to an increase in resistance of platelets or to some chemical alteration of thromboplastin.

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Adrenal Corticoid Activities of 2-Methylhydrocortisone Acetate and 2-Methyl-9α-Fluorohydrocortisone Acetate. (22171)

WILLIAM W. BYRNES, LESTER E. BARNES, BARBARA J. BOWMAN, WILLIAM E. DULIN, ERVING H. MORLEY, AND ROBERT O. STAFFORD.

Department of Endocrinology, The Upjohn Co., Kalamazoo, Mich.

Biological activity of hydrocortisone is markedly increased by halogenation of C-9 (1-5) and by dehydrogenation at positions C-1 and C-2(6,7). When both modifications are present in the molecule, glucocorticoid, but not mineralocorticoid, activity of the steroid is further potentiated(8-10). This paper reports the results from biological testing of 2 new analogues of hydrocortisone: 2-methylhydrocortisone and 2-methyl-9 α-fluorohydrocortisone. These compounds have greatly enhanced gluco- and mineralocorticoid activities, as determined by subcutaneous administration on four different assays.

Materials and methods. Steroids. Steroid suspensions were made by grinding the compounds with carboxymethyl cellulose vehicle (8) in a ground glass tissue homogenizer. Compounds used were: 11 β, 17 α, 21-trihydroxy-4-pregnene-3,20-dione(F), 21-hydroxy-4-pregnene-3,20-dione, 21 acetate (DOCA), 9α-fluoro-11 β, 17α, 21-trihydroxy-4-pregnene-3,20-dione, 21-acetate (FF ac), 2-methyl-11 β, 17 α, 21-trihydroxy-4-pregnene-3,20-dione, 21-acetate (methyl F ac), and 2-methyl-9 α-fluoro-11 β, 17α, 21-trihydroxy-4-pregnene-3,20-dione, 21-acetate (methyl FF ac). The latter 2 new steroids were recently reported by Hogg, Lincoln, Jackson and Schneider(11). *Anti-Inflammatory Test.* This test measures the effect of steroids on granuloma formation in the rat by the cotton pellet assay described by Meier, Schuler and Desaulles(12) as modified by Dulin(9).

TABLE I. Anti-Inflammatory Assays Comparing Methylhydrocortisone Acetate, Methylfluorohydrocortisone Acetate, Hydrocortisone, and Hydrocortisone Acetate.

Compound*	Dose (mg/day)	No. rats	Mean granuloma dry wt (mg)	Potency ratio†
Vehicle	—	10	15	—
HA	.3	10	13.9	MA is
	.9	9	11.9	6.4 × HA
MA	.2	10	10.6	(3.4-10.7)
	.6	10	9.4	
Vehicle	—	8	14.2	
H	.3	8	11.7	MA is
	.9	8	8.4	4.5 × H
MA	.1	8	11.0	(2.6-7.2)
	.3	8	7.1	
Vehicle	—	10	15.3	
H	.2	9	10.6	MFA is
	1.0	8	6.3	9 × H
MFA	.02	9	9.4	(7.1-11.4)
	.1	8	7.3	

* HA = Hydrocortisone acetate; MA = Methylhydrocortisone acetate; H = Hydrocortisone; and MFA = Methylfluorohydrocortisone acetate.

† Figures in parentheses indicate limits of potency ratio at P = .95.

TABLE II. Comparisons of Methylhydrocortisone Acetate with Fluorohydrocortisone Acetate by the Liver Glycogen Technic.

Assay	Potency ratio* (methylhydrocortisone acetate/fluorohydrocortisone acetate)
1	.63 (.56– .71)
2	.97 (.80–1.17)
3	.85 (.65– .96)
4	.48 (.44– .54)
5	1.16 (.97–1.39)
Mean	.82

* Figures in parentheses indicate limits of potency ratio at P = .95.

Glycogen Deposition Test. The technic used was a modification(8) of the method of Pabst, Sheppard and Kuizenga(13). *Muscle Work Assay.* This assay was essentially that of Ingle(14,15) except that in one experiment electronic counters were substituted for mechanical work recorders. The steroids were injected in equal amounts twice in a 24-hour period. At the end of 24 hours, the experiment was terminated even though in some cases the rats were alive and still working. The work performed is reported in terms of revolutions of the counters. *Sodium Retention Test.* This test(8), a modification of the method of Marcus, Romanoff, and Pincus (16), measures the effect of compounds on sodium excretion in the adrenalectomized, salt- and water-loaded rat within four hours after administration. Since F is almost inactive on this assay(8), the reference standard was DOCA. Data obtained from these assays were pooled and submitted to an analysis of potency ratios with respect to DOCA. Potency ratios in this and the preceding assays were calculated by the method of Irwin(17).

Results. Table I presents data from anti-inflammatory assays comparing methyl F ac with F and F ac, and methyl FF ac with F. The potency of methyl F ac is calculated at 6.4 x F ac and 4.5 x F, while methyl FF ac is approximately 9 x F. By these same assay procedures, FF ac has been reported as being 7 x F(9).

Potency ratios of methyl F ac to FF ac in the glycogen deposition assay are given in Table II. The value of .82 represents the mean of 5 quantitative assays. Since FF ac has been found to be 13 x F by this assay(8), the potency of methyl F ac can be calculated to be approximately 10 x F. In Table III are presented data which indicate that methyl FF ac is 38 x as potent as F on this test.

In the muscle work test, methyl F ac was 6-7 x as potent as F (Table IV). Between this assay and the evaluation of methyl FF ac, electronic counters were substituted for mechanical calculators on the Ingle Fatigue Apparatus and, therefore, the values obtained on the latter assay cannot be directly compared with those presented for methyl F ac. The data obtained from comparing methyl

TABLE III. Glycogen Deposition Activity of Methylfluorohydrocortisone Acetate.*

Compound	Dose (μg)	% liver glycogen
Hydrocortisone	300†	.5
	600	1.2
	900†	1.7
Methylfluorohydrocortisone acetate	5	.6
	10	.8
	15	1.3
	30	1.6
	45	1.9
	50	1.8
	90	2.4
	150	2.5

* Pooled data from 3 experiments.

† 10 rats/dose. All other values are means of 5 rat dosage groups. Calculated potency ratio is 38.0 × hydrocortisone. Limits of error are 97-104%.

TABLE IV. Comparative Activity of Methylhydrocortisone Acetate, Methylfluorohydrocortisone Acetate, and Hydrocortisone on the Muscle Work Assay.

Treatment	Dose	Revolutions*	No. rats
Untreated controls	—	8244	34
Hydrocortisone	1 mg	20050	13
Methylhydrocortisone acetate	125 μg	18630	16
	150	23280	14
	250	26500	4
	500	29680	4
Untreated controls	—	3895	10
Hydrocortisone	150 μg	6624	14
Methylfluorohydrocortisone acetate	10 μg	6247	13
	15	7921	10
	50	10427	6

* Mechanical counters were used in assaying methylhydrocortisone acetate, and electronic counters for methylfluorohydrocortisone acetate.

TABLE V. Sodium Retention Activity of Methylhydrocortisone Acetate and Methylfluorohydrocortisone Acetate as Compared with Desoxycorticosterone Acetate.

Compound	Dose (μg)	No. rats	Sodium excretion (% of control)	Potency* (× DOCA)
DOCA	1	24	69	1.0
	2.5	6	59	
	5	12	48	
	10	126	48	
	20	12	35	
	40	12	27	
Methylhydrocortisone acetate	.44	6	90	2.6
	.88	6	68	(2.16–3.10)
	1.0	12	68	
	1.5	6	78	
	1.75	6	57	
	2.0	12	52	
	3.0	6	42	
	3.5	6	46	
	4.0	12	41	
	6.0	6	34	
Methylfluorohydrocortisone acetate	.001	6	87	90.0
	.01	18	79	(68–123)
	.1	24	41	
	1	18	24	
	10	6	24	

* Figures in parentheses indicate limits of error at $P = .95$.

FF ac with F on this assay are also presented in Table IV and indicate that this steroid has between 10 and 15 x the potency of F.

Comparison of potencies of methyl F ac and of DOCA by the sodium retention assay was difficult. Variation in results with methyl F ac made it necessary to conduct 5 multi-dose assays of this steroid. These data are presented in Table V. Methyl F ac is calculated at about 2.6 x DOCA. This steroid, therefore, has less mineralocorticoid activity than FF ac, which has been found to be 5 x DOCA on this assay(8). Data from assays of sodium retention activity of methyl FF ac are also presented in Table V and reveal the remarkable potency of this compound. Statistical analysis of the data shows methyl FF ac to have approximately 90 x the sodium-retaining potency of DOCA.

Discussion. Methylation of F at C-2 increases the mineralocorticoid and anti-inflammatory properties of this hormone to a lesser extent than fluorination at C-9, but these two activities are in about the same ratio in methyl F ac and FF ac. This is in contrast with the Δ^1 analogue of F in which the anti-inflammatory potency is increased out of proportion to the electrolyte activity(9).

The glycogen deposition activity of FF ac is 13 x F and that of 11 β, 17 α, 21-trihydroxy-1, 4-pregnadiene-3,20-dione, 21-acetate (Δ^1F ac) is 3 x F; both changes in the molecule (Δ^1FF ac) resulted in this activity being 49 x F(8). The anti-inflammatory effect of

TABLE VI. Comparative Corticoid Activities of Methylhydrocortisone, Fluorohydrocortisone and Methylfluorohydrocortisone Acetates.

Assays	Steroids assayed: Methylhydrocortisone acetate	Fluorohydrocortisone acetate	Methylfluorohydrocortisone acetate
Anti-inflammatory	4.5 × hydrocortisone	7 × hydrocortisone*	9 × hydrocortisone
Glycogen deposition	10 "	13 " †	38 "
Muscle work	6-7 "	not determined	10-15 "
Sodium retention	2.6 × DOCA	5 × DOCA †	90 × DOCA

* Dulin(9). † Stafford *et al.*(8).

FF ac is 7 x F and that of Δ^1F ac is 3 x F; the Δ^1-9 α-fluoro analogue has 14 x the potency of F(9).

In these experiments, a methyl group at C-2 and fluorine at C-9 in the hydrocortisone molecule did not cause as great a potentiation of glucocorticoid properties as both Δ^1 and fluorine in the same steroid nucleus. Regarding mineralocorticoid potency, however, quite the opposite is the case. Δ^1F, like F, is almost inactive on the sodium retention assay used to evaluate these corticoids, and the Δ^1-9 α-fluoro analogue of F has no more mineralocorticoid activity than FF ac(8). Whereas substitution of fluorine for hydrogen at C-9 and methyl for hydrogen at C-2 each causes a great increase in sodium retention activity of F, the tremendous mineralocorticoid potency of methyl FF ac is considerably greater than would be expected on the basis of the electrolyte activity of methyl F ac or FF ac.

Summary. (1) Two new analogues of hydrocortisone, 2-methylhydrocortisone acetate and 2-methyl-9 α-fluorohydrocortisone acetate, have been tested for corticoid activity on the following assays: anti-inflammatory, glycogen deposition, muscle work, and sodium retention. A summary of the data is presented in Table VI. (2) The sodium-retaining activity of 2-methyl-9 α-fluorohydrocortisone acetate is 90 x desoxycorticosterone acetate and is, therefore, one of the most potent mineralocorticoids.

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Effect of Ultraviolet Radiation on Iron-binding Capacity of Plasma. (22172)

ARTHUR L. SCHADE AND JIRO OYAMA.

Laboratory of Infectious Diseases, U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Microbiological Institute, Bethesda, Md.

In applying our recently developed methods for the determination of plasma iron(1) to ultraviolet-irradiated plasmas (pooled blood-bank ACD plasmas, "UVR-sterilized"), we found significantly lower values for the iron-binding capacity of these plasmas compared with non-irradiated samples. The present study was initiated to investigate this finding and to determine the extent to which siderophilin, the protein responsible for the specific iron-binding capacity of plasma(2,3) was affected by UVR.

Materials and methods. Plasmas obtained from blood-bank ACD-treated blood samples