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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Received December 2, 1955. P.S.E.B.M., 1956, v91.

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

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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

TABLE I. Alteration of Siderophilin Iron-Binding Capacity with Irradiation.

Test material	UVR time (sec)	TIBC* (μg Fe/ml)	% TIBC remaining	C† $\times 10^2 \times$ sec^{-1}
(a) ACD plasma—a‡ = 67, BI§ = 0.98 μg Fe/ml	0	2.53	100	
	25	2.06	81.5	.82‖
	50	1.57	62.0	.95
	75	1.46	57.7	.73
	100	1.35	53.4	.63
	150	1.31	51.8	.44
(b) Fraction IV-7—a = 10.1, BI = 0.26 μg Fe/ml	0	3.91	100	
	10	3.03	77.5	2.5
	25	2.23	57.0	2.2
	50	1.37	35.0	2.1
	100	.86	22.0	1.5
	150	.84	21.5	1.0
(c) ACD plasma-Fraction IV-7 mixture—a = 56.7, BI = 0.29 μg Fe/ml	0	3.04	100	
	15	2.59	85.2	1.1‖
	25	2.22	73.0	1.3
	50	1.70	55.9	1.2
	100	1.41	46.3	.77

* TIBC = Total iron binding capacity of 1 ml of test solution.

† $C = \frac{2.3}{t} \log \frac{\text{TIBC at } t = o}{\text{TIBC at } t}$.

‡ $a = \frac{2.3 \times \text{optical density}}{\text{Light path, cm}} \times$ dilution. Optical densities of appropriate dilution of test solutions of 1 cm depth were determined at 254 mμ with a Beckman spectrophotometer.

§ BI = Bound iron contained in 1 ml of test solution.

‖ These early values (0-20% siderophilin inactivation) are the least reliable since they are most affected by experimental error involved in determination of TIBC.

(1.84 g dextrose, 1.65 g sodium citrate, 0.6 g citric acid in 75 ml of water added to 500 ml of blood) and concentrated siderophilin, (plasma fraction IV-7, Cohn*), assaying 41% on the basis of its specific iron-binding capacity, were employed in this study. The irradiations were performed with an apparatus† having 2 Westinghouse cold-cathode Sterilamps as the UV light source. Their emission at 2537 A represents approximately 95% of the total UV output. A glass-bottomed, plastic-sided tray (31 x 6 x 1.3 cm) was centered 2.5 cm below the outer surface of the lamps and parallel to their longitudinal axes. The average integrated radiation intensity at this distance was 11.3 $\pm$ 1.1 milliwatts/cm^2 as measured by the uranyl-oxalate actinometric method(4). A 3-ml sample of the test solution was uniformly spread over the tray to a constant depth of 0.016 cm and exposed directly to the UV lamps at room temperature for exposure periods ranging from 10 to 150 seconds. Following the irradiation, each sample was removed from the tray and tested for its total iron-binding capacity (TIBC). The TIBC is a measure of the active siderophilin concentrations in plasma and represents the sum of both the iron-complexed and iron-free siderophilin species as determined by the bound iron (BI) and the unsaturated iron-binding capacity (UIBC) test methods, respectively(1). A decrease in the TIBC was considered a measure of siderophilin inactivation.

Results. Pooled plasma, fraction IV-7, and various plasma fraction IV-7 mixtures were irradiated according to the procedure described and their total iron-binding capacities determined. Table I presents the data obtained from typical experiments. It illustrates the quantitative relation between the amount of UVR received by the test siderophilin con-

* Courtesy of Dr. D. M. Surgenor, Harvard University, Boston, Mass.

† We are indebted to Dr. John T. Tripp of the Laboratory of Biologics Control, NMI, for the use of this apparatus and for generous donations of various plasma samples.

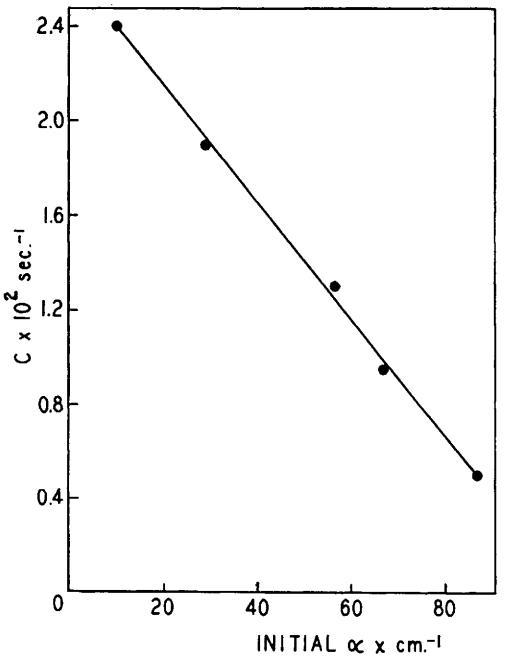

FIG. 1. Relation between rate of inactivation of siderophilin and absorption coefficient of the test solution.

taining solution and the decrease in its TIBC. The C values listed are the first order rate constants calculated on the basis that the effective radiation intensity was constant. It will be observed, however, that the absolute values of C are different for each of the several siderophilin solutions tested and that throughout the period of exposure of any one solution, the C values changed. Hence, these values are not true rate constants.

The magnitude of the C value for a given test solution is dependent upon the absorption coefficient (a) of that solution—the higher the a value the lower the C value. The relationship between C and a is illustrated in Fig. 1 in which the inactivation rates are plotted for solutions containing 2-3 mg siderophilin per ml and exhibiting a range of initial a values from 10.1 to 87. The solutions of intermediate a values were prepared by the appropriate dilutions of ACD plasma with the fraction IV-7 solution. The apparent rate constants were obtained by determining the time required for 50% inactivation ($t_{1/2}$) from a plot of $\log \frac{\text{TIBC at } t = o}{\text{TIBC at } t}$ versus time t (seconds) and substituting the derived $t_{1/2}$ in $C = \frac{0.693}{t_{1/2}}$. It was necessary to extrapolate the linear plot to obtain the $t_{1/2}$ in cases where deviation from linearity occurred before this point was reached. The progressively decreasing rate of inactivation of siderophilin in the test solutions of increasing a value can be ascribed to the enhanced "internal screening" (5) of the effective radiation by the non-siderophilin plasma proteins.

The fact that the value of C did not remain constant during the period of irradiation of any one solution suggested that its initial a value changed during the exposure period. Experiments to elucidate this point showed that the a value of a given test solution did increase linearly for at least the first 50 seconds of exposure, *e.g.*, a plasma sample of initial a value of 67 increased 0.125 a unit/second while a fraction IV-7 solution with an initial a of 10.1 increased 0.11 a unit/second. For longer periods than 50 seconds, additional factors probably played a role in determining the amount of radiation absorbed. One such factor was the progressive formation of a visible surface film of coagulated protein clearly evident after 150 seconds exposure.

Since approximately one-third of the siderophilin present in normal plasma is iron saturated, experiments were performed to determine whether there was any difference in sensitivity to UVR of the iron-saturated and iron-free siderophilin in blood plasma. Table II illustrates the UV inactivation of siderophilin-enriched plasma solutions, 25 and 100% iron-saturated. The inactivation of the iron-saturated sample was measured by determination of the iron dissociated from the complex under conditions comparable to those used for determining the unsaturated iron-binding capacity (UIBC). Subject to the limitations of the methods employed, the inactivation rates of the iron-free and iron-saturated siderophilin species were the same.

Summary. 1. Ultraviolet irradiation of ACD plasma and of plasma fraction IV-7 solutions inactivates the siderophilin component as measured by the loss of its specific iron-binding capacity. 2. The initial inactivation

TABLE II. Effect of UVR on Iron-Saturated and Iron-Unsaturated Plasma Siderophilin.

UVR time (sec)	Iron-saturated—100% TIBC, μg Fe/ml	% TIBC	C × 10² × sec⁻¹	Iron-free— 74.4% Iron-saturated—25.6% TIBC, μg Fe/ml	% TIBC	C × 10² × sec⁻¹
0	3.94	100		3.94	100	
25	2.99	75.9	1.10	2.93	74.5	1.18
50	2.61	66.3	.82	2.76	70	.71
100	2.16	54.8	.60	2.06	52.3	.65
150	1.82	46.2	.52	1.93	49.1	.47

rates, whose magnitude was correlated with the absorption coefficients of the irradiated siderophilin solutions, followed essentially first order kinetics. 3. The UV inactivation rates of iron-free and iron-saturated siderophilin were found to be the same.

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Received December 5, 1955. P.S.E.B.M., 1956, v91.

Effect of New Adrenal Steroids on Electrolyte and Water Excretion. (22173)

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Recent clinical work has indicated that the Δ^1-analogues of hydrocortisone and cortisone, namely prednisolone and prednisone, are in certain respects more potent than the parent compounds and differ from them qualitatively in that they cause little or no sodium and water retention at therapeutically effective doses(Bunim, *et al.,* 1, 2; Spies, *et al.,* 3; and others). Pechet and Bartter(4) found that in Addisonian patients prednisone would cause sodium loss whereas cortisone caused sodium retention. This action was correlated with a marked ability of the Δ^1-compounds to increase the glomerular filtration rate. In animal studies, Perlman and Tolksdorf(5) found the Δ^1-steroids more potent than their natural counterparts in causing liver glycogen deposition, eosinopenia and life-maintenance, but their effects on electrolyte excretion were unchanged. In tests designed particularly to measure sodium-retaining potency, Stafford, *et al.*(6) found prednisolone to have "very slight" activity.

It is well known that adrenal cortical hormones are necessary for the maintenance of a normal rate of water diuresis and that deficiencies in this respect are repaired effectively by hydrocortisone and cortisone, but weakly, if at all, by desoxycorticosterone. We considered it of interest to determine, therefore, the relative activity of hydrocortisone, desoxycorticosterone (DC), prednisone* and prednisolone in this test. Excretion of sodium and potassium was measured also in the water-loaded test animals. Under the experimental conditions used, hydrocortisone and cortisone are known to increase sodium and potassium as well as water excretion.

Methods. Fasted male rats weighing approximately 200 g were tested 18 hours after adrenalectomy. At the beginning of an ex-

* Prednisone (Meticorten) and prednisolone (Meticortelone) were generously supplied to us by Schering Corp.