Potentiation of Androgen by Aqueous Aluminum Phosphate Suspension. (18004)

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Carlinfanti, D'Alo, and Cutolo(1,2) applied to testosterone, with striking results, the Holt(3) aluminum phosphate suspension method used in the preparation of water-soluble diphtheria toxoid. Their data indicated that a depot effect also occurs when small quantities of aluminum phosphate are added to water-insoluble testosterone. Characteristic of the depot effect of aqueous testosterone-aluminum phosphate suspensions was a 2- to 3-fold potentiation, and increase in duration of activity. As an outcome of the reports by these authors, experiments were undertaken in an attempt (1) to confirm the work of Carlinfanti and his associates in the guinea pig; (2) to extend the data to another species, the albino rat.

Immature male guinea pigs obtained from Manor Farms and immature Sprague-Dawley male rats were castrated, and immediately injected with a single dose of the various androgen preparations. In order to study the degree and duration of androgenic stimulation, groups of animals were sacrificed and examined at predetermined intervals. In both species, growth response of the seminal vesicles was the end-point in evaluating potency. The androgen preparations assayed were (1) an aqueous testosterone-aluminum phosphate (ATAP) suspension prepared according to the method described by Carlinfanti *et al.*, (2) an aqueous testosterone suspension, and (3) testosterone propionate oil solution. Each preparation contained 25 mg/cc of testosterone or testosterone propionate, and was administered on a body weight basis.

Exp. 1. Guinea pigs weighing 200 to 300 g were injected after castration with a single subcutaneous dose of 25 mg of androgen per 100 g of body weight under the skin of the back. Groups (3 to 5 guinea pigs per preparation) were autopsied at days 3, 6, 13, 23, 31, and 35 after injection. Body weights were recorded; seminal vesicle, adrenal, liver, kidney, and pituitary weights also were taken, and sections of these organs were prepared for histological examination. Table I summarizes and Fig. 1 illustrates the outcome of this experiment. The values closely resemble those reported by Carlinfanti for the guinea pig. Examination of the time response curves obtained by Carlinfanti and by us in the guinea pig suggests that the effect of ATAP suspension lasts at least twice as long as that of testosterone propionate oil solution.

Exp. 2. Immature rats, 27 days old, were castrated and injected intramuscularly with a single dose of 25 mg of androgen per 100 g of body weight. Groups (4 rats per preparation) were killed on days 3, 7, 14, 24, and 32. At autopsy, body weights were taken. The seminal vesicles, ventral prostates, adrenals, livers, and kidneys were weighed and portions of these organs preserved for histological examination. The data obtained with rats show that $AlPO_4$ in aqueous suspension potentiates the androgenic effect of testosterone (Fig. 2). Seminal vesicle response to aqueous testosterone suspension with aluminum phosphate was more than twice that found for aqueous suspensions containing testosterone alone. Maximum effect was obtained 14 days after injection as compared with a maximum at 7 days for aqueous testosterone suspension. Approximately 20 days after injection of ATAP suspension, the seminal vesicle weight still equaled the maximum obtained with an aqueous testosterone suspension on day 7. Apparently a 2- to 3-fold increase in duration of action occurred.

Exp. 3. As a further test of the effectiveness of the new preparation, vials of a commercially available aqueous testosterone sus-

1. Carlinfanti, E., D'Alo, F., and Cutolo, L., *Riv. ist. sieroterap. ital.*, 1948, v23, 151; Abstr. *Chem. Abst.*, 1949, v43, 3085.

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

3. Holt, L. B., *Lancet*, 1947, v1, 282.

TABLE I.
Androgenic Response of Immature Castrate Guinea Pigs to Aqueous Testosterone Aluminum Phosphate Suspension.

	Carlinfanti	Schering
Onset of potentiation	10-15 days	13 days
Max. response	30 "	31 "
Potency ratios:		
Testosterone $AlPO_4$ vs. testosterone propionate in oil	1.9	2.9
Testosterone $AlPO_4$ vs. controls	5.8	8.3
Testosterone propionate in oil vs. controls	3.1	2.8

pension were purchased and divided into 2 lots. One lot was treated with aluminum phosphate using a modified Carlinfanti suspension method; the other remained unaltered. Again, there was a significant potentiation and prolongation of androgenic activity with the aluminum phosphate-treated product when compared with the commercial preparation containing no aluminum phosphate (see Fig. 3).

It has been demonstrated that esters of testosterone with short-chain fatty acids elicit a greater biological response than free testosterone when administered in oil (Miescher, Wettstein, and Tschopp)(4). Kochakian(5) and others have found enhanced biological response to testosterone in experimental animals when given as an aqueous suspension. If the increase in activity of testosterone esters in oil over that of free testosterone in oil depends merely on

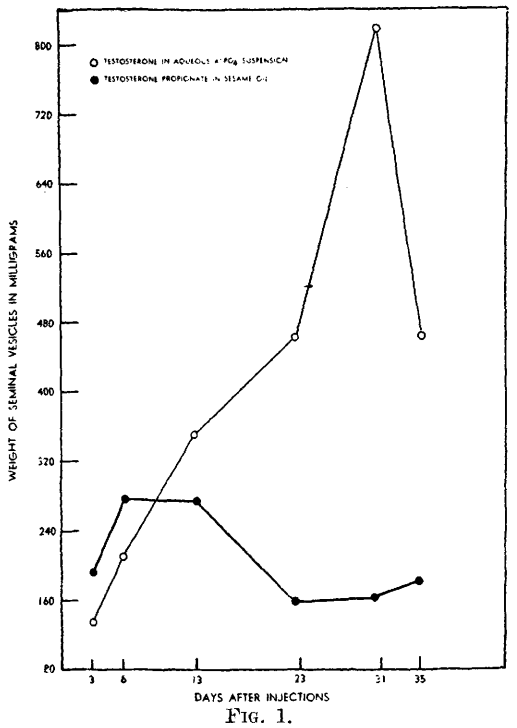

FIG. 1.
The depot effect of ATAP suspension. Seminal vesicle response in immature guinea pigs to a single subcutaneous injection of androgen.

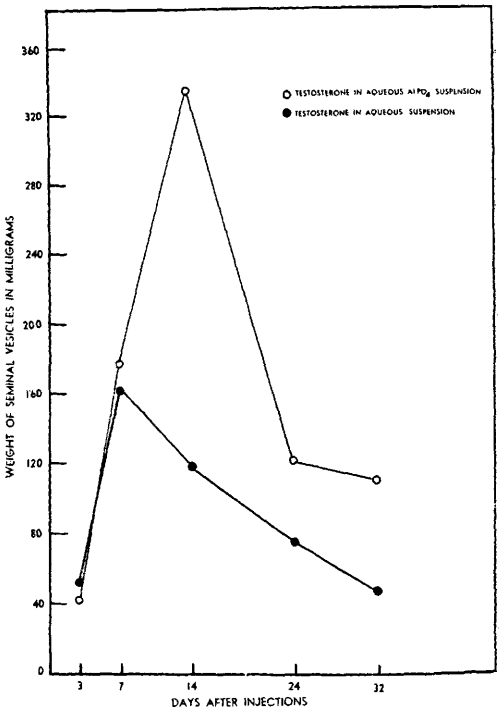

FIG. 2.
The depot action of ATAP suspension. Seminal vesicle response in immature castrate rats to a single intramuscular injection of testosterone.

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

5. Kochakian, C. D., *Endocrinology*, 1938, v22, 181.

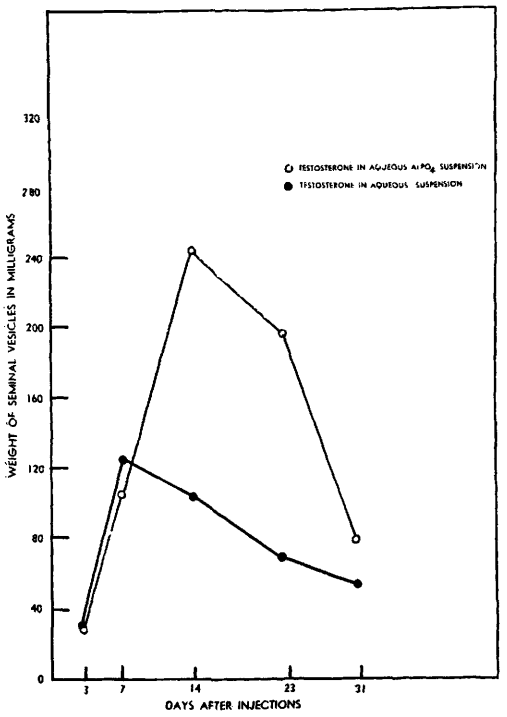

FIG. 3.

Potentiation of a commercial aqueous testosterone suspension by aluminum phosphate. Seminal vesicle response of immature rats to a single intramuscular injection.

slower release from the depot site, it follows that a depot of micro-crystalline testosterone, subcutaneously or intramuscularly, should be ideal for slow absorption. As yet, there is no experimental demonstration of the mechanism by which aqueous $AlPO_4$ suspension of testosterone elicits a greater androgenic response than the aqueous suspensions without $AlPO_4$.

Summary. The experimental data confirm the depot effect described by Carlinfanti and his co-workers following subcutaneous injection of ATAP suspensions in the guinea pig. The effect also has been demonstrated in immature castrate rats after intramuscular injection. In both instances marked increase in response and duration of biological activity were obtained. Potency ratios and indices of duration of action indicate that each milligram of testosterone in aqueous aluminum phosphate suspension is (1) two or more times as active, and (2) acts approximately two to three times as long as the same quantity of testosterone in aqueous suspension alone.

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Inhibition of the Phagocytic Action of Leucocytes by Mumps and Influenza Viruses. (18005)

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In previous experiments(1-3), it has been shown that mumps virus has a destructive effect on the erythrocytes from a number of species which results in hemolysis. It was therefore of interest to determine if this virus would damage other cells. Polymorphonuclear leucocytes of the guinea pig were selected for study since their ability to engulf killed bacteria offered a ready test of their intact functional activity for which a quantitative technic has been developed for direct observation with the microscope(4). The erythrocytes of this species are very susceptible to the hemolytic action of mumps virus (3).

The effect of influenza virus on the phagocytosis of bacteria was also studied since reports(5) indicated that this virus caused

1. Morgan, H. R., Enders, J. F., and Wagley, P. F., *J. Exp. Med.*, 1948, v88, 503.

2. Chu, L. W., and Morgan, H. R., *J. Exp. Med.*, 1950, v91, 393.

3. Chu, L. W., and Morgan, H. R., *J. Exp. Med.*, 1950, v91, 403.

4. Merchant, D. J., *J. Inf. Dis.*, in press.