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-

sone, 11β, 17α, 21-trihydroxy-4-pregnene-3,20-dione (F); Δ^1-hydrocortisone, 11β, 17α, 21 - trihydroxy-1, 4-pregnadiene - 3,20-dione (Δ^1-F); desoxycorticosterone acetate, 21-hydroxy-4-pregnene-3,20-dione, 21-acetate (DOCA); and 6-methyl-Δ^1-hydrocortisone, 11β, 17α, 21-trihydroxy-6-methyl-1, 4-pregnadiene-3,20-dione (6-methyl-Δ^1-F). The latter steroid was recently reported from our laboratories(7).

Animals. Rats from the Upjohn colony (Sprague-Dawley ancestry) were used in these experiments. In the glycogen deposition and sodium retention assays, male rats were adrenalectomized at a body weight of 140-160 g and fed stock laboratory diet (Purina Laboratory Chow) and 1% NaCl as a drinking fluid. In the anti-inflammatory assay, the test animals were intact female rats weighing 150-160 g.

Anti-inflammatory test. The granuloma pouch technic of Selye(8) as modified by Robert* was used to assess anti-inflammatory activity. In this assay, 25 ml of air are injected subcutaneously into rats, followed by 0.5 ml of a sterile 1% solution of croton oil in cottonseed oil. On the second day after formation of the pouch, the air is removed by vacuum; on the third day the pouch is compressed manually to prevent the formation of adhesions; on the fourth day the pouch is opened and the exudative fluid is measured in graduated cylinders. The test measures the ability of steroids to inhibit inflammatory exudation of fluid into the granuloma pouch.

Glycogen deposition test. This assay for glucocorticoid activity is a modification(6) of the method of Pabst, Sheppard and Kuizenga(9).

Sodium retention test. This test(6), based on the method of Marcus, Romanoff, and Pincus(10) measures the effect of compounds on sodium excretion in the adrenalectomized, salt- and water-loaded rat within 4 hours after administration.

Results. Fig. 1 depicts the comparative activities of 6-methyl-Δ^1-F, Δ^1-F and F in inhibiting fluid exudation into the granuloma pouch of rats following subcutaneous injection of the steroids suspended in .2 ml CMC vehicle. These data indicate the potency of 6-methyl-Δ^1-F to be about 6 times F and twice Δ^1-F by this route of administration.

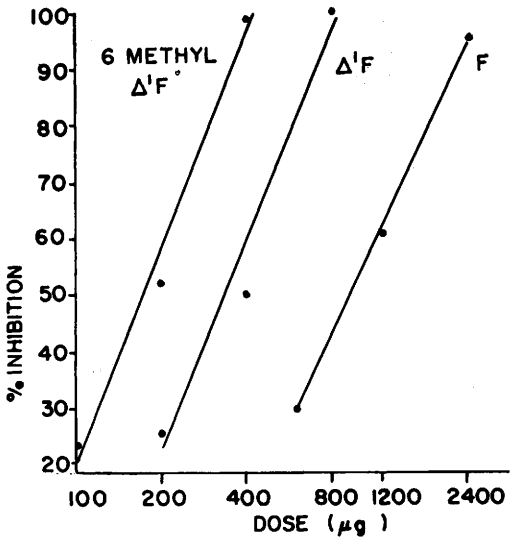

FIG. 1. Effect of F, Δ^1-F and 6-methyl-Δ^1-F in inhibiting fluid exudation in granuloma pouch following subcutaneous administration of steroids. Each point is the mean value for 6 or 7 rats.

6-Methyl-Δ^1-F and Δ^1-F were each compared with F for liver glycogen deposition activity by both subcutaneous and oral routes. 6-Methyl-Δ^1-F was found to be about 10 times as potent as F subcutaneously and 16 times F orally, and Δ^1-F to be 3 times F subcutaneously and more than 5 times F orally (Table I). 6-Methyl-Δ^1-F is therefore cal-

TABLE I. Oral and Subcutaneous Glycogen Deposition Activity of F, Δ^1-F and 6-Methyl-Δ^1-F. Potency ratios calculated according to Irwin(11).

		Liver glycogen deposition activity Potency ratio	
		Δ^1-F	6-Methyl-Δ^1-F
No. rats	Route of admin.	F	F
30	Oral		18.5
30			18.1
45		4.4	11.6
45		6.3	16.4
Mean		5.4	16.2
30	Subcutaneous	2.2	
30		3.6	
30		2.7	
30			10.6
30			8.5
Mean		2.9	9.6

* Personal communication.

TABLE II. Comparison of Effects of 6-Methyl-Δ^1-F and DOCA on Sodium and Water Excretion in Adrenalectomized Salt-Loaded Rats. Six rats were used for each determination.

Compound	Dose (μg)	Urine vol (cc)	Urine sodium (mg)
None		6.1	28.5
DOCA	10	6.9	24.9
	20	5.8	21.5
	40	4.9	16.0
6-Methyl-Δ^1-F	10	8.8	35.6
	50	10.6	35.2
	100	12.0	37.6
	200	11.2	32.2
	500	11.8	33.0
	1000	11.3	30.5

culated at about 3 times the potency of Δ^1-F by either route of administration. Both steroids show enhanced oral:parenteral activity ratios when compared with F. Table II presents data obtained from the subcutaneous injection of 6-methyl-Δ^1-F and DOCA in the sodium retention assay. 6-Methyl-Δ^1-F not only failed to induce sodium and water retention, but actually acted as a diuretic and natriuretic agent in the adrenalectomized rat.

Discussion. Chemical modifications of F frequently alter glucocorticoid, anti-inflammatory and mineralocorticoid activities in an unpredictable manner. Table III is a summary of data obtained in this laboratory from testing 6 analogues of F, each with one or two modifications present in the molecule. It will be noted, for example, that fluorine at C-9 increases sodium retention activity to a far greater degree than it does glucocorticoid potency, while in contrast, a double bond between C-1 and C-2 enhances the latter without affecting electrolyte activity. It is also of interest that whereas 2-methyl-9α-fluorohydrocortisone has only about 70% the glucocorticoid and anti-inflammatory activity of Δ^1-9α-fluorohydrocortisone, it is at least 20 times more potent in retaining sodium.

The electrolyte activity of these analogues is of special interest. Although the analogues of F with fluorine at C-9 or a methyl group at C-2 have greater anti-inflammatory potency than F or its Δ^1 analogue, their remarkable salt retaining activity limits clinical usefulness as therapeutic agents in inflammatory diseases. 6-Methyl-Δ^1-F, on the other hand, has increased glucocorticoid effectiveness as compared with F or Δ^1-F without exhibiting salt retention activity. In fact, the diuresis of sodium and water in rats treated with this steroid deserves comment. Animals receiving 100 μg doses of 6-methyl-Δ^1-F doubled the excretion of urine and increased urinary sodium loss by 30% as compared to untreated rats. Since F fails to cause sodium retention in our test animals contrary to its effect in man, it can be presumed that this assay may exaggerate the salt losing activity of 6-methyl-Δ^1-F. The natriuretic activity of this new analogue of F will be the object of further investigation.

Summary. Comparisons are presented on relative potencies of Δ^1-F and a new analogue, 6-methyl-Δ^1-F, on 3 different assays for corticoid activity: anti-inflammatory (granuloma pouch technic), liver glycogen deposition, and sodium retention. 6-Methyl-Δ^1-F is 2 times as potent as Δ^1-F on the anti-inflammatory test and about 3 times Δ^1-F on

TABLE III. Comparative Corticoid Activities of Analogues of Hydrocortisone.

Steroids	Glycogen deposition (× hydrocortisone)	Anti-inflammatory* (× hydrocortisone)	Sodium retention (× DOCA)
Hydrocortisone	1	1	Very slight activity†
Δ^1-Hydrocortisone	3†	3	*Idem*
6-Methyl-Δ^1-hydrocortisone	10	6	Na and H_2O diuresis
9α-Fluorohydrocortisone acetate	13†	7‡	5†
Δ^1-9α-Fluorohydrocortisone acetate	49†	14‡	5†
2-Methyl-hydrocortisone acetate	10§	5§	3§
2-Methyl-9α-fluorohydrocortisone acetate	38§	9§	90§‖

* Cotton wad technic used except for assay of Δ^1-hydrocortisone and 6-methyl-Δ^1-hydrocortisone. † Stafford *et al.*(6). ‡ Dulin(12). § Byrnes *et al.*(5). ‖ More recent testing in this lab indicates somewhat greater potency.

the glycogen deposition assay. 6-Methyl-Δ^1-F, like Δ^1-F, does not induce sodium and water retention on the assay for electrolyte activity, but in contrast to Δ^1-F, this steroid acts as a diuretic and natriuretic agent.

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Reaction of Splenic Tissue in Culture to *Listeria monocytogenes*. (22886)

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A study of *Listeria monocytogenes* in tissue culture was conducted because of the peculiar behavior and wide host susceptibility in experimental animals(1,2) as well as in natural hosts(3-8). Listeric infections in rabbits cause a monocytosis with a generalized infection while ruminants usually manifest a severe encephalitis. Abortions due to *L. monocytogenes* have been reported in cattle, sheep, goats, swine and rabbits(9,7). In recent years several reports of abortion, and early deaths in infants due to *L. monocytogenes* have come from both sectors of Germany(10-12). The disease has been called *granulomatosis infantiseptica* and is characterized by a granulomatous reaction associated with capillary endothelium(10,13). Gray *et al.*(14) reported that maceration of tissues followed by refrigeration increased the probability of recovery of the organism from infected tissues; other workers have reported similar results. Because of this strange behavior inoculated tissue cultures were studied for this same phenomenon.

Materials and methods. The culture of *L. monocytogenes* used was isolated from the brain of a calf with listeric encephalitis. Previously, Gray *et al.*(16) reported that the concentration of bacteria obtained from a suspension made to match the 0.5 tube of a MacFarland nephelometer was adequate to produce symptoms and lesions typical of *L. monocytogenes* infection in rabbits. This concentration of bacteria did not suppress growth of tissue in culture. The bacterial suspension was prepared by washing the 24-hour growth off tryptose agar (Difco) slant with sterile saline. This suspension was diluted with saline until the density matched the 0.5 tube of a MacFarland nephelometer when read in a Cenco Sheard Sanford photelometer. An inoculum of 0.01 ml of this bacterial suspension was added to the tissue cultures from tip of 27 gauge needle. The double coverslip method of Maximow(15) was used throughout. Explants of adult rab-

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