3.50	56	12.9	8.2	15.2	
[a]Androstane-3,17-dione	(3)	3.50	69	122.3	12.3	18.0	.05
[a]Δ^{4}-Androstene-17(α)-ol-3-one-acetate	(3)	3.50	63	33.2	11.4	15.0	
[a]$\Delta^{3,5}$-Androstadiene-3,17-dione-3-enol ethyl ether	(3)	5.25	68	56.1	10.3	15.1	
[a]$\Delta^{5,16}$-Pregnadiene-3(β)-ol-20-one acetate	(3)	3.50	69	10.7	7.5	12.0	
[a]Allopregnane 17(α)-ol-3,20-dione	(3)	3.50	72	43.2	8.3	14.7	
[a]Allopregnane-3(β),17(α),21-triol-20-one-3,21-diacetate	(3)	3.50	68	11.0	8.6	14.8	
[d]17-Iso-5-pregnene-3 β, 17 β-diol	(3)	1.75	64	15.4	9.4	15.2	

* Administered subcut. once/day for 7 days.

† Ratio of increase in levator ani to increase in ventral prostate. (Included only if levator ani response showed a highly significant increase, $P < .01$, over controls.)

‡ Figures in parentheses represent No. of animals used.

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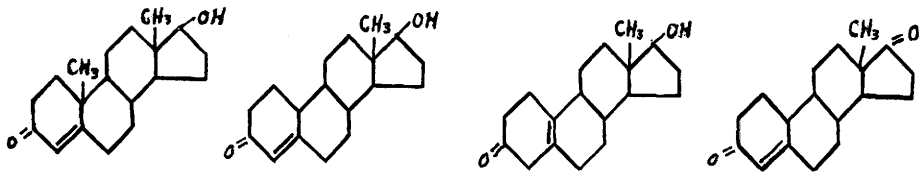

Testosterone 17 β-Hydroxy-5(10)-estren-3-one

19-Nortestosterone 19-Norandrostenedione

the experimental levator ani weight of 15.7 mg minus the castrated control weight of 12.2 mg gave an *increase* in muscle weight of 3.5 mg. Likewise, the experimental ventral prostate response of 21.5 mg minus the control weight of 9.7 mg gave an *increase* of 11.8 mg for the prostate. The ratio of these two increases, 3.5/11.8 = 0.30, is the l.a./v.p. ratio. The l.a./v.p. ratios for testosterone propionate range between 0.20 and 0.30 at all doses studied. Using this method of comparison any relative increase in levator ani response would be reflected in an increase in the l.a./v.p. ratio.

Since these assays were primarily intended for assessing myotrophic activity, the myotrophic/androgenic ratios in Tables I and II were calculated for only those dose-levels which produced a highly significant ($P < 0.01$) increase in levator ani weight. Levator ani/seminal vesicle ratios were not calculated because the seminal vesicles are less sensitive than the ventral prostate to small quantities of most androgens and to all dose levels of some well known androgens, *e.g.* androsterone(13). Furthermore, the seminal vesicle weights are believed to be affected more by non-androgenic steroids(14). Although steroids are often known to produce hydration of the tissues, there was no evidence of hydration of the levator ani muscles in this experiment as shown by the dry weight determinations. Therefore all myotrophic/androgenic ratios are taken directly from the wet weights of the organs.

Using this method, l.a./v.p. ratios were calculated for testosterone, testosterone propionate, and methylandrostenediol, which have been recognized as myotrophic agents. The l.a./v.p. ratios found for methylandrostenediol were of the same order of magnitude as those found for testosterone propionate. This does not support the reports that methylandrostenediol is more valuable than testosterone propionate as a protein-anabolic compound (10,15).

A group of compounds lacking the C-19 methyl group was of special interest to us (16). The group included 19-nortestosterone, 17 β-hydroxy-5(10)-estren-3-one, and 19-norandrostenedione, all of which are analogues of familiar androgens. It can be seen from Table I that 19-nortestosterone, 17 β-hydroxy-5(10)-estren-3-one, and 19-norandrostenedione were compounds which showed l.a./v.p. ratios greater than those for testosterone propionate and for unesterified testosterone. The l.a./v.p. ratios for testosterone propionate and free testosterone were very similar, while the ratios for 19-nortestosterone were much higher. 17 β-hydroxy-5(10)-estren-3-one (a double-bond isomer of 19-nortestosterone) showed even higher l.a./v.p. ratios, while 19-norandrostenedione, at the single dose level given, showed a ratio which was not as striking as for the other 19-nor compounds but was greater than for the other standard androgens tested. It is evident that a minimum of androgenic activity is coupled with definite myotrophic activity in the 19-nor compounds.

Since the preparations of 19-nortestosterone and its isomers were not esterified the responses to these compounds should be compared to those for free testosterone rather than testosterone propionate. Equal doses by weight of free testosterone and 19-nortestosterone produced approximately equal myotrophic responses. An examination of the androgenic activity in response to equal doses of the 2 compounds reveals, however, that the increases in ventral prostate weights for testo-

sterone treated groups are much greater than the increases following 19-nortestosterone administration. Thus, while the *myotrophic* activities of the 2 compounds are approximately equal, the *androgenic response* to 19-nortestosterone is markedly lower than the androgenic response to testosterone. Chick comb assays obtained in this laboratory (unpublished) indicate that testosterone is approximately 4 times as active androgenically as 19-nortestosterone, while the ventral prostate responses in the rat show testosterone to be about 10 times as active androgenically in this species.

The myotrophic activity of these preparations suggests that the muscle response is limited to androgens and to analogs of androgenically active compounds, which may possess a minimum of androgenic activity *per se*. A number of hormonally active non-androgenic compounds which do not elicit the myotrophic response have been included in Table I for comparison. Progesterone, 19-norprogesterone, pregnenolone acetate, estrone, estradiol dipropionate, cortisone acetate, and desoxycorticosterone acetate were shown to have no significant stimulatory effect on the levator ani muscle when the total doses indicated in Table I were administered. Additional evidence for the specificity of the myotrophic response to androgens and their isomers is found in Table II where the results of tests on a number of other compounds are tabulated. Only 2 compounds in Table II (17-vinyl Δ^4-androsten-3-one-17 β-ol and androstane-3,17-dione) showed myotrophic activity ($P < 0.01$) and in each case androgenic activity was sufficient to produce a l.a./v.p. ratio similar to the testosterone ratios.

Although to date steroidal myotrophic activity has been found associated with androgens or analogs of androgens, there is a distinct lack of parallelism between myotrophic and androgenic activities. This finding is in agreement with the data of Eisenberg and Gordon(9). The 19-nor analogs which have been tested (Table I) show high myotrophic/androgenic ratios while, on the other extreme, androsterone (Table I) and androstane-3,17-dione (Table II) exhibit very low myotrophic/androgenic ratios. The favorable myotrophic/androgenic ratios shown for the 19-nor compounds tested would suggest that the 19-nor series of steroids may be valuable for their protein-anabolic activity.

Summary. An 8-day rat levator ani muscle assay for myotrophic activity is presented. Preliminary steroid screening results indicate that 19-nortestosterone and other 19-nor analogs of androgens promise to be effective protein-anabolic, and relatively weak androgenic agents. Using the same dose levels of 19-nortestosterone and testosterone, equal myotrophic responses were produced, yet 19-nortestosterone showed only weak androgenic activity and testosterone exhibited strong androgenic activity. Androsterone possesses relatively strong androgenic and only weak myotrophic activity.

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