Androgenic Activity of C21 Steroids.* (17635)

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Previous studies by Pfiffner and North(1,2) have indicated that 17(α)-hydroxyprogesterone is an androgen and possesses an activity approximately equal to that of androsterone on the immature castrate rat seminal vesicle and prostate. When administered intramuscularly to capons this compound was less than one half as active as androsterone on the comb. These studies were done with a limited quantity of material isolated from the adrenal gland. With the availability of this and related compounds as the result of partial synthesis it has become possible to study the androgenic activity of 17(α)-hydroxyprogesterone and related compounds in more detail. This communication is concerned with the influence of 17 (α)-hydroxyprogesterone, Δ^{16}-dehydroprogesterone, Δ^{4}-pregnenetriol-17(α), 20 (β), 21-one-3-diacetate-20,21 and 17-hydroxy-11-desoxycorticosterone acetate (Compound "S" acetate, Reichstein) on the chick's comb.

Experimental. Androsterone† (used as the reference compound) and the 4 steroids, 17 (α)-hydroxyprogersterone,‡ Δ^{16}-dehydroprogesterone,§ Δ^{4}-pregnenetriol - 17(α),20(β), 21-one-3-diacetate-20,21,‡ were dissolved in corn oil so that the daily dose was contained in 0.05 cc. The material was applied to the comb once daily for 7 days starting 2 days after the male white leghorn chicks‖ were hatched. Twenty-four hours after the last applications comb and body weight were determined. The results are expressed as the ratio of the comb weight to body weight. In 2 instances the steroids were administered by subcutaneous injection, the daily dose being contained in 0.1 cc of corn oil. All other details were similar to that described for the direct comb application technic.

Results and conclusions. The results are tabulated in Table I. Δ^{16}-Dehydroprogesterone, applied directly to the comb, in a dosage of 500 μg, produced in 2 experiments significant increments of 97 and 124% in comb weight. A total dose of 940 μg produced a comb weight increase of 190%. By direct comb application this C21 steroid, Δ^{16}-dehydroprogesterone, was found to be about 6% as active as androsterone.

17(α)-Hydroxyprogesterone was studied by direct comb application, at the 80, 320, and 640 μg dosage levels. Neither 80 nor 640 μg produced any significant changes in comb weight, but at 320 μg a statistically significant increase of 29% was observed ($t = 3.90$). When a total dose of one mg was injected a statistically significant decrease in comb ratio of 18% ($t = 2.78$) was found.

Δ^{4}-Pregnenetriol-17(α),20(β),21-one-3 diacetate-20,21 was applied to the comb in total doses of 500 and 840 μg. Statistically significant decreases in comb ratio were observed in both instances. At the lower dose level a decrease of 26% was found and at the higher dose level a decrease of 21% was observed. The fact that local application inhibited the growth of the chick's comb speaks for direct antagonism to endogenous androgen stimula-

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† Androsterone was kindly supplied by Ciba Pharmaceutical Products, Inc.

‡ Δ^{4}-Pregnenediol-17(α),21-one - 3 - acetate-21 (compound "S" acetate, Reichstein) was part of a gift from Mr. Adrian Joyce of the Glidden Co. 17(α)-Hydroxyprogesterone and Δ^{4}-pregnenetriol-17(α),20(β),21-one-3-diacetate-20,21 were purchased from the Glidden Co.

§ Δ^{16}-Dehydroprogesterone was kindly supplied by Ayerst, McKenna, and Harrison Limited in collaboration with Syntex, S.A.

‖ The chicks were purchased from the Kerr Chickeries, Frenchtown, N.J.

TABLE 1.
Action of Various C-21 Steroids and Androsterone on Chick's Comb.

Exp. No.	Compound (Method of administration)	Total dose μg	No. of Chicks	Comb ratio* Mean ± S.E.	Change in comb ratio* %	"t"
1.	0	0	22	35 ± 1	--	—
	Androsterone (Application)	20	14	62 ± 5	+ 77	—
		40	15	88 ± 7	+151	—
		80	15	125 ± 10	+257	—
	17(α)-Hydroxyprogesterone (Application)	80	15	35 ± 1	0	—
		320	16	45 ± 2	+ 29	3.90
		640	15	37 ± 2	+ 6	—
	Δ^{16}-Dehydroprogesterone (Application)	500	15	69 ± 5	+ 97	
	Δ^{4}-Pregnenetriol-17(α),20(β),21-one-3-diacetate-20,21 (Application)	500	16	26 ± 2	- 26	4.23
	Δ^{4}-Pregnenediol-17 (α),21-dione-3,20-acetate-21 (Compound "S" acetate, Reichstein) (Application)	500	16	27 ± 2	— 23	3.73
2.	0	0	12	38 ± 1	—	—
	Δ^{16}-Dehydroprogesterone (Application)	500	9	85 ± 6	+124	—
		940	9	110 ± 14	+190	
	Δ^{4}-Pregnenediol-17(α),21-dione,3,20-acetate-21 (Injection)	1000	10	27 ± 1	- 29	6.36
	Δ^{4}-Pregnenetriol-17(α),20(β),21-one-3-diacetate-20,21 (Application)	840	11	30 ± 2	- 21	3.20
	17(α)-Hydroxyprogesterone (Injection)	1000	12	31 ± 2	- 18	2.78

* Comb Ratio $= \frac{100 \times \text{Comb wt (mg)}}{\text{Body wt (g)}}$

tion. Δ^4-Pregnenediol-17(α),21-dione-3,20-acetate-21 (compound "S" acetate, Reichstein) inhibited the growth of the comb when applied directly or when administered subcutaneously. When 500 μg was applied to the comb a decreased comb ratio of 23% ($t = 3.73$) was found, while one mg administered subcutaneously produced a decrease of 29% ($t = 6.36$).

Summary. Δ^{16}-Dehydroprogesterone exhibited androgenic activity by direct application to the chick's comb. This steroid has approximately 6% of the activity of androsterone. Δ^4-Pregnenetriol-17(α),20(β), 21-one-3-diacetate-20,21, and Δ^4-pregnenediol-17(α),21-dione-3,20-acetate-21 (compound "S" acetate, Reichstein) inhibited the growth of the comb probably by direct antagonism of endogenous androgens. 17(α)-Hydroxyprogesterone produced no consistent effect. By direct comb application 80 and 640 μg levels were without effect while the 320 μg dose produced a 29% comb ratio increment which was statistically significant. When this steroid was injected subcutaneously at a dose of 1000 μg a significant comb inhibition was observed.

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Complement-fixing and Neutralizing Antibody Studies on Humans Vaccinated Against Rabies. (17636)

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Many workers have reported that neutralizing antibodies develop in the serum of animals and of human beings treated with live or killed rabies vaccines(1-5). It also has been shown that complement-fixing antibodies develop to a high titer in rabbits, mice, guinea pigs and dogs hyperimmunized by repeated injections of living virus(6). The somewhat meager evidence to date indicates that the two antibodies are not identical. Thus Habel(7) found that monkeys developing rabies showed no complement-fixing antibodies although virus neutralizing antibodies were present. Similarly, in experiments carried out in guinea pigs, Nachtigal(8) and Bernkopf and Nachtigal(9) showed that complement-fixing antibodies reached a maximal titer about a week after the last injection, diminished to one-eighth the maximal titer after one month, and disappeared completely within three months. On the other hand, neutralizing antibodies reached maximal titers after one month and showed no decrease in titer during the subsequent three months of observation.

This communication reports on the results obtained in determining to what extent neutralizing and complement-fixing antibodies are induced in human beings vaccinated with the Semple-type, phenol-killed rabies vaccine(1).

Materials and Methods. Vaccinations. All vaccinations were made with the Semple-type,

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