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Prevention by Certain Steroids of Testicular Atrophy Usually Elicited by Small Doses of Testosterone.*

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An important argument against the clinical use of testoids† in patients suffering from eunuchoidism is that such compounds may aggravate the testicular atrophy when they are given in doses just sufficient to restore the accessory sex organs to the normal size. It is known that gonad atrophy is produced only with moderate doses which suffice to inhibit the hypophyseal gonadotropic hormone production, but not to exert a direct gonadotropic effect. Very high doses—which maintain the spermatogenic epithelium even in the absence of the pituitary—cause no testis atrophy in the intact organism since their direct gonadotropic effect fully compensates for the inhibition of hypophyseal function.[1] However, such high doses could hardly be given in clinical cases of eunuchoidism since they would lead to an excessive development of the secondary sex characteristics. As it has recently been shown that the testis atrophy caused by estradiol can be inhibited by testosterone and progesterone[2, 3] the question arose whether the testis atrophy normally elicited by "therapeutic doses" of testosterone might not in its turn be inhibited by steroids devoid of testoid activity. It was felt that if this could be accomplished testosterone might be given in combination with such gonadotropic steroids without the fear of aggravating testis atrophy or having to give enormous doses which would lead to excessive masculinization.

In order to study this problem 4 groups each consisting of 6 male albino rats weighing between 105 and 145 g (average 121 g) were used. Group I acted as untreated controls; Group II received 1.0 mg

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† This term is used as synonymous with "male hormone-like" or "androgenic" in accordance with the recently proposed pharmacological classification of the steroid hormones.[4]

1 Selye, H., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 142.

2 Selye, H., *Canad. Med. Assn. J.*, 1940, **42**, 113.

3 Albert, S., in press.

4 Selye, H., *Nature*, 1941, **148**, 84.

of testosterone daily; Group III received daily 1.0 mg of testosterone and 10.0 mg of Δ^5-androstene-3(β),17(α)-diol (m.p. 182-183°C), which will henceforth be referred to as androstenediol; Group IV received 1.0 mg of testosterone and 10.0 mg of 17-ethyl-Δ^5-androstene-(β)-ol-20-one (m.p. 186°C) which will henceforth be referred to as pregnenolone. The daily dose was administered in 2 subcutaneous injections of 0.1 ml each in all cases. Testosterone was dissolved in peanut oil (5 mg/ml) while pregnenolone and androstenediol were given as finely ground crystals suspended in the same material (50 mg/ml). All animals were killed and their gonads weighed on the day following the tenth day of treatment. The average testis weight in the controls was 2.138 g and under the influence of testosterone alone declined to 1.418 g. In the group receiving androstenediol the testicular atrophy otherwise produced by testosterone was apparently inhibited since the average testis weight was 1.925 g. In the group receiving pregnenolone the atrophy was completely prevented, the average testis weight being 2.174 g.

The significance of the apparent differences between the testis weights of the testosterone-treated rats and the controls was evaluated by "Student's" method for small samples and was expressed in terms of probability estimated by graphic interpolation in Fisher's[5] table of t. It is generally agreed that the differences may be regarded as significant if P is smaller than 0.05. In this case P was smaller than 0.01. Similarly, the testes of the groups receiving androstenediol and pregnenolone respectively were significantly larger than those receiving testosterone alone, since in both cases the increase in testis weight in comparison with the last mentioned group gave a P of less than 0.01.

Since androstenediol possesses a weak testoid activity its gonad-protecting effect might be regarded as, at least partly due to this property. Pregnenolone, however—as our earlier experiments on castrate rats have shown—is devoid of any demonstrable testoid action.

Summary and Conclusions. Experiments on post-pubertal albino rats indicate that androstenediol and pregnenolone prevent the testis atrophy otherwise produced by small doses of testosterone. Since androstenediol possesses only a weak "male hormone" or testoid action and pregnenolone is completely devoid of it, it is evident that the gonad-protecting power of a steroid compound is independent of

[5] Fisher, R. A., *Statistical Methods for Research Workers*, 6th Edition, Edinburgh, 1936, p. 128.

its testoid action. The experiments suggest, furthermore, that by combining moderate doses of testosterone with a non-testoid compound such as pregnenolone, therapeutic doses of testosterone may be given in cases of eunuchoidism without the danger of producing testis atrophy.

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Comparative Distribution and Retention of Crystalloid and Colloid Fraction of Arsphenamine and Neoarsphenamine.

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Wright, *et al.*,[1] have separated both arsphenamine and neoarsphenamine, by dialysis in an inert atmosphere, into a crystalloid and a colloid fraction. The crystalloid fraction is less toxic and more curative for trypanosomiasis in rats than either the whole drug or colloid fraction. Death from toxic doses of either the crystalloid fraction or whole drug was due to delayed arsenical poisoning. The colloid fraction produced acute toxic symptoms, such as extreme respiratory embarrassment and death from respiratory failure.

We have investigated the comparative distribution and retention of the whole drug and the crystalloid and colloid fractions of arsphenamine and neoarsphenamine in the tissues of the rat following their intravenous administration.

The drugs were injected intravenously in a dose of 15 mg/kg and the animals were sacrificed at intervals of ½, 2, 6, 12, 24, 168, and 336 hours, at which times blood, skin, muscle (skeletal), bone, liver, kidneys, spleen, brain, stomach, small intestine and colon were taken for analysis. The arsenic content was determined by the Gutzeit method of the A.O.A.C.[2] A total of 308 rats was employed

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1 Wright, H. N., Biederman, A., Hanssen, E., and Cooper, C. I., *J. Pharm. Exp. Therap.*, 1941, **73**, 12.

2 Methods of Analysis, Official and Tentative, of the Association of Official Agricultural Chemists, 4th edition, 1935.