Influence of 19-Nortestosterone Cyclopentylpropionate on Urinary Nitrogen of Castrate Male Rat. (21086)

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(Introduced by M. H. Kuizenga.)
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Since the first demonstration of the nitrogen-sparing metabolic action of androgenic steroids(1), there has been much research to determine the activities of many androgens with respect to protein anabolism. High anabolic activity with relatively low androgenic activity has been reported for 17α-methyl androst-5-ene-3β,17β-diol(2,3) 6β-hydroxy-3, 5-cycloandrostan-17-one(4), 17β-hydroxy androstan-3-one(5), 17α-methyl androstan-17β-ol(6), 17β-hydroxy estr-5(10)-en-3-one, 19-nortestosterone(7), and three esters of the latter compound(8). "Anabolic" activity has been attributed to many compounds based on indirect measures—increase in weight of kidney(9), levator ani(10) or temporal muscles (11), increase in body weight, etc. Although these criteria are of great value in preliminary work or for precise quantitative data, we believe that direct evidence of nitrogen retention in the whole animal is the most nearly indisputable evidence for protein anabolic effect. The nitrogen balance of the rat has been used as a test for anabolic activity by Kochakian and his co-workers(12) and by Kassenaar, van Bekkum, and Querido(4). Although such experiments are frequently not adaptable to precise quantitative interpretation, they do indicate order of magnitude of potency.

This report deals with limited studies of the effect of NTCP (19-nortestosterone cyclopentylpropionate*) on the urinary N of the castrate rat in comparison with TP (testosterone propionate), 19-nortestosterone, MAD (17α-methyl androst-5-ene-3β, 17β-diol), and androstanolone (17β-hydroxy androstan-3-one).

Methods. The rats used were large males from the Upjohn colony castrated for long periods of time before treatment. They were housed in individual metabolism cages and force-fed twice daily with the Ingle medium-carbohydrate diet† 28 g/rat/day (390 mg nitrogen). This diet caused weight gain and in the earlier experiments the animals were all in positive N balance. In the later experiments growth had virtually stopped and the rats were in balance. Water was given *ad libitum*. Twenty-four hour urine specimens were collected 3 times weekly and analyzed for total N(13). Results were calculated as mg N/24 hours. The steroids were given by subcutaneous injection of .1 ml cottonseed oil solution. The details presented above are common to all experiments; other conditions which varied from one experiment to another are described below.

Results. Comparison of effects of NTCP and TP. Rats were castrated at 25 days of age and kept, untreated, for 67 days before beginning force-feeding and transfer to metabolism cages. At the beginning of force-feeding, the average weight was 300 g. The transition from stock diet to controlled intake of liquid diet was accomplished by beginning with 10 ml/rat/day and increasing the amount 2 ml/day to a level of 26 ml/rat/day. Steroid treatment was begun 24 days after the maximum feeding level was attained, when the rats weighed an average 350 g. Weight gain con-

* A paper describing preparation, 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.

† Composition of this diet is as follows:

Cellu Flour	180 g
Salt Mixture No. 2 Nutritional Biochemicals	120
Brewer's yeast	300
Casein	480
Starch	600
Dextrin	570
Sucrose	600
Water	3330 cc
Corn oil	570
Cod liver oil	30
Wheat germ oil	30
Vit. K, 0.5% sol. in cottonseed oil	30

tinued so that after 30 days of steroid treatment mean body weight was 410 g and 3 weeks after cessation of treatment was 460 g. The weight increase was the result of the restricted activity and more-than-adequate diet combining to cause the rats to become obese. In addition to fat there was deposition of protein, as the rats were in positive N balance at all times. Two groups of rats were treated with TP in doses of .25 mg or 1 mg and 2 groups with NTCP in doses of .5 mg or 2 mg/rat/day for 30 days. Fig. 1 and 2 show the N excretion and weight changes of the 4 groups. The base-line for the graphs is the mean pre-injection N excretion. The lines drawn through the body weight curves are projections of the pre-treatment growth rate, not intended to represent the expected growth for an untreated animal, but as baselines for evaluating changes in weight during treatment. Both doses of both compounds caused substantial N retention initially. After

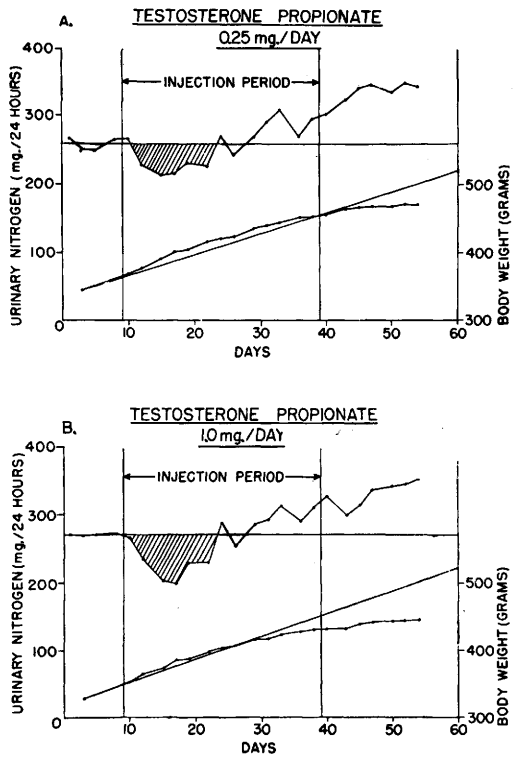

FIG. 1. Urinary total nitrogen and body weight changes of rats treated for 30 days with .25 and 1 mg TP. Shaded areas indicate significant nitrogen retention during injection period.

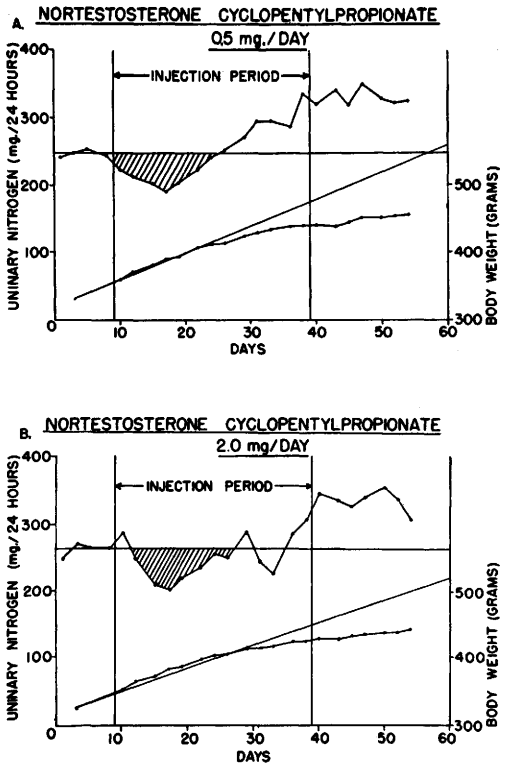

FIG. 2. Urinary total nitrogen and body weight changes of rats treated for 30 days with .5 and 2 mg NTCP. Shaded areas indicate uninterrupted nitrogen retention during injection period.

a time N excretion increased in spite of continued treatment, continuing to rise at about the same rate after cessation of treatment. The rate of increased N excretion after the wearing off of the steroid effect was the same as that of untreated control animals.

Three indices of potency have been used in evaluation of these data: The "greatest daily retention" is the difference between the lowest daily N value after beginning of treatment and the pre-injection average. The "total N retention" is the summation of the differences between pre-injection excretion and the daily values during the retention period. The values of the days on which N was not determined are estimated as being the mean of the experimentally determined values before and after. The "number of days in the retention period" is the period between the last day on which N was equal to or higher than the pre-injection average and the last day

TABLE I. Summary of Nitrogen Excretion of Rats Treated with Varying Doses of Testosterone Propionate and 19-Nortestosterone Cyclopentylpropionate.

	Testosterone propionate		Nortestosterone cyclopentyl-propionate	
	0.25 mg/day	1 mg/day	0.5 mg/day	2 mg/day
No. of rats	5	5	4	5
Total N retained, mg	398	604	507	483
No. of days in retention period	16	20	16	16
Greatest single daily retention, mg	44	73	54	60

before two consecutive values equal to or higher than the pre-injection average were obtained.

Table I lists the values of these indices in this experiment. NTCP at .5 mg/day produced a greater response than TP at .25 mg, but a lesser response than TP at 1 mg, indicating that NTCP is more than half but less than twice as potent as TP. NTCP produced essentially the same response at 2 mg as at .5 mg. Until some explanation is found for this lack of proportionality between dose and response, it is not possible to derive a firm potency ratio for the two compounds. From examination of the data it appears reasonable to conclude that the N-retaining potencies of NTCP and TP are of the same order of magnitude. The weight change curves reveal that both compounds produced slight acceleration of weight gain (Fig. 1 and 2). This is consistent with experience of other workers(14) with hormones causing retention of N.

Short courses of treatment with NTCP, MAD, and androstanolone. The rats were the same animals used in the experiment described above. The beginning of treatment in this experiment was 67 days after the last injection in the previous experiment, a period presumed

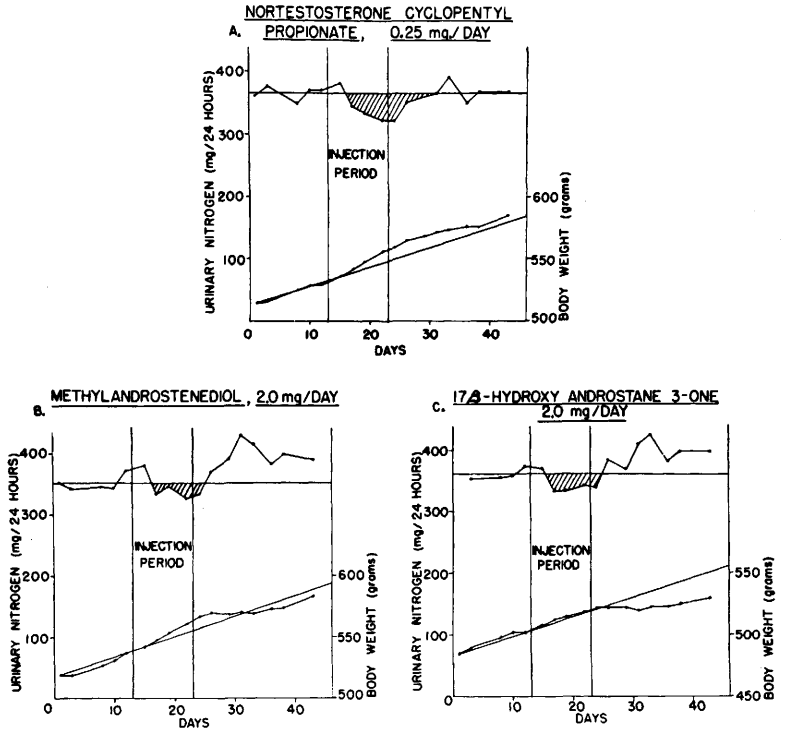

FIG. 3. Urinary total nitrogen and body weight changes of rats treated for 10 days with .25 mg NTCP, 2 mg methylandrostenediol, or 2 mg androstanolone. Shaded areas indicate nitrogen excretion less than that of fore-period.

TABLE II. Nitrogen Excretion of Rats Treated with Nortestosterone Cyclopentylpropionate, Methylandrostenediol, and Androstanolone.

	Nortestosterone cyclopentylpropionate, 0.25 mg/day	Methylandrostenediol, 2 mg/day	Androstanolone, 2 mg/day
Total N retained, mg	342	128	196
No. of days in retention period	14	9	9
Greatest single daily retention, mg	43	24	27

sufficient to permit recovery from the previous treatment. At the beginning of the injection period the mean body weight was 530 g. Food intake had been maintained at a constant level of 28 g/day (390 mg nitrogen) for about 4 months. For 30 days prior to the beginning of treatment the mean daily N excretion had been 364 mg. Postulating a fecal N of 8-9% of intake(14), the rats were in balance. The slow weight gain that continued was probably due to addition of fat with no appreciable protein deposition.

Comparison of the anabolic potencies of androstanolone and MAD with that of NTCP was undertaken because of recent interest in these compounds as anabolic steroids(3,5). Four rats were injected for 10 days with 2 mg androstanolone once daily, 4 with 2 mg MAD, and 4 with .25 mg NTCP. All 3 compounds caused decreases in N excretion lasting throughout the injection period (Fig. 3). Following withdrawal of treatment all animals returned to pre-injection N levels, and the androstanolone and MAD treated rats had transitory periods of N loss before leveling off at pre-injection levels. The relative magnitudes of the changes are seen in Fig. 3 and Table II.

NTCP was the most potent of the 3 compounds tested. The N retention effect of NTCP persisted for several days after withdrawal, while the rats receiving the other 2 compounds returned to or above pre-injection levels immediately following withdrawal. The total N retained by the NTCP-treated rats was about 1.7 times that retained by the androstanolone-treated animals and 2.7 times that retained by those receiving MAD.

Comparison of single injections of NTCP and nortestosterone. It has been shown(8) that NTCP has greater myotrophic activity than unesterified nortestosterone when the rat levator ani is used as an activity index. To confirm this difference by another test was desirable for characterization of NTCP and also for establishing parallelism between 2 animal tests.

An experiment was therefore performed in which N excretion was followed in 2 groups of animals treated with single injections of NTCP and nortestosterone. Two groups of 4 castrate male rats weighing a mean 424 g (372-542) at the beginning of the fore-period were treated with 2 mg of NTCP or nortestosterone in .1 ml cottonseed oil. A single injection of nortestosterone failed to produce a positive response. The single injection of NTCP produced a substantial depression of N excretion for about 10 days (Fig. 4).

Discussion. NTCP appears from these and previous(8) data to be one of the most potent anabolic steroids reported to date. Although it is difficult definitely to fix its N-retaining potency in comparison with TP because of the lack of differential in response to graded doses, one can speculate that it is roughly equivalent to TP. NTCP is more potent than androstanolone, a result consistent with Kochakian's findings(12,15) that androstanolone had less N-retaining potency than TP. NTCP is obviously a more potent N-retaining agent that MAD.

The fact that esterification with cyclopentylpropionic acid greatly improves the anabolic activity of nortestosterone is firmly established. The present data confirm the earlier levator ani data from this laboratory(8).

Summary. 1. In the adult castrate rat, oil injections of 19-nortestosterone cyclopentylpropionate produced decreases in urinary N excretion comparable with those obtained with testosterone propionate. 2. This compound caused greater retention of N than was observed with parenteral administration of me-

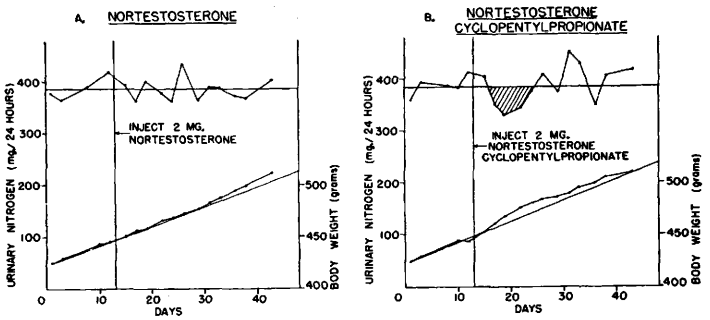

FIG. 4. Urinary total nitrogen and body weight changes of rats given single injections of (A) nortestosterone or (B) NTCP. Shaded area indicates nitrogen retention.

thylandrostenediol or androstanolone when given at 1/8 the dose of the 2 latter compounds. 3. 19-nortestosterone cyclopentylpropionate in a single injection of 2 mg produced retention of N lasting several days; unesterified nortestosterone, similarly given, did not result in significant N retention.

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Effect of Aluminum Hydroxide Gels on Experimental Hypercholesterolemia and Atheromatosis in Chicks.* (21087)

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It has been reported that aluminum hydroxide gel reduced cholesterolemia, delayed the onset of atheromatosis, and produced muscular weakness in chicks fed cholesterol(1,2). These effects were attributed to possible adsorption phenomena by alumina gel in the small intestine. The significance of these observations made it of interest to further investigate them.

Method. The regimen for producing atheromatosis was that of Dauber and Katz(3). One hundred White Leghorn cockerels certified to be free of salmonella infection were

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