The Singularly Brief Anabolic Activity of Testololactone, A Compound Lacking Androgenicity. (19157)

IRVING SHEMANO, GILBERT S. GORDAN, AND EUGENE EISENBERG.

From the Division of Pharmacology and Experimental Therapeutics and the Division of Medicine, University of California School of Medicine, San Francisco.

In the course of studies designed to screen compounds for anabolic activity, with the hope of finding potent anabolic agents with little or no androgenicity, it has previously been shown that anabolic activity does not necessarily parallel androgenic potency(1). The anabolic activity of testololactone,* a nonandrogenic steroid derivative, provides the basis for this report.

Methods. The screening test for anabolic activity was growth of the levator ani muscle of the castrate immature male rat. Testololactone, in a dose of 5 mg per rat per day, produced levator ani muscles weighing 45 ± 1.0 mg in the standard screening test previously described(1). In control animals, the levator ani muscles weighed 33 ± 2.9 mg. To test further the anabolic potential of this compound, its effect upon urinary nitrogen excretion was carried out by the method previously described(2,3). Two such studies were conducted, one using 6 castrate male rats and the other, 5 "plateaued" adult female rats of the Long-Evans strain. These animals were treated with testololactone (10 mg per rat per day). An additional group of 6 castrate male rats was subjected to similar treatment in order to determine the effects of a smaller dose of testololactone (5 mg per day). After the animals were decapitated at the end of the period of administration, the levator ani muscles and seminal vesicles of the castrate males were dissected and weighed by the methods described elsewhere(1).

Experimental data. The data relating to urinary nitrogen excretion are graphed in

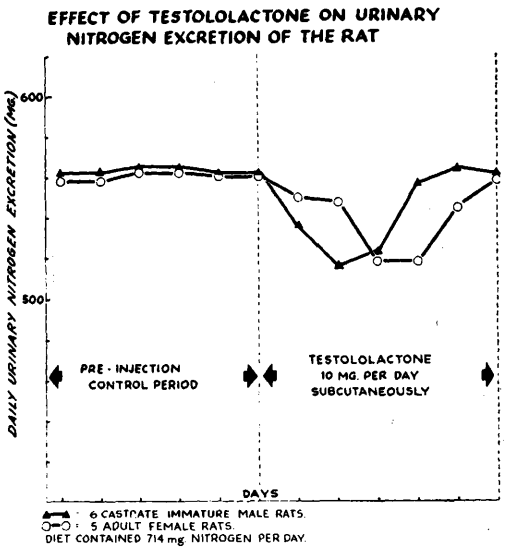

FIG. 1.

Fig. 1, and the weights of the levator ani muscles are reported in Table I. Body weight was not significantly altered. It will be noted that in all cases, testololactone produced a moderate but evanescent decrease in urinary nitrogen excretion, amounting at the time of peak effect to 32 to 42 mg per rat per day ($p < 0.01$). Despite continued administration of the agent, the animals did not show continued retention of nitrogen after the fifth day of treatment, and the urinary nitrogen content rebounded to pretreatment levels.

Based on the assumption of complete conversion of the retained nitrogen to protoplasm, an increase of body weight of not more than 2 or 3 g would be anticipated in view of the short duration of the period of nitrogen retention. Such changes in body weight are not assessable in rapidly growing castrate males, nor even in relatively "plateaued" adult females.

In contrast to the lack of change of general body weight, there was a statistically significant increase in the weight of one tissue, the

1. Eisenberg, E., and Gordan, G. S., *J. Pharm. and Exp. Therap.*, 1950, v99, 38.

* Generously furnished by Dr. Irwin Winter, G. D. Searle & Co., Chicago, Ill.

2. Gordan, G. S., Evans, H. M., and Simpson, M. E., *Endocrinology*, 1947, v40, 375.

3. Gordan, G. S., Bennett, L. L., Li, C. H., and Evans, H. M., *Endocrinology*, 1948, v42, 153.

TABLE I. Effect of Testololactone on Levator Ani Muscle and Seminal Vesicle of Immature Male Castrate Rat.

No. of rats	Group	Levator ani wt, mg ± 6 m	Seminal vesicle wt, mg ± 6 m
6	Control castrates	20 ± 2.1	8.5 ± 1
5	Testololactone, 5 mg/day	31 ± 2.7	8 ± .8
6	Testololactone, 10 mg/day	38 ± 2.2	12.5 ± 2.7

levator ani muscle. The weight of this muscle in untreated and treated animals in this experiment in which dietary restriction was imposed is less than has previously been noted for untreated and treated animals, respectively (1) on *ad lib.* dietary intake.

Androgenic activity, as judged by the weight of the seminal vesicles, was completely lacking, despite the relatively large amounts of testololactone administered for periods of 7 to 9 days.

Discussion. It is typical of the protein anabolic steroids that a retention of nitrogen is produced, followed by an eventual wearing off of the effect or "rebound"(4). Testololactone manifests the most rapid wearing-off of effect of any protein-anabolic compound reported so far.

Kochakian in 1950(4) attributed the "wearing-off effect" of testosterone propionate to a loss of body fat and a shift of carcass protein to the continued synthesis of new tissue at other sites in the body, especially the accessory sex organs. In the case of testololactone, however, such a mechanism is most improbable. Since the effect noted was brief and the rebound during the administration period was rapid, it seems unlikely that the rat would exhaust its body fat in so short a time. Also, testololactone produces no androgenic activity at all under the conditions of this experiment, so a shift of carcass protein to the accessory sex organs cannot be invoked to account for the wearing-off effect of the compound.

Alternative hypotheses for the wearing-off effect immediately suggest themselves. For example, an adaptive process may be taking place so as to activate an enzyme system which inactivates the compound. It is known from studies *in vitro* that enzymatic adaptation may take place in bacteria within a matter of hours. Thus, it is feasible that the wearing-off effect could take place in as short a space of time as observed in this experiment.

The steric configuration of the D ring of testololactone is as yet unknown(5). Inasmuch as all steroids with protein-anabolic activity studied so far have polar groups both at the 3 and 17 positions, it may be suggested that the ketone group of the D ring is likewise in the 17 position. In this connection, we have noted that two other steroids with ketone groups in the 3 and 17 positions produce significant growth of the levator ani muscle without increase in the weight of the seminal vesicles. These are androstadiene △ 1-2, 4-5 dione 3, 17 and androstadiene △ 4-5, 6-7 dione 3, 17.†

Summary. Testololactone has been demonstrated to have protein-anabolic activity, having passed both the levator ani screening test and a confirmatory nitrogen balance study. This compound showed a rapid effect, remarkably evanescent, which after quickly wearing off was followed by the rebound of nitrogen excretion in the urine to the basal level when testololactone treatment was continued. Testololactone is completely nonandrogenic at the very high dose of 10 mg per day.

4. Kochakian, C. D., Robertson, E., and Bartlett, M. N., *Am. J. Physiol.*, 1950, v162, 332.

5. Jacobsen, R. P., Picha, G. M., and Levy, H., *J. Biol. Chem.*, 1947, v171, 81.

† Generously furnished by Dr. Gordon Grant of Ayerst, McKenna and Harrison, Ltd., Rouses Point, N. Y.

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