Comparison of Myotrophic and Androgenic Activities of 19-Nortestosterone Cyclopentylpropionate, Androstanolone, and Methylandrostenediol. (21276)

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A recent publication from these laboratories (1) described the effect of 19-nortestosterone and some of its esters on causing increase in size of the rat levator ani muscle with relatively little stimulation of the seminal vesicles. Of the esters, 19-nortestosterone cyclopentylpropionate was considered most worthy of further study on the basis of potency and preferential myotrophic activity. The present paper describes the comparison of 19-nortestosterone cyclopentylpropionate (NTCP)* with 2 other steroids reported to have preferential anabolic activity, methylandrostenediol (MAD)† and androstanolone‡ (2,3), with regard to their abilities to stimulate seminal vesicles and levator ani in the castrate male rat.

Method. The test method was described in detail previously (1). Weanling male rats are castrated and the test compounds are injected subcutaneously in oil for 21 days. Seminal vesicle and levator ani muscle weights (4) are used as indices of androgenic and myotrophic activity. The dose-response data are plotted on semilogarithmic graph paper and potency ratios calculated by the method of Irwin (5). In calculating the data we choose straight-line portions of the dose-response curve of the unknown steroid which are most nearly parallel to the dose-response curve of the standard, testosterone propionate (TP).

Results. Methylandrostenediol. MAD was tested at a dosage range of from 50 to 1500

TABLE I. Comparison of Activities of Testosterone Propionate, 19-Nortestosterone Cyclopentylpropionate, Methylandrostenediol, and Androstanolone on the Seminal Vesicles and Levator Ani Muscles of Castrate, Weanling Male Rats.

Treatment	Daily dose (μg)	No. animals		Body wt		Organ wt (mg/100 g body wt) Sem. ves.		Lev. ani	
		Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Oil	—	14	15	142	153	5	6	23	24
Testosterone propionate	25	5	5	163	157	46	39	39	36
	50	10	10	160	166	94	85	57	55
	100	8	10	168	172	134	137	77	73
	250	10	10	171	169	193	179	80	81
	500	5	5	168	166	196	204	98	92
19-Nortestosterone cyclopentylpropionate	12.5	5	5	154	157	6	6	36	34
	25	5	5	156	158	5	6	52	47
	50	10	10	166	167	10	9	69	61
	100	5	5	163	171	25	36	90	88
	125	5	5	167	163	22	22	85	73
	250	5	5	171	174	67	66	86	89
	500	5	5	167	166	125	137	93	104
Methylandrostenediol	50	1		158		4		26	
	100	2		154		5		23	
	250	10		154		12		28	
	500	10		157		39		35	
	1000	5		201		78		47	
	1500	5		160		106		69	
Androstanolone	50		10		154		13		34
	100		10		155		33		39
	250		10		158		55		53
	500		10		158		106		78

* Paper describing preparation and physical and chemical properties of this compound is in press (*J. Am. Chem. Soc.*): Babcock, J. C., R. A. Donia, B. A. Johnson, and A. C. Ott.

† 17α-methyl androst-5-ene-3β,17β-diol.

‡ 17β-hydroxy androstan-3-one.

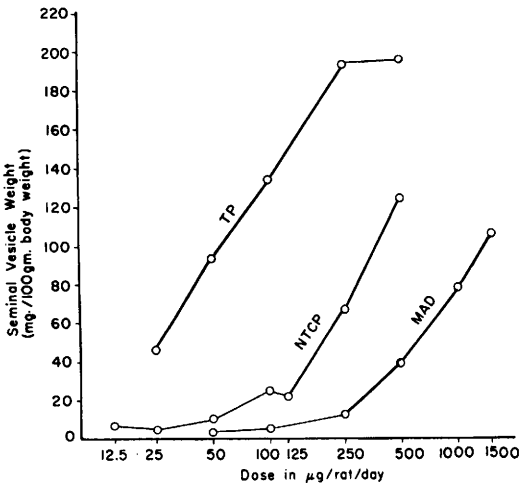

FIG. 1. Seminal vesicle weights of rats treated with testosterone propionate (TP), methylandrostenediol (MAD), and 19-nortestosterone 17β-cyclopentylpropionate (NTCP).
Portions of data used in statistical analysis presented in bold lines in all 4 figures.

μg/day. At doses less than 250 μg there was essentially no androgenic or myotrophic response (Table I, Fig. 1 and 2). At doses above 500 μg (5 mg/cc) the steroid crystallized from the oil and it was necessary to give it as a suspension. Statistical analysis of the results of these tests showed MAD to have a myotrophic potency§ of 2.2% and an androgenic potency of 2.1% giving a myotrophic/androgenic ratio of 1.0. NTCP studied simultaneously had potencies of 160% and 15% giving a ratio of 10.7 (Table II). These data indicate that MAD has no preferential myotrophic activity, since both myotrophic and androgenic activities are of the same low order.

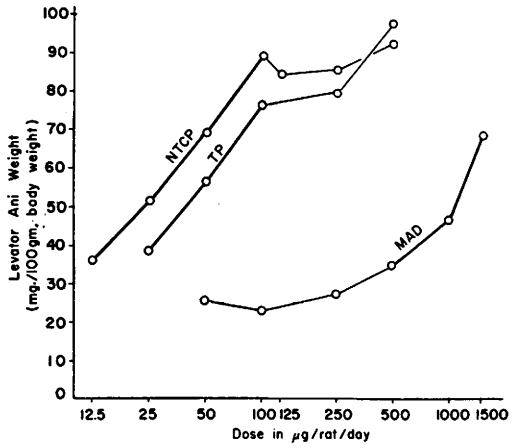

FIG. 2. Levator ani muscle weights of rats treated with testosterone propionate (TP), methylandrostenediol (MAD), and 19-nortestosterone 17β-cyclopentylpropionate (NTCP).

Androstanolone. This compound showed activity at a dosage range of from 50 to 500 μg/day (Table I, Fig. 3 and 4). Statistical analysis of the data from these tests showed androstanolone to have a myotrophic potency of 21.8% and an androgenic potency of 13.2% with a resultant myotrophic/androgenic ratio of 1.7. NTCP on parallel tests had a ratio of 8.5‖. Thus of the two steroids currently available for therapy where anabolic effect is desirable and "virilizing" effects are undesirable, androstanolone had higher potency than MAD, as well as a more favorable myotrophic/androgenic ratio. NTCP, on the other hand, while causing only slightly more seminal vesicle stimulation than androstanolone, produced about 6 times greater myotrophic response and thereby a much higher ratio of activities. NTCP had a mean ratio of 9.5 for the two comparison assays.

The use of the levator ani muscle as an indicator of general anabolic activity has been open to some question because of the slight possibility of its being stimulated only as a "secondary sex organ." That it does reflect the state of the whole organism with respect to protein build-up or break-down is strongly indicated by the recent work of Eisenberg and Gordan(6) who showed levator ani atrophy in states associated with protein catabolism. Stafford, Bowman, and Olson(7) showed that the nitrogen retention activity of the steroids tested in this study is in the same order as the levator ani-stimulating activity reported here. It is believed that these circumstances materially strengthen the case for the levator ani as an index of protein metabolism.

Although the nitrogen retention experiments (7) are not subject to the same quantitative analysis as the levator ani data, it is interest-

§ Results expressed as % of similar activity of standard (TP).

‖ Five multidose assays comparing NTCP and TP have been performed. Anabolic/androgenic ratios ranged from 7.7 to 10.8 with a mean ratio of 9.3.

TABLE II. Myotrophic and Androgenic Potencies and Myotrophic/Androgenic Ratios of 19-Nortestosterone Cyclopentylpropionate, Methylandrostenediol, and Androstanolone.

Test substance	Androgenic* potency	Myotrophic* potency	Myotrophic/androgenic ratio
Testosterone propionate	100	100	1.0
Methylandrostenediol	2.1	2.2	1.0
19-Nortestosterone cyclopentylpropionate	15	160	10.7
Androstanolone	13.2	21.8	1.7
19-Nortestosterone cyclopentylpropionate	17.6	150	8.5

* Myotrophic and androgenic potencies expressed as percentage of activity of testosterone propionate.

ing to consider their results in light of the androgenic activity reported in this study. The doses used in the nitrogen retention series were NTCP .25 mg, androstanolone 2.0 mg, methylandrostenediol 2.0 mg. Considering the seminal vesicle stimulation reported here, the relative androgenicity of these doses is: NTCP .03, androstanolone .32, methylandrostenediol .04. Since NTCP caused much greater retention of nitrogen than either of the other compounds, while having the lowest androgenic potency, it is obvious that by this criterion, as well as by the levator ani end-point, NTCP is a more effective anabolic compound with low androgenic activity than either MAD or androstanolone.

Summary. 1. Three steroids with protein anabolic activity were tested in parallel with testosterone propionate to compare their relative properties of stimulating the levator ani muscles and seminal vesicles of weanling castrate male rats when injected subcutaneously in oil solution or suspension. The androgenic and myotrophic potencies of the test compounds, as well as their myotrophic/androgenic ratios, were calculated by analysis of significant portions of the data. 2. The androgenic potencies of these steroids, expressed as percentages of the potency of testosterone propionate, were: methylandrostenediol 2.1, androstanolone 13.2, 19-nortestosterone cyclopentylpropionate 16.2. 3. The myotrophic potencies, expressed as percentages of the potency of testosterone propionate, were: methylandrostenediol 2.2, androstanolone 21.8, 19-nortestosterone cyclopentylpropionate 155. 4. The myotrophic/androgenic ratios of these compounds are, therefore: methylandrostene-

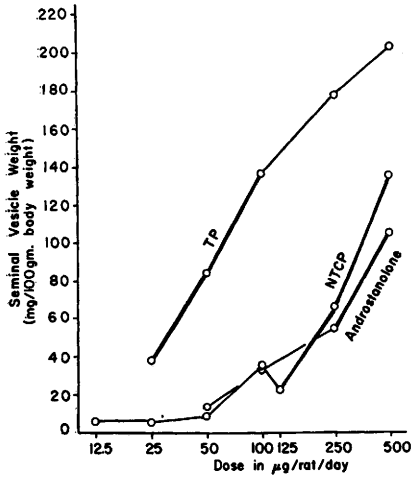

FIG. 3. Seminal vesicle weights of rats treated with testosterone propionate (TP), androstanolone, and 19-nortestosterone 17β-cyclopentylpropionate (NTCP).

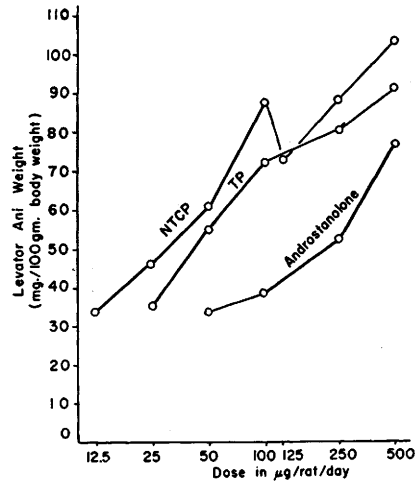

FIG. 4. Levator ani muscle weights of rats treated with testosterone propionate (TP), androstanolone, and 19-nortestosterone 17β-cyclopentylpropionate (NTCP).

diol 1.0, androstanolone 1.7, 19-nortestosterone cyclopentylpropionate 9.5.

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Vitamin B_{12} Serum Levels and Diabetic Retinopathy.* (21277)

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Methods for the determination of vit. B_{12} activity in serum have been reported(1,2,3), although the measured activity may not be due to this vitamin *per se*. Recently, a modified procedure was described(4) which permits the estimation of as little as 10 micromicrograms of activity of this crystalline vitamin. The availability of this method prompted us to compare the serum vit. B_{12} levels of diabetics with or without retinal lesions. These findings which will be reported in this communication, should be of particular interest since we have shown earlier(5) that following an intramuscular test dose of vit. B_{12}, diabetic subjects with retinopathy excrete significantly more of the vitamin than non-diabetics; while diabetics without retinopathy excrete considerably less than non-diabetics.

Procedure. All patients used in this investigation suffered from diabetes mellitus of different degrees of severity. They were drawn from the Diabetic Clinic of The Wilmer Institute of Johns Hopkins Hospital. When the diagnosis of diabetes had been established and the absence or presence of retinopathy† recorded, venous blood specimens were drawn from all subjects and allowed to clot. Sera were separated by centrifugation and were kept frozen in a deep-freeze until assay. The procedure of assay will be published in detail. Samples were identified by code numbers at Johns Hopkins Hospital and analyzed. Only after completion of the assay was the ophthalmoscopic diagnosis of the patients compared with the analytical results.

Results. Over a period of 6 months involving at least 10 different assays, blood specimens from 16 patients with retinopathy (5 males and 11 females) and from 18 patients (7 males and 11 females) were analyzed. The vit. B_{12} activity in sera expressed as micromicrograms per ml was plotted against age of the patients. It can be seen from the scatter-diagram (Fig. 1) that the B_{12} levels of the 2 groups of diabetics are distinctly different. If a line were drawn parallel to the horizontal axis at the level of 200 mg/ml, it would divide the observations clearly into 2 groups, with only one exception in the non-retinopathic group and none in the retinopathic group. The mean values of serum B_{12} levels with the standard errors of the mean are 162 ± 18 and 292 ± 24 $\mu\mu$g/ml for the non-retinopathic and retinopathic groups, respectively. The difference in the serum levels is statistically significant with $P < 0.001$.

Although the role of vit. B_{12} in metabolism

* The authors wish to acknowledge with thanks the U. S. Public Health Service Grant No. A435.

† All diabetic patients were examined with a giant ophthalmoscope following mydriasis. Those cases were included in which the fundi could be well visualized and in which no ocular disease was present. Only the eyes with no evidence whatsoever of capillary aneurysms, exudates, or hemorrhages were classified as nonretinopathic.