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Adrenal Corticoid Activities of Δ^1, 9α fluoro-hydrocortisone 16α, 17α isopropylidenedioxy.*† (25341)

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Important chemical transformations which augmented the mineralo- and/or glucocorticoid actions of hydrocortisone include: halogenation at C-6(1), C-9(2) and C-12(3); introduction of a double bond between C-1 and C-2(4); and methylation at C-2(5), C-6 (6) and C-16(7). Bernstein and associates (8-12) dissociated the undesirable sodium-retentive activity of certain potent glucocorticoids by inclusion of 16α hydroxyl group. Fried, *et al.*(13) recently reported that 16α, 17α diol groups of Δ^1, 16α hydroxy, 9α fluoro-hydrocortisone reacted with various aldehydes and ketones to form cyclic acetals and ketals, which exhibit greater glucocorticoid activity than the parent steroid. This report details the biological activities of Δ^1, 9α fluoro-hydrocortisone, 16α, 17α isopropylidenedioxy (triamcinolone acetonide).

Materials and methods. A 3-day bioassay was employed in which both liver glycogen deposition and thymus involution were determined in each of a group of adrenalectomized immature male rats. Urine volume and electrolyte excretion also were measured. Immature male rats of Sherman strain, weighing 40-60 g, were bilaterally adrenalectomized. Animals were maintained with Purina Lab. Chow and 1% NaCl drinking fluid *ad lib.* Twenty-four hours after adrenalectomy the rats were injected subcutaneously or given by gavage graded doses of steroid suspended in 0.2 ml of a modified carboxymethylcellulose vehicle (14). Control animals were injected with vehicle only. One-half hour after administration of the steroid the rats were injected intraperitoneally with 3 ml of 0.9% saline and 3 rats placed in each metabolism cage without food or water. Five hours later urine volume was measured and the cages rinsed with distilled water. The animals were then removed from the metabolism cage and returned to holding racks containing the stock diet and saline drinking fluid. Total milliequivalents of sodium and potassium in urine samples were determined by flame photometry. Steroid was administered daily for the next 2 days. The

* The generic name of this steroid is triamcinolone acetonide. It is currently being marketed as a topical anti-inflammatory agent under trademarks Aristocort® Triamcinolone Acetonide Cream (Am. Cyanamid Co.) and Kenalog® (E. R. Squibb & Sons).

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rats were fasted 15 hours prior to and 7 hours after the last injection to insure maximum depletion of liver glycogen. Animals were then anesthetized with sodium pentobarbital and the livers quickly excised and hydrolyzed in 30% potassium hydroxide(15). Thymi were removed and weighed on Roller-Smith Torsion balance. Liver glycogen was measured by the anthrone method of Seifter, *et al.*(16). Data were statistically analyzed by fitting straight lines by the method of least squares and computing the corresponding equation. Life-maintenance and growth of adrenalectomized, immature male rats were studied by a method previously described(4). However, since analyses of data indicated that survival time of adrenalectomized rats treated with corticosteroids did not follow a normal distribution, results were plotted on *log*-probability paper and expressed as median survival time.

Results. Triamcinolone acetonide and triamcinolone were each compared with hydrocortisone for liver glycogen deposition and thymolytic activities by both subcutaneous and oral routes (Table I). Triamcinolone acetonide was 21 times as potent as hydrocortisone subcutaneously and 167 times hydrocortisone orally (Table II). Subcutaneously administered triamcinolone was 6 times more potent than hydrocortisone and 42 times hydrocortisone orally. These values for triamcinolone are somewhat lower than previously reported(14); however, this apparent disparity is presumably due to differences in assay procedures.

Table I depicts comparative activities of triamcinolone acetonide and triamcinolone on thymus involution. Triamcinolone acetonide was 29 times more potent than hydrocortisone subcutaneously, and 79 times hydrocortisone orally (Table II). Relative potencies of triamcinolone to hydrocortisone on subcutaneous and oral assays were calculated to be 4 and 5, respectively.

Triamcinolone acetonide and triamcinolone exhibited marked ability to promote sodium, potassium and water excretion when administered subcutaneously (Fig. 1). Similar results were obtained when the steroid was given orally. Potassium and water excretion appeared to have increased over control levels to a greater degree than did sodium excretion.

TABLE I. Oral and Subcutaneous Liver Glycogen Deposition and Thymus Involution Activity of Hydrocortisone, Triamcinolone and Triamcinolone Acetonide.

Compound	Dose (μg/rat)	Liver glycogen* (mg/g liver)	Thymus wt* (mg/100 g body wt)
		Subcut.	
Control		2.03 ± .12†	407 ± 18†
Hydrocortisone	200	7.37 ± .82	302 ± 21
	400	12.82 ± .88	215 ± 16
	700	26.83 ± 2.78	124 ± 7
	1000	54.60 ± 4.23	83 ± 5
	1300	57.80 ± 2.46	75 ± 8
Triamcinolone	25	6.52 ± .81	341 ± 15
	50	11.80 ± 1.05	284 ± 11
	100	35.07 ± 5.63	184 ± 13
	200	68.12 ± 3.04	94 ± 4
	400	77.60 ± 4.19	80 ± 3
Triamcinolone acetonide	6.25	8.41 ± 3.20	221 ± 22
	12.5	10.20 ± 1.20	179 ± 13
	25	16.48 ± 2.22	131 ± 9
	50	45.42 ± 1.85	79 ± 5
	100	69.51 ± 5.25	68 ± 5
		Oral	
Control		2.10 ± .20	390 ± 21
Hydrocortisone	500	4.24 ± 1.11	274 ± 18
	1000	6.52 ± .86	211 ± 21
	2000	16.06 ± 1.86	137 ± 15
	4000	21.77 ± 6.97	123 ± 8
	8000	28.51 ± 4.22	88 ± 4
Triamcinolone	100	15.18 ± 1.87	296 ± 20
	200	26.03 ± 3.75	226 ± 23
	400	40.27 ± 4.43	146 ± 16
	800		110 ± 7
Triamcinolone acetonide	12.5	15.39 ± 1.82	207 ± 20
	25	24.90 ± 3.72	156 ± 15
	50	29.36 ± 3.72	106 ± 6
	100	36.72 ± 5.96	80 ± 3
	200	52.17 ± 5.28	68 ± 6

* 10-18 rats/dose. † Mean ± stand. error.

A graphic representation of life-maintenance data is shown in Fig. 2. Doses of steroids resulting in median survival time of 10 days were as follows: desoxycorticosterone, 187 μg (95% confidence limits 129 and 271); triamcinolone, 19.9 μg (14.5 and 27.3); and triamcinolone acetonide, 1.4 μg (1.0 and 1.9). The potencies of triamcinolone and triamcinolone acetonide relative to desoxycorticosterone were 9 and 132, respectively (Table II).

Discussion. The reaction of triamcinolone and acetone to form the cyclic derivative, triamcinolone acetonide, results in a steroid possessing greater glucocorticoid activity than

TABLE II. Comparative Adrenocorticoid Activities.

Steroid	Route of admin.	Relative potencies: Glycogen deposition (× hydrocortisone)	Relative potencies: Thymus involution (× hydrocortisone)	Life maintenance (× DOC)
Hydrocortisone	Subcut.	1	1	
Desoxycorticosterone				1
Triamcinolone		6 (5 & 7)*	4 (3 & 4)	9 (5 & 19)
" acetonide		21 (18 & 24)	29 (25 & 34)	132 (71 & 244)
Hydrocortisone	Oral	1	1	
Triamcinolone		42 (29 & 61)	5 (3 & 6)	
" acetonide		167 (114 & 244)	79 (56 & 112)	

* 95% confidence limits.

the parent compound. Compared to hydrocortisone by subcutaneous liver glycogen assay method the following activities were determined: triamcinolone, 6 (95% confidence limits 5 and 7) and triamcinolone acetonide, 21 (18 and 24). Thymolytic activities were:

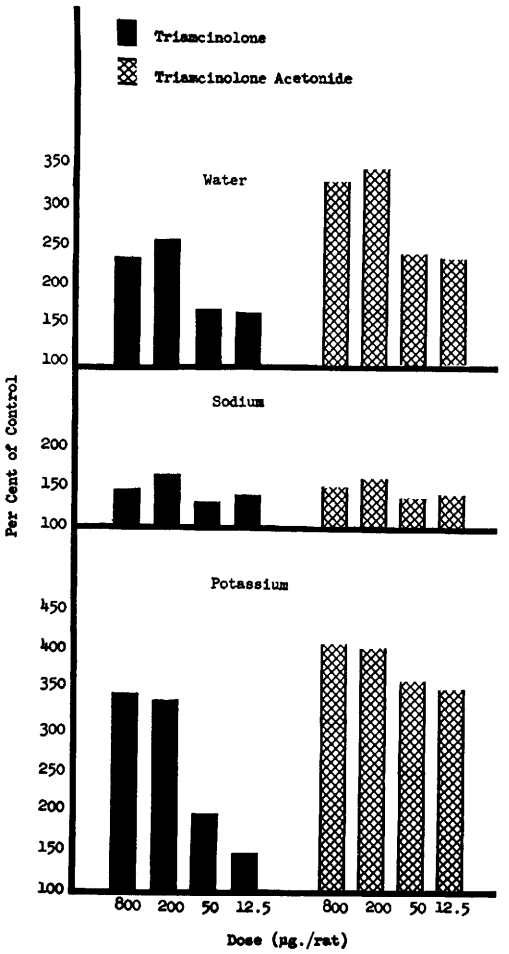

FIG. 1. Diuretic, natriuretic and kaliuretic activities of triamcinolone and triamcinolone acetonide.

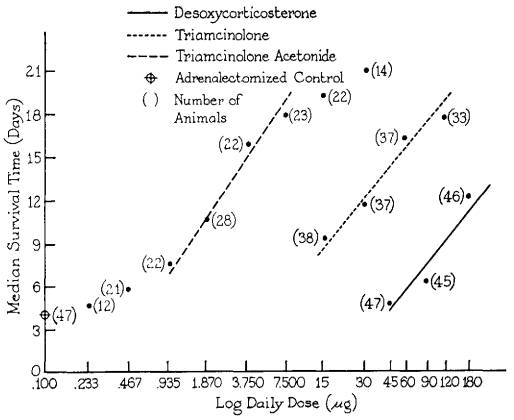

FIG. 2. Life-maintenance activities of desoxycorticosterone, triamcinolone and triamcinolone acetonide.

triamcinolone 4 (3 and 4) and triamcinolone acetonide 29 (25 and 34). Oral administration of triamcinolone and the cyclic derivative resulted in a further enhancement of the parenteral liver glycogen deposition activity. Table II shows that there was no increase in thymolytic activity of triamcinolone when given orally, while triamcinolone acetonide showed a marked increase. At present, there is no rationale for this observation.

Though in animal assays, orally administered triamcinolone acetonide is appreciably more active than the parent steroid, clinical investigations have shown that triamcinolone acetonide, given orally to patients with diseases that ordinarily respond to steroid therapy, was equivalent in activity, on a weight basis, to triamcinolone free alcohol (Lederle Labs., personal communication). Nevertheless, dermatological studies have shown that 0.1% triamcinolone acetonide cream was more efficacious than 1% hydrocortisone

cream(17). These clinical results further emphasize the disparity which may occur between assessments of corticosteroid activity in human subjects and laboratory animals.

Although the ketal analogue of triamcinolone possesses greater glucocorticoid activity than the parent steroid, effects on water, sodium and potassium diureses are comparable. The diuretic and natriuretic potencies of triamcinolone acetonide have been corroborated using normal intact rats and dogs (Cummings, J. J., personal communication).

Survival of adrenalectomized immature male rats following multiple injections of steroids indicates that triamcinolone is 9 (95% confidence limits 5 and 19) and triamcinolone acetonide is 132 (71 and 244) times more active than desoxycorticosterone. Triamcinolone acetonide is 14 (9 and 23) times more effective than triamcinolone. Desoxycorticosterone proved superior to either triamcinolone or triamcinolone acetonide in promoting body weight gain. These results are similar to those previously reported for desoxycorticosterone and triamcinolone(14).

Summary. Glucocorticoid properties of subcutaneously and orally administered triamcinolone acetonide were assayed in immature adrenalectomized rats by liver glycogen deposition and thymus involution. Subcutaneously, triamcinolone acetonide is about 3 times as potent as triamcinolone on liver glycogen assay and 7 times triamcinolone on the thymolytic assay. Oral activities of triamcinolone acetonide also were greater than those of the parent steroid. Triamcinolone acetonide was capable of inducing a diuretic, natriuretic and kaliuretic response in adrenalectomized rats. Triamcinolone acetonide was more active than either desoxycorticosterone or triamcinolone for maintaining the life of immature, adrenalectomized male rats.

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