Andro-stanazole, A New Orally Active Anabolic Steroid. (25184)

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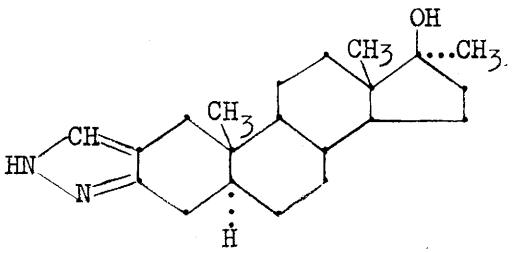
Testosterone and its esters have been used clinically as anabolic agents(1,2) but usage has been severely limited by early appearance of undesirable masculinization. Numerous steroids have been synthesized in search of an anabolic agent, preferably active by oral administration, which would have a more favorable ratio of anabolic to androgenic activity. Estimation of anabolic activity in rats has usually been based upon quantitative increase in weight of the levator ani muscle(3,4) or a decrease in urinary nitrogen(5). We have utilized the sensitivity of castrated male rats to anabolic agents for quantitative evaluation of nitrogen retaining properties(6) as well as for measurement of androgenic activity. It has been found that 17β-hydroxy-17α-methyl-androstano-[3,2-c] pyrazole (I, hereafter referred to as andro-stanazole), described by Clinton *et al.*(7), has pronounced anabolic properties(8) with a relative reduction of androgenic activity when administered orally or parenterally. In the following paragraphs, the anabolic and androgenic activities of andro-stanazole are compared with established reference androgens.

Procedure. (1) As suggested by Kochakian (6), 200 g castrated male rats were brought essentially to weight and nitrogen equilibrium. The initial feed allotment of 14 to 15 g per day was decreased gradually until body weights of the rats reached a plateau. The rats were used after 3 to 4 months at which time the feed allotments were 10 to 11 g per day. In general, the prepared rats were used only once in 6 weeks, and under these conditions they have been utilized up to 2 years. The diet used was composed of: cerelose 33.67, dextrin 33.67, yeast (Fleischmann's 2019) 9.2, hydrogenated vegetable oil (Primex) 7.4, lactalbumin 6.0, methyl cellulose 5.0, salt mixture(9) 3.7, choline dihydrogen citrate 0.9, cod liver oil (2000 A, 250 D) 0.25, wheat germ oil 0.16, and liver extract (Wilson's fraction 0) 0.05. Bulk was supplied by adding 5 parts alphacel per 100 parts diet. This diet supplied 1.4% nitrogen, equivalent to 9.35% protein. At feed intake of 10 to 11 g per day the rats received 150 to 175 mg nitrogen per rat. Urinary nitrogens of the experimental animals generally averaged 100 mg per rat per day with individual values varying 10-15 mg of nitrogen per rat per day on either side of the group average. Fecal nitrogens were not determined because they are constant under conditions of daily weighed feed allotments(1,2). The rats were housed in metabolism racks, and control urinary nitrogen values were determined over a 3-day collection period (Sunday to Wednesday). The urine and daily cage washings were filtered through glass wool into collecting bottles, and samples were diluted to 500 ml preparatory to macro-Kjeldahl nitrogen determinations(10) using 25 ml aliquots. Premedication urinary nitrogen excretion values and body weights provided the basis for distribution of rats with urinary nitrogen excretion of 85 mg of nitrogen or more into uniform groups. The test compounds were administered over a 5-day period (Sunday to Thursday) following the control period. Urine collections were made over the last 3 days of the medication period (Tuesday to Friday) to permit 2 days for response. (2) Androgenicity of the test agent was evaluated by its effect on weight of the ventral prostate gland of 22 day-old castrated rats(11). Starting 7 days after castration, the test materials were administered subcutaneously or



(I)

TABLE I. Relative Effectiveness of Andro-stanazole, Testosterone Propionate and Methyltestosterone on Nitrogen Retention by Castrated Male Rats.

Compound	Dose, mg/rat/day	No. of animals	Body wt, g		Urinary N, mg/rat/day			Relative activity
			Initial	Final	Pre-test	Test	Retained	
Methyltestosterone (oral)	6.0	4	274	278	109.6	103.9	5.7	
	24.0	7	282	285	112.0	97.8	14.2	1
Andro-stanazole (oral)	.1	4	275	277	106.9	104.0	2.9	
	.4	6	285	289	110.7	99.9	10.8	
	1.6	7	277	282	112.0	91.2	20.8	
	6.4	6	269	273	113.6	94.1	19.5	30
Andro-stanazole (s.c.)	.1	4	254	257	111.6	108.4	3.2	
	.4	7	275	279	111.8	97.9	13.9	
	1.6	5	266	271	113.2	95.8	17.4	
	6.4	7	281	290	114.6	89.3	25.3	30
Methyltestosterone (oral)	2.0	8	323	326	96.2	84.5	11.7	
	6.0	7	316	321	98.9	84.4	14.5	
	18.0	6	301	303	99.5	80.4	19.1	1
Andro-stanazole (oral)	.2	6	326	328	100.2	84.7	15.5	30
Testosterone propionate (s.c.)	.025	6	292	298	96.4	81.6	14.8	
	.079	6	298	302	103.1	83.2	19.9	
	.25	6	292	298	101.8	73.2	28.6	1
Andro-stanazole (s.c.)	.45	7	294	295	100.0	86.7	13.3	
	1.42	7	291	293	100.3	79.8	20.5	.05
Testosterone propionate (s.c.)	.02	6	305	309	98.4	93.6	4.8	
	.08	4	314	320	97.4	78.3	19.1	
	.32	4	296	302	104.9	75.3	29.6	1
Andro-stanazole (s.c.)	1.0	5	302	306	98.7	83.3	15.4	.06
Testosterone propionate (s.c.)	.02	5	290	291	98.8	92.0	6.8	
	.08	6	294	296	98.2	82.3	15.9	
	.32	7	299	302	99.4	68.1	31.3	1
Andro-stanazole (s.c.)	1.0	7	299	299	99.1	87.9	11.2	
	4.0	6	300	302	101.2	80.8	20.4	.05

orally for 5 days. The vehicle was cottonseed oil, containing ethanol, 10% W/V. The daily dose was contained in 0.2 ml of vehicle when injected subcutaneously or in 0.4 ml when given by stomach tube. The rats were sacrificed on the sixth day, 24 hours after the last medication. The ventral prostates were excised, blotted and weighed on a micro-torsion balance, and compared with the organ weight of appropriate control animals.

Results. The nitrogen-retaining activity of andro-stanazole was compared with that of methyltestosterone by oral administration and testosterone propionate by subcutaneous administration (Table I). On the basis of the log dose : response formula(12), andro-stanazole orally was 30 times more active than methyl-testosterone in effecting nitrogen retention; subcutaneously, andro-stanazole was one-twentieth as active as testosterone propionate. In general, andro-stanazole was about as effective orally as parenterally. This suggests that andro-stanazole becomes available to the organism as rapidly following oral administration as following parenteral administration.

As indicated by Kochakian(6) castrated male rats are quite sensitive to the effects of parenterally administered testosterone propionate. They respond poorly to orally administered methyltestosterone, which cannot be evaluated at higher levels in this test procedure because the rats refuse to eat when the dose is increased above 20 to 25 mg per day. The adverse effect of methyltestosterone at elevated dose levels has been noted by others (13).

The results of comparative tests of the androgenicity of andro-stanazole, methyltestosterone and testosterone propionate are sum-

TABLE II. Relative Androgenic Activities of Andro-stanazole, Methyltestosterone and Testosterone Propionate. 6 rats/group.

Compound	Dose, mg/kg/day	Body wt, g Initial	Body wt, g Final	V. prost. wt, mg	Relative activity
None		77	106	7.3	
Andro-stanazole (oral)	42.0	76	97	20.2	
	84.0	77	97	31.3	
	168.0	"	89	40.5	.25
Methyltestosterone (oral)	10.5	78	107	17.4	
	21.0	77	104	31.4	
	42.0	"	102	42.7	1
None		77	106	5.7	
Andro-stanazole (s.c.)	7.0	76	107	13.6	
	14.0	77	108	24.9	
	28.0	76	102	27.5	.025
Testosterone propionate (s.c.)	.175	"	107	15.9	
	.35	"	109	20.5	
	.70	"	107	28.0	1

marized in Table II. Over comparable ranges of ventral prostate responses, andro-stanazole was about ¼ as androgenic as methyltestosterone when each was given orally. Parenterally andro-stanazole was about 1/40 as androgenic as testosterone propionate. Andro-stanazole was about 3 times as androgenic given parenterally as orally.

Discussion. For guidance in seeking evidence of separation of anabolic activity from androgenic properties of steroids, the quantitative retention of urinary nitrogen by medicated equilibrated rats was deemed to be a more specific index of anabolic action of an androgen than is the increased weight of the levator ani muscle. On the basis of comparative data obtained in rats, it was inferred that andro-stanazole may be clinically useful as an orally active weak androgen with marked anabolic activity. Howard, Norcia, Peter and Furman(14) have confirmed that this compound, administered orally at varied dose levels to androgen sensitive patients, is capable of inducing significant retention of urinary nitrogen without appearance of masculinizing side effects under the conditions of their experiment. The known properties of this compound suggest that it will be possible to obtain significant conservation of protein nitrogen with oral doses sufficiently small that androgenic side effects will be absent in short-term regimens, and delayed as well as minimal under conditions of more chronic administration.

Summary. Andro-stanazole (17β-hydroxy-17α-methylandrostano[3,2-c] pyrazole) has been evaluated for its nitrogen-retaining and androgenic activities in castrated male rats. Orally, it appears to be 30 times more anabolic and ¼ as androgenic as methyltestosterone. Parenterally, it appears to be 1/20 as anabolic and 1/40 as androgenic as testosterone propionate. Andro-stanazole is clearly an agent which merits further investigation for oral administration to patients under conditions where maximum anabolic action with minimum androgenic side effects is desired.

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Demonstration of Gamma Globulin in Vascular Lesions of Experimental Necrotizing Arteritis in the Rat.* (25185)

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In renal and vascular lesions of human periarteritis nodosa, presumably of antigen-antibody character, gamma globulin has been demonstrated by fluorescence microscopy(1). In a few instances of other human and experimental arteritis of similar origin, gamma globulin has also been demonstrated(2,3). It appeared therefore interesting to look for gamma globulin in the walls of vessels with necrotizing arteritis, which is not considered to be due to immunological causes. Necrotizing arteritis was produced in rats by unilateral nephrectomy followed by administration of DOCA(4). These lesions were examined for presence of gamma globulin(5,6).

Material and methods. Thirty-nine female Sprague-Dawley rats weighing approximately 150 g were divided into 3 groups: *Group 1,* consisting of 16 rats, were subjected to unilateral nephrectomy under nembutal and ether anesthesia. Subsequently they were given 1.5 mg of DOCA† intramuscularly 3 times a week for 5 weeks. The dosage of DOCA was then increased to 2.5 mg daily. One % NaCl was provided for drinking water. The diet consisted of standard pellets. This regimen was continued for varying periods to 7½ months after which the animals were sacrificed. From some animals, heart blood was drawn for determination of serum gamma globulin(7) immediately before death. The animals were weighed at beginning, occasionally during, and at end of experiment. Weight loss and a general deterioration, reflected in ruffled fur, lethargy and loss of appetite, were used as criteria for selecting time of sacrificing. Some rats succumbed spontaneously. *Group 2* consisted of 13 animals treated as Group 1 but sacrificed within the first 5 weeks (before increase in dosage of DOCA). Eight died spontaneously within 7 to 10 days after operation. *Group III* consisted of 10 rats which only had unilateral nephrectomy and received an ordinary diet and tap water. Immediately after death, tissues were removed from all organs except central nervous system and skeleton, and prepared in the following ways: Blocks of tissue were fixed in 10% neutral formalin for preparation of paraffin sections. Blocks from various organs were snap-frozen at −70°C in a dry ice-isopentane mixture, stored at −30°C and sectioned in a cryostat at approximately 4 μ. Both paraffin and cryostat sections were stained by the conventional staining methods. Staining with fluorescein-labelled serum was carried out, generally following the method of Coons(5,6): Rabbits were immunized with rat gamma globulin (RGG)(8) and bled from the heart. Gamma globulin was precipitated out with 18% Na_2SO_4(9), purified and conjugated with fluorescein-isothiocyanate(10,11). Cryostat sections of rat tissues were then stained with this labelled rabbit anti-RGG serum globulin solution. Controls included: (1) testing the system with non-fluoresceinated rabbit immune serum for blocking of the reaction, and (2) testing the system with normal rabbit serum

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