tection lasted several hours (3 experiments).

Discussion and summary. The various mercurials studied show both quantitative and qualitative differences in their cardiac action. In the case of the mercurial diuretics the aminophylline containing compounds produce an increase in the sinus rate as well as ventricular tachycardia while the free mercurials produce only a ventricular tachycardia (Fig. 2). The evidence presented makes it probable that the differences in response of the heart rate are due to the theophylline content of these preparations.

The ST and T wave changes described occurred somewhat prior to the QRS changes. ST and T wave changes are probably related to alterations in the process of repolarization of cardiac muscle while the QRS changes probably indicate mainly changes in intraventricular conduction. The mercurials studied are powerful inhibitors of enzymes containing essential SH groups(4,5). Thus the biochemical processes responsible for repolarization of cardiac muscle seem to be more sensitive to these SH inhibitors than the processes involved in intraventricular conduction.

The action of p-chloromercuribenzoate is of interest. It affects only the ST and T wave and thus probably interferes mainly with repolarization of the muscle but has no effect on intraventricular conduction processes. However, it does interfere with atrioventricular conduction and thus, with this enzyme poison it seems possible to inhibit selectively atrioventricular conduction. Differences in the histology and staining qualities of the atrioventricular and intraventricular conductile system have been described by Robb *et al.*(6) and pharmacological differences are thus not surprising.

4. Pitts, R. F., and Sartorius, O. W., *J. Pharmacol. Exp. Therap.*, 1950, v98, 161.

5. Handley, C. A., and Lavick, P. S., *J. Pharmacol. Exp. Therap.*, 1950, v100, 115.

6. Robb, J. S., personal communication.

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Prolongation and Potentiation of Anabolic and Androgenic Effects of Steroids: Testosterone and Methylandrostenediol.*† (18505)

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Carlinfanti *et al.*(1,2) have reported the potentiation and prolongation of the androgenic activity of testosterone in castrated male guinea pigs by the addition of aluminum phosphate to an aqueous suspension of the steroid. Margolin *et al.*(3) produced similar results in the albino rat. Both groups of workers have limited their reports to the androgenic effects. By use of a recently described assay procedure for anabolic activity (4), methylandrostenediol (MASD) has been found to be an anabolic agent which, in ordinary doses, has little androgenic activity (5). It was the purpose of our investigation to compare the duration and degree of action of the androgenic and anabolic properties of aqueous suspensions of MASD and of testo-

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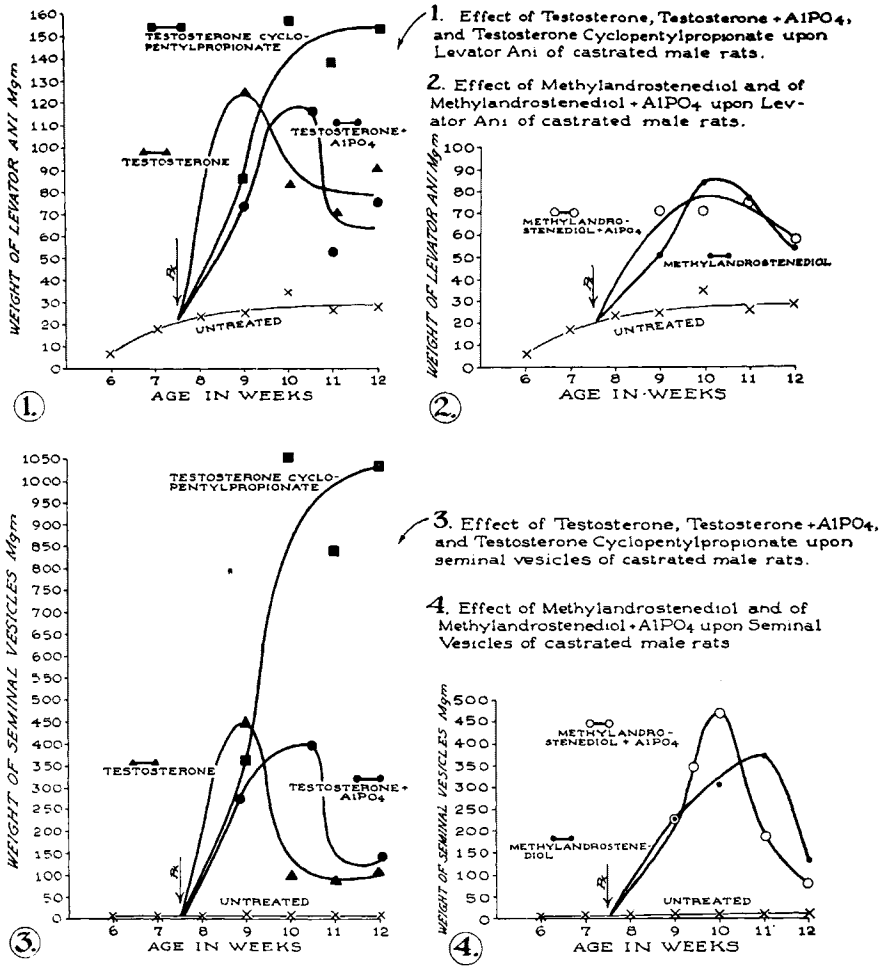
1. Carlinfanti, E., D'Alo, F., and Cutolo, L., (Abstr.) *Chem. Abstr.*, 1949, v23, 3085.

2. Carlinfanti, E., D'Alo, F., and Cutolo, L., *Lancet*, 1949, v1, 479.

3. Margolin, S., Lee, S. W., and Tislow, R., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 649.

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

5. Gordan, G. S., Eisenberg, E., Moon, H. D., and Sakamoto, W., *J. Clin. Endocrinol.*, in press.



sterone, with and without added aluminum phosphate 3 mg and aluminum stearate 5 mg per 25 mg of steroid. Testosterone cyclopentylpropionate in sesame oil (TCP) was also studied because of the reported depot effect of this esterified form of testosterone (6), and for comparison with the aluminum phosphate suspensions.

Method. Male rats of the Long-Evans strain were castrated at the age of 4½ weeks. At the age of 7½ weeks, each was injected subcutaneously with a single dose of 25 mg of the agent. Groups, consisting usually of 5 animals but occasionally of 7, were autopsied at the ages of 9, 10, 11, and 12 weeks. Control groups of castrate untreated rats were autopsied at 6, 7, 8, 9, 10, 11, and 12 weeks of age to observe the sequence of events following castration. Androgenic activity was assayed by the weight of the seminal vesicles, and anabolic potency was estimated by the weight of the levator ani muscle, using the technique described elsewhere(4).

Experimental data and discussion. The results of this experiment are presented in Fig. 1-4.

It will be noted that in the absence of gonadal steroids (castrate untreated animals), the weight of the seminal vesicles remains plateaued, indicating that androgenic activity is lost. In contrast, the levator ani muscle continues to grow in the growing castrate ani-

6. Ott, A. C., Kuizenga, M. H., Lyster, S. C., and Johnson, B. A., personal communication.

mal but more slowly than in the intact animal(4).

Comparing the anabolic effects of the various agents in the dose employed in this study, it will be noted that esterification of testosterone with cyclopentylpropionic acid (Fig. 1) increases and prolongs anabolic activity to the greatest degree. With this agent (TCP) the anabolic effect is still maximal a month after injection. Aluminum phosphate delays both the measured actions of testosterone (Fig. 1 and 3) but does not significantly potentiate or prolong either effect of this agent. In the case of MASD (Fig. 2) there is no significant difference between the curves obtained with and without aluminum phosphate, probably because the steroid is highly insoluble. All the agents employed in this assay produce a great increase in the size of the levator ani muscle.

Androgenic activity is manifested by all these agents in the very large dose used. Aluminum phosphate does not delay, potentiate, or prolong the androgenic action of MASD (Fig. 4), presumably because of the solubility properties of the agent itself. Aluminum phosphate delays and somewhat prolongs the androgenic action of testosterone (Fig. 3); however, no great potentiation, *i.e.* increased efficacy, of this suspension is observed. Testosterone cyclopentylpropionate is the most potent agent in this group (Fig. 3). Its increased activity cannot be explained on the basis of slower release of the active constituent from the injection site alone, since there is no greater delay in growth of the levator ani muscle and seminal vesicles with the cyclopentylpropionic acid ester than with the aluminum phosphate suspension of testosterone. Inasmuch as the increased efficacy of this ester cannot be accounted for on the basis of delayed absorption, it seems likely that the long side chain delays the inactivation of the compound. This conclusion is in harmony with that of Miescher *et al.*(7) that the longer the carbon side chain, the more protracted is the effect. However, TCP differs from the esters studied by Miescher *et al.* in that prolongation of effect is not combined with decreased intensity of effect.

Caution must be exercised in transferring these observations to other species since, as Miescher *et al.* have pointed out, rats are more sensitive to the action of testosterone esters than are capons.

Summary. 1. Testosterone cyclopentylpropionate shows the greatest anabolic and androgenic efficacy of the agents studied. Its increased efficacy cannot be explained on the basis of delayed absorption, suggesting a delayed inactivation of this ester. 2. Aluminum phosphate delays the onset and decline of the anabolic and androgenic actions of testosterone in aqueous suspensions, but produces no such effect upon the actions of methylandrostenediol.

7. Miescher, K., Wettstein, A., and Tschopp, E., *Biochem. J.*, 1936, v30, 1977.

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Purification and Identification of the Antistiffness Factor.* (18506)

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In a previous study(1) evidence was presented that the infra-red absorption spectrum of the antistiffness factor obtained from sugar cane resembled the spectrum of stigmasterol more closely than any other spectrum of a

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1. Rosenkrantz, H., Milhorat, A., Farber, M., and Milman, A., *Fed. Proc.*, 1951, in press.