Myotrophic (Anabolic) Activity of 4-Substituted Testosterone Analogs. (23105)

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The screening in castrated rats of a group of new steroids recently synthesized in this laboratory(1) showed that some of the substituted testosterone analogs increased the weights of levator ani muscle without modifying to any great extent that of the ventral prostate. This suggested that an anabolic effect was occurring and induced us to investigate the biologic activity of these steroids. Thus, in the absence of potent androgenic activity they may have therapeutic value as anabolic agents.

Method. Myotrophic and androgenic activity were determined by the method of Herschberger, Shipley and Meyer(2) using groups of 7 or more previously castrated albino rats weighing 30-40 g. The steroids were either prepared in oil or as aqueous suspensions with 0.5% carboxymethylcellulose, 0.4% Tween 80, 0.9% benzyl alcohol and physiological salt solution and injected subcutaneously daily for 7 days. Twenty-four hours after the last injection, the animals were autopsied, the levator ani muscle, ventral prostate and seminal vesicles were removed and their wet weights recorded. In preliminary studies, the dry weights were also recorded and since there was no evidence of hydration, the wet weights were used throughout the experiment. For comparing the anabolic and androgenic activity, the following ratio was established:

$$\frac{\text{Exp. levator ani wt} - \text{Control levator ani wt}}{\text{Exp. prostate wt} - \text{Control prostate wt}}.$$

Other hormonal activities were evaluated as follows: life maintaining(3), glycogen deposition and Na retention(4), anti-inflammatory and water diuresis(5), proinfectivity(6), progestational(7) and estrogenic(8).

Results. I. *Myotrophic and androgenic activity of the new steroids.* The effects produced by various doses of the testosterone analogs are shown in Table I and are compared with those due to testosterone propionate, methylandrostenediol and 19-nor-17-α-ethyltestosterone. All weights differ significantly from those of the controls ($P<.01$). The data show that 4-chloro analogs (4-chlorotestosterone acetate, 4-chloro-19-nortestosterone acetate or cyclopentyl propionate, and 4-chloro-17-α-methyl-19-nortestosterone) and 4-hydroxy-19-nortestosterone acetate produce myotrophic effects similar to that of testosterone propionate. The average weights of the levator ani muscle following these agents are comparable. Investigation of the androgenic activity shows that the 4-chloro analogs have weak stimulating action on prostate and seminal vesicles whereas testosterone propionate and methylandrostenediol increase weights of the accessory sex organs of male rats. The weight of the prostate following 4-chlorotestosterone acetate or 4-chloro-19-nortestosterone acetate is significantly less ($P<.01$) than that due to testosterone or methylandrostenediol. These analogs, therefore, have therapeutic indexes (levator ani wt: ventral prostate wt) which exceed those of testosterone or methylandrostenediol. Since similar results were obtained with 19-nortestosterone cyclopentyl propionate, it appears that the presence of the chlorine atom at C-4 or the absence of the 19-methyl group decreases androgenic potential of testosterone without reducing the myotrophic action. Thus, the 4-chloro-19-nortestosterone acetate or the cyclopentyl propionate which show both changes in the same molecule have the highest indexes.

As to the mechanism of 4-substitution, it is noteworthy that 4-hydroxytestosterone is a myotrophic agent but has less androgenic potency than testosterone. This results in an elevated index. On the other hand, 4-substitution with Br or F results in less active analogs.

II. *Other hormonal effects of 4-chloro analogs.* Neither 4-chlorotestosterone nor 4-

TABLE I. Myotrophic (Anabolic) and Androgenic Activity of New Testosterone Analogs.

Treatment	No. rats	Dosage (μg/day)	Mean wt in mg ± S.E.* Levator ani	Ventral prostate	Seminal vesicles	Therapeutic index (lev. ani/ prostate)
Castrated controls	174		8.6 ± .33	9.3 ± .41	5.4 ± .48	—
Testosterone propionate	52	100	22.2 ± 1.05	62.5 ± .81	50.6 ± 1.78	.26
	19	250	27.9 ± 2.22	87.1 ± 6.72	73.4 ± 3.27	.25
	25	500	31.3 ± 1.73	91.9 ± 4.17	87.9 ± 4.20	.27
Methylandrostenediol	7	"	23.5 ± 1.51	63.7 ± 3.70	53.1 ± 4.66	.28
19-nortestosterone cyclopentyl propionate	19	"	40.8 ± .57	59.1 ± 2.71	59.3 ± 2.59	.65
19-nor-17α-ethyltestosterone	7	"	26.2 ± 3.39	41.0 ± 5.66	37.1 ± 3.99	.55
4-fluorotestosterone acetate	7	"	14.0 ± 1.50	20.2 ± 1.46	8.3 ± .81	.50
4-chlorotestosterone acetate†	37	100	11.5 ± .84	20.6 ± 1.47	9.4 ± .53	.26
	27	250	26.7 ± 1.69	35.7 ± 1.50	20.4 ± 1.01	.68
	56	500	37.2 ± 1.31	55.7 ± 1.43	44.3 ± 1.61	.62
4-chlorotestosterone propionate	7	"	27.4 ± 2.52	43.1 ± 3.19	40.1 ± 7.30	.56
4-bromotestosterone	12	"	14.4 ± 1.20	25.9 ± 1.45	16.7 ± 1.40	.34
4-chloro-11β-hydroxytestosterone acetate	7	"	21.1 ± .64	41.4 ± 1.79	31.7 ± 1.30	.39
4-hydroxytestosterone acetate	7	"	20.5 ± 4.98	32.0 ± 4.68	25.2 ± 3.12	.52
4-chloro-19-nortestosterone acetate	23	100	21.2 ± 1.25	20.4 ± 1.20	12.8 ± 1.26	1.13
	10	300	27.1 ± 2.25	26.7 ± 1.26	30.4 ± .97	1.06
	8	500	34.1 ± .96	31.1 ± 1.96	21.9 ± 1.64	1.16
4-chloro-19-nortestosterone cyclopentyl propionate	15	100	11.2 ± 1.35	12.5 ± 1.34	7.9 ± .60	.81
	18	250	27.1 ± 2.12	18.4 ± 1.67	14.8 ± 1.69	2.03
	8	500	27.3 ± 3.31	18.2 ± 1.53	19.6 ± 1.28	2.10
4-hydroxy-19-nortestosterone acetate	7	"	29.6 ± 1.13	47.7 ± 3.80	54.0 ± 4.65	.55
4-chloro-17α-methyl-19-nortestosterone	7	"	24.7 ± 2.03	31.2 ± 1.15	34.6 ± 3.12	.73

* Stand. error $= \sqrt{\frac{\Sigma d^2}{n(n-1)}}$. † Aqueous suspension.

chloro-19-nortestosterone has estrogenic or corticoid activity. Neither increases glycogen content of the liver nor affects the survival rate of adrenalectomized rats. Moreover, they do not cause Na retention, K loss, increase output of urine or aggravate experimental infection. Although the former has no progestational action, the latter is about ½ to ¼ as active as progesterone.

Summary. 1) C-4 substituted testosterone analogs possess potent myotrophic and weak androgenic activity as measured by increase in weight of levator ani muscle and ventral prostate gland, respectively. 2) Daily subcutaneous injections of 0.5 mg 4-chlorotestosterone acetate induce greater anabolic and lesser androgenic effects than similarly administered testosterone propionate; the anabolic/androgenic ratios are 0.62 for the former and 0.27 for the latter. 3) The anabolic activity of the 4-chloro-19-nortestosterone acetate and 4-chloro-19-nortestosterone cyclopentyl propionate is equivalent to that of testosterone propionate, but their effect on prostate growth is less. The anabolic/androgenic ratios are 1.16 and 2.10 respectively. 4) Neither 4-chlorotestosterone actetate nor 4-chloro-19-nortestosterone acetate has estrogenic or corticoid activity. Only the latter, however, has progestational activity.

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A Modified Seroflocculation Reaction in Relationship to Invasive Neoplasms.* (23106)

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In a previous communication(1) a seroflocculation reaction was described as a laboratory aid in diagnosis of invasive cancer in humans. This reaction consisted of mixing serum with ethyl choladienate under controlled conditions and analyzing the resulting flocculation reaction. While a preponderance of positive reactions was obtained with sera from histologically proven malignancies, a considerable number of positive reactions was given by sera from normal individuals and from some patients with non-malignant pathological conditions. This is to report on a modification in the procedure whereby the incidence of "non-specific" positives can be significantly reduced. As a result of an investigation by Kidd(2), who noted that non-specific reactions to the Brown-Pearce tumor antigen by normal rabbit sera could be eliminated through heating the sera at 65°C for 30 minutes, the following study was made to determine whether a similar treatment of human sera would reduce the incidence of non-specific positives in the flocculation test for malignancies. Preliminary studies, in which sera were heated at various temperatures ranging from 56°C to 65°C, showed that 64°C was optimum. Higher temperatures tended to coagulate certain sera. Sera from 2,747 individuals were tested (Table I); 220 of these individuals had biopsy proven cancer (Table II), 1,822 were normal blood donors, and 705 had non-malignant diseases (Table III). The results clearly indicate that this modified procedure reduces the number of false positives.

Materials and methods. Blood was obtained by venipuncture under aseptic precautions and stored overnight at temperatures between 4°-6°C. Serum was separated from the clot by decantation and centrifugation. Each serum was then divided into approximately 2 equal volumes. One was heated at 56°C and the other at 64°C for 30 minutes simultaneously. *For testing, all sera and solutions were maintained at 15°C (iced water bath).* The following solutions were required: (A) Buffered saline solution(3) consisting of 6.5 ml of 0.1M citric acid, 43.5 ml of 0.2M anhydrous disodium phosphate, 42.5 ml of 5% sodium chloride and then diluted to 250 ml with water; (B) 0.1 mg percent of bovine albumin solution (Fraction V Armour Laboratories) in buffered solutions; (C) 1% cholesterol in absolute ethanol; (D) Ethyl choladienate 15 mg per ml in 95% ethanol. The *antigen suspension* was prepared by depositing at the bottom of a tube 0.013 ml of the cholesterol solution (C), plus 0.1 ml of the antigen solution (ethyl choladienate solution D) and mixed by rapid rotation of the tube. To the alcoholic solutions C and D was added 0.4 ml of the citrate phosphate buffered nor-

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