and May), no natural challenge of disease occurred. This was unfortunate since one group of serologically-susceptible patients had been prepared for this natural challenge by stimulation of demonstrable antibodies. Furthermore, it is possible that the group of serologically-converted patients in whom antibodies could no longer be detected might not have exhibited clinical signs of illness when exposed to virulent measles virus, but may have reacted with the production of antibodies. In contrast to the experience in the community at large, the institution where this study was carried out was free of measles and no cases were clinically recognized. It is possible that the non-occurrence of measles actually reflects the immunity of the population as suggested by the survey and by the program of vaccination.

Conclusions. A survey of 302 patients from a total population of approximately 2600 patients indicated serologic susceptibility of 20 (7%) as defined by an absence of measles virus neutralizing antibodies. A chemically inactivated (ethylene oxide) measles virus vaccine was administered according to an arbitrary vaccination schedule (1.0 ml subcutaneously at intervals of 4 weeks for a total of 3 injections) and, in 10 of 12 serologically-susceptible patients the formation of measles antibodies was stimulated (serological conversion was accomplished). Patients who had detectable antibodies prior to vaccination showed sharp elevation of titers in response to a single injection of vaccine. In all patients tested, antibody titers promptly declined when the antigenic stimulus was not repeated. However, revaccination of the originally serologically-susceptible individuals resulted in significant increases in antibody titers. Since no natural challenge in the form of measles occurred in the population during the period of study, there was no opportunity to evaluate the *protective* value of these antibody demonstrations.

Addendum. Since this manuscript was prepared, studies concerning successful vaccination with another inactivated-measles virus-vaccine preparation have been reported. (Feldman, H. A., *et al.*, and Winkelstein, W., *et al.*, *J.A.M.A.*, Feb. 10, 1962, v179).

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Comparative Thymolytic Activities of 11β-Hydroxy and 11-Keto Adrenocortical Steroids. (27453)

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Although cortisone and hydrocortisone exhibit similar anti-inflammatory activity, the former has never been shown to be effective *per se*. There is evidence suggesting cortisone is biologically inert and that transformation to the 11β-hydroxy analogue is obligatory for

TABLE I. Thymus Involution Dose Response Data.*

Substituent (s)	Hydrocortisone (F) Total dose (μg/rat)	Hydrocortisone (F) Thymus wt (mg/100 g final body wt)	Hydrocortisone (F) Potency† (95% confidence limits)	Cortisone (E) Total dose (μg/rat)	Cortisone (E) Thymus wt (mg/100 g final body wt)	Cortisone (E) Potency† (95% confidence limits)
	300	271 ± 10 (51)‡	1	300	268 ± 16 (12)	1.0 (.9– 1.3)
	600	224 ± 10 (52)		600	170 ± 17 (12)	
	900	155 ± 7 (52)		1200	129 ± 12 (12)	
	1200	139 ± 6 (52)		1800	95 ± 6 (12)	
16α-hydroxy	1200	237 ± 22 (16)	.3 (.2– .4)		No response at levels up to 100 mg	
	2400	218 ± 27 (16)				
	4800	156 ± 16 (15)				
16α, 17α-isopropylidenedioxy	100	245 ± 12 (16)	2.1 (1.4– 3.1)		*Idem*	
	200	182 ± 11 (16)				
	400	162 ± 16 (16)				
	800	126 ± 5 (16)				
Δ¹, 9α-fluoro	25	247 ± 17 (8)	12.0 (9.4–15.4)	12.5	281 ± 20 (8)	19.0 (14.8–24.4)
	50	153 ± 10 (8)		25	207 ± 22 (8)	
	100	133 ± 10 (8)		50	136 ± 20 (8)	
	200	85 ± 3 (8)		100	111 ± 6 (7)	
Δ¹, 9α-fluoro, 16α-hydroxy	50	289 ± 8 (24)	3.4 (2.4– 5.1)	100	265 ± 22 (12)	1.8 (1.3– 2.4)
	100	237 ± 7 (24)		200	214 ± 30 (12)	
	200	171 ± 8 (24)		400	178 ± 15 (12)	
	400	111 ± 4 (24)		800	150 ± 28 (11)	
Δ¹, 9α-fluoro, 16α, 17α-isopropylidenedioxy	12.5	239 ± 11 (28)	26.5 (22.1–31.8)	100	271 ± 21 (11)	2.0 (1.6– 2.5)
	25	189 ± 7 (28)		200	213 ± 15 (11)	
	50	150 ± 9 (28)		400	163 ± 12 (11)	
	100	102 ± 6 (28)		800	117 ± 8 (11)	

Control, 320 ± 11 (52).

* Pooled data minimum of 2 replications for each steroid. † All potencies relative to hydrocortisone.
‡ Mean ± stand. error (number rats).

glucocorticoid activity. It has been demonstrated that conversion of 2α-methylcortisone to the corresponding 11β-hydroxy analogue is blocked by the presence of the 2α-methyl substituent(1,2). 2α-Methylhydrocortisone is biologically active while the 11-keto analogue is without effect(3). Addition of either 12α-bromo or 12α-chloro substituents to 11-keto progesterone reduced glucocorticoid activity (4). This was rationalized as being due to the inhibitory action of the bromo or chloro substituents on the enzymatic reduction of the 11-keto group.

In the present study, attention was directed to the effects of 16α-hydroxyl and 16α, 17α-isopropylidenedioxy groups on the comparative thymolytic activities of hydrocortisone, cortisone and their analogues.

Materials and methods. Intact immature female rats (Sherman strain, 40-60 g) were given a single subcutaneous injection of steroid suspended in 0.2 ml of carboxymethylcellulose vehicle(5), with benzyl alcohol excluded. Control animals received vehicle alone. The use of 6 dose levels (4 rats per level) permitted elimination of responses not on the linear portion of the curve and left a sufficient number of points to provide a least squares estimate of the line equation. Forty-eight hours after injection the rats were autopsied and thymus and body weights determined. Results were expressed as thymus weight (mg) per 100 g final body weight. By utilizing the aforementioned procedure a significant involution of the thymus gland was obtained with 300 μg of hydrocortisone.

Results. The data in Table I show that the thymolytic activity of cortisone is equivalent to hydrocortisone. 16α-Hydroxylation of hydrocortisone decreased activity to one-third that of the nonhydroxylated parent steroid; however, both the 16α-hydroxy and the 16α, 17α-isopropylidenedioxy analogues of cortisone were inactive at doses up to 100 mg. The latter steroids failed to induce a significant decrease in the relative thymus weight at 333 times the minimum effective dose of hydrocortisone. In contrast, formation of the

16α, 17α-isopropylidenedioxy derivative of hydrocortisone doubled the thymolytic activity.

Hydroxylation of the 16α position of Δ^1, 9α-fluorohydrocortisone reduced activity 72%, whereas a similar substitution on Δ^1, 9α-fluorocortisone reduced activity 90%. The difference in potency between Δ^1, 9α-fluorohydrocortisone and Δ^1, 9α-fluorocortisone is not statistically significant. Though ketalization of Δ^1, 16α-hydroxy-9α-fluorohydrocortisone increased activity, ketalization of Δ^1, 16α-hydroxy-9α-fluorocortisone did not enhance thymolytic activity, but resulted in a potency comparable to that elicited by Δ^1, 16α-hydroxy-9α-fluorocortisone. Whereas 16α-hydroxylation and 16α, 17α-ketalization reduced thymolytic activity of cortisone to an unmeasurable level, similar substitutions of Δ^1, 9α-fluorocortisone lowered activity only 90%.

Discussion. From these data we could hypothesize that 16α-hydroxylation or 16α, 17α-ketalization affects thymolytic activity by blocking the conversion of the steroid 11-keto to 11-hydroxyl configuration. However, if these substituents are effectively inhibiting this conversion, it is apparent that the degree of inhibition can be influenced by additional steroid substitutions, *e.g.*, 1, 2 unsaturation and 9α-fluorination.

Until recently, 11-oxygenation of the steroid nucleus has been regarded as a prerequisite for glucocorticoid activity, Robinson *et al.*(6) have reported that 9α, 11β-dichloro-1, 4-pregnadiene, 17α, 21-diol-3, 20-dione is 8.5 times more active than prednisolone 21-acetate in the granuloma pouch assay. Moreover, Bianchi *et al.*(7) have shown 6α-methyl-pregna-1, 4-diene-3, 20-dione, 16α, 17α-isopropylidenedioxy to be a potent anti-inflammatory agent which approaches prednisolone in activity. Thus, it is possible that steroids lacking the 11-oxygen function may display anti-inflammatory activity; however, for those steroids possessing such a function, biological activity is contingent on the availability of the 11β-hydroxylated configuration.

Potentiation of glucocorticoid activity by 16α, 17α-acetonide formation is well documented(8,9,10). The data presented herein suggest that potentiation requires the 11β-hydroxyl configuration of the steroid nucleus.

Summary. Evidence is presented to show that 16α-hydroxylation or 16α, 17α-ketalization of cortisone produces a profound loss in thymolytic activity. In contrast to the 11-hydroxylated analogue, formation of the 16α, 17α-acetonide of Δ^1, 16α-hydroxy-9α-fluorocortisone does not potentiate biological activity.

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