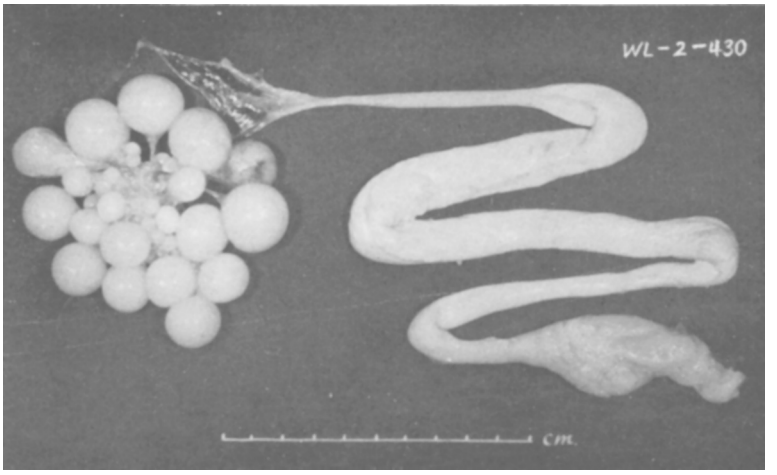

FIG. 2. Ovary and oviduct from FSH-treated fasting hen No. 2–430 (Table II), sacrificed 12 hr following inj. of 2.0 mg LH. Note absence of full-sized follicles.

gonadotrophin-treated 7-day starved hens. 2. 0.1 mg of the LH preparation used in this experiment is asumed to be the minimum dose for forced ovulation. 3. About 8 hours is the time interval between LH administration and rupture of follicles in such treated hens. 4. It was not possible to induce ovulation of small immature follicles by 2 mg of LH.

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Anticortisol Action of 2-Methyl-9(α)-Fluorocortisol.* (22478)

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It has been shown recently that 2-methyl-9(α)-fluorocortisol acetate (Me-F-COLA) possesses extraordinarily intense mineralocorticoid but comparatively slight glucocorticoid activity(1). This raised the question whether this new steroid, like other potent mineralocorticoids, can also block the antiphlogistic, thymolytic, splenolytic and catabolic actions of a glucocorticoid, such as cortisol. It will be recalled that aldosterone, the most active known naturally occurring mineralocorticoid, does possess such anticortisol actions(2). To verify this point, an experiment was performed with Me-F-COL, under essentially the same experimental circumstances under which aldosterone proved to inhibit cortisol.

Materials and methods. Forty female

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TABLE I. Anticortisol Action of 2-methyl-9 (α)-fluorocortisol.

Treatment	Final body wt (g)	Wt gain (%)	Exudate (ml)	Thymus (mg)	Spleen (mg)
COLA	145 ± 3.1	−9	10.4 ± 1.3	114 ± 12.7	454 ± 36.4
COLA + Me-F-COL*	163 ± 1.9	+2	19.5 ± 1.4	158 ± 20.1	607 ± 21.7
COLA + Me-F-COL†	150 ± 2.5	−6	11.2 ± 1.9	61 ± 8.6	416 ± 33.7
COLA + DOCA	169 ± 1.9	+6	22.8 ± 1.8	233 ± 18.3	654 ± 31.8

* 10 μg. † 100 μg

Sprague-Dawley rats, with an average initial body-weight of 160 g (range: 154-166 g) were bilaterally adrenalectomized and subdivided into 4 groups, as indicated in Table I. Throughout the observation period these rats were maintained exclusively on "Purina Fox Chow" and tap water, without special salt supplements. Hormone treatment was initiated immediately after adrenalectomy. Cortisol acetate (COLA†) was given at the dose of 400 μg/day (Groups I-IV), Me-F-COL† at the dose of 10 μg (Group II) and 100 μg (Group III), and desoxycorticosterone acetate (DOCA†) at the dose of 100 μg/day (Group IV). The latter, a known active anticortisol compound, served merely as a control. All steroids were administered as microcrystals, subcutaneously, the daily dose being contained in 0.2 ml of aqueous suspension medium.

For the quantitative assessment of inflammation "granuloma pouches"(3) were prepared 48 hours after initiation of the hormone treatment, by the injection of 25 ml of air under the dorsal skin; this was immediately followed by the injection of 1.0 ml of 1% croton oil (in corn oil) into the air-space so created. All animals were killed on the 13th day after adrenalectomy. At this time the exudate was measured in milliliters, by aspiration into a graduated syringe, while the thymus and spleen were weighed after fixation in Susa solution.

Results. It is evident from Table I that, under these conditions, 10 μg of Me-F-COL was almost as effective as 100 μg of DOCA, in significantly inhibiting the loss of body-weight, the suppression of exudate formation and the involution of the spleen, which occur under the influence of COLA alone. Thymus involution was also inhibited, though not significantly. On the other hand, 100 μg/day of Me-F-COL proved to be virtually ineffective in all these respects.

Discussion. At first sight, it may appear to be paradoxical that Me-F-COL possesses a clear-cut anticortisol effect at low, but not at high, dose-levels. However, this is quite in agreement with what has been termed "the law of intersecting dose-effect curves." It had been found that, when a solution containing fixed proportions of COLA and DOCA is administered to adrenalectomized rats, the cortisol action (catabolism, inhibition of exudation, thymolysis and splenic atrophy) predominates at high and the opposite, desoxycorticosterone-type of activity, predominates at low dose-levels. This was ascribed to the fact that the DOCA activity rises rapidly to its optimum level, but then a "ceiling" is reached and raising the dose further will not increase the effect. The cortisol-type of activity, on the other hand, rises more slowly, but does not flatten out until it far exceeds the "ceiling" of its antagonist(4). Since Me-F-COLA is known(1) to possess considerable glucocorticoid activity (about 39 times that of cortisol), in addition to its much more evident mineralocorticoid action (about 90 times that of DOCA), it was to be expected that small doses of the compound would be prophlogistic, anticatabolic and stimulating to thymic and splenic development, while large doses would act inversely. In fact, it is conceivable that even smaller doses of Me-F-COL would have to be administered in order to bring out the maximum anticortisol action of this compound. In any event, our observations furnish additional evidence of some

† The authors gratefully acknowledge generous supplies of "Cortril" (COLA) from the Pfizer Laboratories, of 2-methyl-9(α)-fluorocortisol from Upjohn Co., and of "Cortate" (DOCA) from Schering Corp.

close relationship between mineralocorticoid and prophlogistic (anticortisol) potency. They demonstrate furthermore, by comparison with our earlier findings concerning aldosterone(2), that Me-F-COL—a synthetic steroid not known to occur in nature—at least equals, and probably exceeds, the anticortisol activity of the most potent natural mineralocorticoid.

Summary. In adrenalectomized rats bearing a "granuloma pouch," it has been demonstrated that 10 μg/day of 2-methyl-9(α)-fluorocortisol (Me-F-COL) definitely antagonizes the catabolic, antiphlogistic, thymolytic and splenic atrophy-producing actions of 400 μg/day of cortisol acetate (COLA). Higher doses of Me-F-COL are less effective in these respects. Since Me-F-COL possesses some glucocorticoid potency, in addition to its strong mineralocorticoid effect, the comparatively low anticortisol activity of this compound, when it is given at high dose-levels, is in agreement with expectations based on the "law of intersecting dose-effect curves."

Addendum. Since this manuscript went to press we were able to show that Me-F-COL is more than 100 times as active as DOCA in producing nephrosclerosis and cardiovascular hyalinosis in suitably conditioned rats (Selye, H., and Bois, P., *Endocrinology,* in press).

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Diffusion Coefficient of Urate for Human Connective Tissue Membrane. (22479)

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Determinations of the diffusion coefficient of urate for connective tissue have not as yet been reported. The present study was carried out on 10 samples of the cerebellar tentorium, obtained fresh at autopsy, using the diffusion method of Johnsen and Kirk(1). In this procedure the diffusion rate of a compound from one fluid phase to another through a membrane is measured under sterile conditions and at a constant total pressure.

Methods. The experiments were conducted at 37°C. A solution of sodium urate (190-197 mg %) in Krebs' phosphate buffer, pH 7.4, was used in the donor compartment of the apparatus, and phosphate buffer in the recipient compartment. The urate solution was prepared fresh daily by addition of 20 ml 0.1 N NaOH solution to 200 mg of uric acid contained in a beaker. The beaker was placed in a water bath at 60°C, and the solution stirred vigorously. 60 ml of Krebs' phosphate buffer, preheated to 60°C, were then added; after adjustment to pH 7.4 the solution was made up to 100 ml with phosphate buffer, and, after cooling to 25°C, was filtered through a Whatman No. 2 filter paper. The purpose of the cooling and subsequent filtration was to insure against precipitation of urate from the solution during the experiment. For the diffusion experiment the tentorium sample was rinsed with sterile buffer solution and a portion of the membrane inserted in the diffusion apparatus. Both the donor and recipient solutions were heated to 37°C before they were introduced into the apparatus. 45 minutes were allowed to elapse for establishment of temperature equilibrium and for initial penetration of urate through the membrane before the first samples were withdrawn for analysis. In each experiment 2 diffusion periods of 90