may have either thromboplastic or antithromboplastic activity; it has been reported that hyaluronic acid has thromboplastic activity (18), whereas sulfonation of hyaluronic acid produces a strong heparin-like anticoagulant(19).

Summary. Most of the radiosulfate in S^{35}-labeled rat blood platelets was recovered in an alkali extract. The extracted material stained with Alcian blue for mucopolysaccharides and was found to behave similarly to chondroitin sulfate when subjected to paper electrophoresis and paper chromatography.

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Effect of 2-Methyl-9 Alpha Fluorohydrocortisone on Internal Distribution of Fluid and Electrolytes of Fasted Adrenalectomized Dogs.* (22884)

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It has been suggested(1-3), that animals lacking adrenal cortical hormones are unable to dilute their blood by mobilizing the water and salt in cells and tissues for transfer to the vascular system to maintain a volume of circulating fluid compatible with life. These early experiments indicated a regulatory influence of the adrenal cortex over the internal distribution of water and certain electrolytes. More recently several investigators(4-8), using DCA, cortisone and ACTH, have obtained convincing evidence for steroid induced water and electrolyte shifts between intra and extracellular compartments. The following experiments in which the potent corticoid 2-methyl-9α-fluorohydrocortisone (2-methyl FF), was employed, seem to afford unequivocal evidence that adrenalectomized dogs, exhibiting severe symptoms of insufficiency, even though deprived of food and water possess sufficient water and salt reserves in their cells and tissues to completely restore normal activity and vigor. However, they are unable to mobilize and redistribute these substances in the absence of adrenal hormones. When potent corticoids are administered i.v., water and electrolytes are withdrawn from tissues and cells in amounts adequate to (1) fully restore the failing circulation; (2) restore the normal electrolyte pattern of the blood; (3) induce renal elimination of the accumulations of K and blood urea nitrogen and (4) reduce the hemocon-

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TABLE I. Effect of 2-Methyl FF on Serum Electrolytes of Fasted Adrenalectomized Dogs during Recovery from Insufficiency.

Dog No.	Day bled	Dose, gamma	BP, mm Hg	Blood urea N, mg %	Hb, g %	Hmct, %	RBC 10^6, mm^3	Blood sugar, mg %	Serum electrolytes Na	Cl	K	*
									mEq/l			
I	1	Initial	100	21.0	13.7	36.6	5.36	86.5	138.0	111.4	4.5	N
	9	400	72	112.0	16.0	37.9	5.76	88.0	130	107.0	8.6	S
	10	1000	82	24.5	13.3	32.7	5.04	86.7				A
	11	0	90	18.0	11.4	29.8	3.10	88.0	148	117.8	3.1	R
II	1	Initial	110	20.5	12.4	32.6	4.50	80.0	144	114.0	4.6	N
	7	400	52	156.0	15.7	44.8	6.02	75.0	131	105.0	7.4	S
	8	1000	88	75.0	15.7	39.5	5.87	85.7				A
	9	0	92	30.0	13.0	39.5	5.18	86.5	144	113.0	3.8	R
III	1	Initial	112	12.0	13.47	38.0	5.34	81.5	142	121.0	4.3	N
	12	1000	62	90.4	20.44	44.2	6.46	72.5	129	103.0	7.8	S
	13	1000	86	96.4	16.23	39.7	6.13	100.0				A
	14	500	120	46.0	14.9	37.8	5.38	91.0	150	116.2	4.4	A
	15	0	120	45.5	13.61	34.3	5.85	96	147	117.4	4.4	R
IV	1	Initial	108	18.0	13.33	37.2	5.17	77.5	140	118.0	4.4	N
	10	1000	58	120.0	20.14	49.7	8.05	60.0	128	94.3	7.0	S
	11	1000	74	67.0	15.78	43.9	7.34	99.0				A
	12	500	90	39.0	14.55	39.3	5.95	102.0	141	105.0	4.2	A
	13	0	102	24.5	13.47	38.5	5.51	104.0	140	111.2	4.2	R
V	1	Initial	110	19.0	12.28	37.5	4.25	76.5	144	114	5.2	N
	8	1000	72	45.0	15.0	42.0	6.94	78.5	129	107.0	7.1	S
	9	1000	91	11.0	13.6	31.3	5.24	90.0				A
	10	0	96	10.5	11.9	30.2	4.54	80.0	136	109.0	2.4	R

* Condition of animal: N = Normal, S = Severe symptoms, A = Active and vigorous, R = Complete recovery.

centration by dilution. These changes occur within 24-60 hours regardless of additional loss of water and salt by ensuing diuresis.

Methods. The 6 dogs used had been adrenalectomized 2-5 years previously and in the interim subjected to repeated cycles of insufficiency and recovery on various types of steroid therapy. Between experiments adequate maintenance was effected by daily i.m. injections of 0.5 mg DCA in oil. The day an experiment started, DCA was withheld and the dogs sampled for study of the blood constituents. These data are included in Table I, designated as initial or control values. No further treatment was given until severe symptoms of adrenal failure became evident 7-12 days later (Table I), at which time arterial pressure and blood samples were again taken. The dogs were immediately given an i.v. injection of 2-methyl FF, in a dosage recorded in Table I, and after voiding urine, placed in metabolism cages without food or water for 48-60 hours. Injections of the steroid were repeated every 12 hours during this period, the total dose varying from 1.4 to 4 mg. The 2-methyl FF was kept as a stock solution in 100% ethanol, diluted shortly before using to 10% with warm distilled water. The total amount of fluid given as a vehicle for the compound was approximately 40 cc, which was counterbalanced by withdrawal of a similar volume of blood for sampling. Final determination of arterial pressure, serum electrolytes and other blood constituents was made at the termination of the experiment. The quantities of Na, Cl and K eliminated in the urine during the recovery period were measured and are recorded in Table II.

Results. The essential data concerning the effect of 2-methyl FF on mobilization and distribution of fluid and electrolytes are shown in Table I. The reaction of all the animals to the steroid was practically identical, the chief differences consisting in variation of the amount of water and salt excreted in the urine during revival from insufficiency. The dogs developed marked symptoms 7-12 days after discontinuing DCA (Table I), with drastic decline in arterial pressure, serum Na and Cl. Serum K rose to 7-8 mEq/l; two animals (1

TABLE II. Effect of 2-Methyl FF on Serum and Urine Electrolytes of Fasted Adrenalectomized Dogs Recovering from Insufficiency.

Dog	Serum electrolyte changes, 0-48 hr, mEq/l			Total urine electrolyte excretion, mEq/48 hr			Urine vol, 0-48 hr, cc
	Na	Cl	K	Na	Cl	K	
1	+19.0	+10.8	-5.5	14.4	33.3	46.8	835
2	+14.0	+ 8.0	-3.7	17.0	25.6	55.3	676
3	+22.0	+13.2	-3.4	5.8	16.2	54.4	534
4	+13.0	+16.9	-2.8	7.0	9.6	62.4	788
5	+ 8.0	+ 2.0	-4.6	7.5	27.5	22.5	310
6*	+ 8.0	+ 7.0	-4.0	19.0	55.0	70.0	575

+ = Increase in serum electrolyte. - = Decrease in serum electrolyte.
* Received 4 mg 2-methyl FF 48 hr.

and 3), exhibited cardiac and muscle difficulties due to hyperpotassemia. For example, Dog 1 was prostrate and unable to use his hind legs when bled. A total i.v. dose of 2-methyl FF varying between 1.4-4 mg. over 36-48 hours in 4-5 doses, restored the serum electrolytes to normal levels; in fact, the serum Na of Dogs 1 and 3 rose considerably above their initial values. Both blood urea nitrogen and serum K declined to low levels following the onset of diuresis. Hemodilution occurred as evidenced by decreases in hemoglobin, hematocrit and erythrocyte count. Blood sugar did not change significantly in Dogs 1 and 2 but did increase in those animals which received the larger doses of steroid. Coincident with restoration of normal values for the blood constituents and steep rise in arterial pressure was a striking disappearance of symptoms and resumption of normal activity. Return of vigor was obvious within a few hours after the first injection. All dogs continued to eliminate fluid and electrolytes during the fast, although the quantities of salt and water excreted showed considerable variation from animal to animal. The amount of urine voided varied from 310 to 835 cc (Table II). Potassium excretion far exceeded that of Na and Cl and the chloride exceeded sodium. Two animals (3 and 4, Table I) were fasted for 60 instead of 48 hours and received 2.5 mg of 2-methyl FF. These 2 dogs raised their arterial pressure to normal or above, whereas dogs given the lesser dosage (1 and 2) did not quite regain their initial level. Dog 6, Table II, received the largest dose of steroid or 4 mg over 48 hours. The quantity of electrolyte excreted in the urine of this fasted animal is noteworthy.

Discussion. The view(9-12), that one locus of action of adrenal cortical hormones upon Na, Cl and water metabolism is the kidney is undoubtedly correct but it is also a fact that internal transfer of these substances can readily be induced by potent steroids. The source of the fluid, Na and Cl that are mobilized is of interest. It has been suggested (5), that sodium stores ordinarily sequestered in certain tissues, *e.g.,* bone and perhaps also connective tissue(7), are made available for exchange when hormone is administered. It seems not improbable however, that all cells and tissues may relinquish a portion of their Na and water under the conditions of these experiments. A small amount of Na may be contributed by residues of unabsorbed food remaining in the alimentary tract at the time the animals were injected though most of the dogs had not ingested food for 16-24 hours before using. The quantity of Na that is redistributed may be large, for as shown in the tables, the serum Na rises to control levels despite (1) continuing loss of the cation and fluid in the urine and (2) obvious hemodilution which should lower serum Na concentration rather than increase it. The quantity of K excreted indicates that this substance was derived from cells in which it had accumulated during insufficiency and from which it was released by adrenal hormones for eventual renal excretion. The rapidity of remission of symptoms and restoration of vigor following injection of 2-methyl FF, coincident with hemodilution and reestablishment of control values for arterial pressure and serum electrolytes in the

absence of exogenous sources of fluid, Na and Cl, supports the view that one of the functions of the adrenal cortex is the regulatory control of the *internal distribution* of water and certain electrolytes between body compartments. The mechanism of action of the cortical steroids in effecting these changes remains an unsolved problem.

Summary. 2-methyl-9α-fluorohydrocortisone will revive adrenalectomized dogs from severe insufficiency and restore them to normal health within 48-60 hours even though they are deprived of food and water during recovery. The steroid fully corrects the distorted fluid and electrolyte equilibria of the blood. The lowered arterial pressure, serum Na and Cl increase to normal levels, the elevated serum K and blood urea nitrogen fall, while accompanying these changes, hemodilution occurs along with a diuresis and further loss of fluid, Na and Cl. When adrenal hormones are lacking, water and salt are not only lost in urine and other excretions but perhaps equally important, are immobilized in cells and tissues. Following administration of potent steroid ample fluid and salt are redistributed to the extracellular and intravascular compartment with complete disappearance of symptoms and return of activity and vigor.

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Adrenal Corticoid Activities of 6-Methyl-Δ^1-Hydrocortisone. (22885)

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Corticoid activity of hydrocortisone is increased by the presence of a halogen at C-9 (1,2), a double bond between C-1 and C-2 (3) and a methyl group at C-2(4,5). Potency of hydrocortisone is further augmented in some instances by the presence of two such modifications in the same molecule as in Δ^1-9-fluorohydrocortisone(6) and 2-methyl-9-fluorohydrocortisone(5). This paper reports biological activity of a new corticoid, 6-methyl-Δ^1-hydrocortisone. This steroid is about 3 times as potent as Δ^1-hydrocortisone in the glycogen deposition assay and twice as potent in the anti-inflammatory assay. It has no measurable sodium or water retention activity —on the contrary, it causes diuresis of sodium and water in the test animal.

Materials and methods. Steroids. Steroid suspensions were made by grinding the compounds with carboxymethylcellulose (CMC) vehicle(6) in a ground glass tissue homogenizer. Compounds used were: hydrocorti-