ing are also in disagreement. No satisfactory explanation can be offered except those of strain difference and climatic conditions.

Adding the values for I.P.-P., Cr.P.-P. and A.T.P.-P. leaves rather small quantities of difficultly hydrolyzable phosphate. Values for this latter fraction do not show the decline exhibited by other fractions but remain quite constant. The trauma to which these tissues are exposed during separation is appreciable, although ortho phosphate content is not excessive.

Wide variation of concentration values for various fractions in the control group is perhaps best accounted for by animals' random eating patterns. Some animals may have just eaten, others not for several hours. As animals are subjected to more uniform treatment, range of concentration decreases. The apparent increase of A.T.P.-P. in the mucosa after 24-hour fast is perhaps accounted for by this mechanism, however this may be an example of transfer of energy-rich phosphate bonds from a storage unit to an active donor. With increased starvation time, even the donor becomes greatly depleted. The brief feeding of 48-hour fasted animals, causing shifts of phosphate values towards control amounts, suggests a mechanism responsible for preparing intestines for receiving and handling of food material. Incomplete data shows a similar picture for 24- and 72-hour fasted animals. Rapidity of these alterations indicates that amounts of energy-rich phosphate compounds are more dependent on activity states than purely nutritional depletion and restoration. A study of this "chemical reflex" may prove of value in determining whether hormonal and/or nervous mechanism is responsible for activation of intestinal tract. Investigation is now underway to determine sensory mechanisms for this reflex.

Summary. 1. Both muscularis and mucosa of rat show significant changes in amounts of energy-rich phosphate compounds when animal is fasted or fasted and briefly fed. 2. With increasing periods of starvation to 72 hours, the easily hydrolyzable phosphate compounds, Cr.P.-P. and A.T.P.P., diminish in both tissues in a predictable manner. 3. The mucosa appears to be more labile of the 2 tissues, although the muscularis shifts in the same direction and to nearly the same magnitude. 4. The assay of either Cr.P.-P. or A.T.P.P. on whole intestinal segments will provide sufficient data as would the independent assay of each, in the major tissues comprising the intestine, to follow the nature of the mechanism involved.

1. Grossman, M. I., *Physiol. Rev.,* 1950, v30, 77.
2. Picchioni, A. J., Edwards, L. D., *J. Am. Pharm. Assn., Sci. Ed.,* 1952, v41, 289.
3. Kroeger, D. C., Edwards, L. D., *ibid.,* 1953, v42, 560.
4. Ernster, L., Zetterstron, R., Lundberg, O., *Acta Chem. Scand.,* 1950, v4, 942.
5. Moses, L. E., *Psychol. Bull.,* 1952, v49, 122.
6. Furchgott, R. F., Shorr, E., *Am. J. Physiol.,* 1950, v162, 88.
7. Walaas, O., Walaas, E., *Acta Physiol. Scand.,* 1950, v21, 18.
8. Born, G. V. R., *J. Physiol.,* 1956, v131, 704.

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Fluorometholone, A Preferentially Anti-Inflammatory Corticoid. (25049)

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Biological activities of hundreds of analogs and derivatives of hydrocortisone synthesized in the past decade have begun to establish certain patterns. Potencies far beyond that of hydrocortisone have become commonplace(1-5) and separation of "glucocorticoid" from "mineralocorticoid" properties has been achieved with several compounds. Many compounds which are potent in bioassays measuring changes in carbohydrate metabolism are also potent in bioassays measuring suppression of experimental inflammation, involution of lymphoid tissue, production of eosinopenia, and suppression of ACTH secretion. This

communication reports the biological profile of a new high-potency corticosteroid in which a marked divergence exists between "glucocorticoid" potency and anti-inflammatory potency in the rat. This compound is 6α-methyl-9α-fluoro-11β, 17α-dihydroxy-1,4-pregnadiene-3,20-dione, or fluorometholone(5,6).*

Methods. Fluorometholone was assayed in several test systems in comparison with appropriate standard compounds. In glycogen deposition assay(7) and granuloma pouch assay(8) it was compared with hydrocortisone, in ulcerogenic assay(9) it was compared with prednisolone, and in "mineralocorticoid" assay(7), the effect on excretion of electrolytes and on urine volume was compared with that of several other corticosteroids. All assays reported were performed with steroids given by subcutaneous route.

Results. Glycogen deposition assay. The calculated ratio of potencies (fluorometholone hydrocortisone) in a typical assay was 24 (Table I). Seven such assays were performed and potency ratios were: 29, 21, 33, 40, 10, 24, 27. Mean potency ratio was 26.

Granuloma pouch assay. Table II shows results of 4 multiple-dose comparisons of fluorometholone with hydrocortisone. Potency ratios calculated from these data indicate the new compound to be 134, 123, 153, and 116 times as potent as hydrocortisone, for a mean ratio of 131.

Ulcerogenic assay. Experiments were performed to estimate ability of fluorometholone to produce ulcers in glandular portion of stomach of the fasting rat (Fig. 1). Prednisolone was the standard compound. Although quantitative relationships are not precise, inspection of the Figure permits an estimate that fluorometholone is about 15 $\times$ as potent as prednisolone in this respect. Since it was previously shown that prednisolone is 2 to 3 $\times$ as potent as hydrocortisone(9), it may be concluded that fluorometholone is 30 to 45 $\times$ hydrocortisone as an ulcerogenic agent.

Mineralocorticoid assay. Effect of graded doses of fluorometholone on urine volume and sodium output of adrenalectomized, salt-loaded rats was tested. Fluorometholone, at doses to 400 μg/rat, caused increased excretion of sodium and increased urine volume. Maximum sodium increase was 30% above control level at 100 μg/rat; increase was somewhat smaller when dose was increased to 400 μg. Maximum observed increase in urine volume was 100% above control, and was seen at 400 μg dose. This pattern is similar to those of prednisolone and 6α-methylprednisolone. It is, however, qualitatively different from that of 6α-methyl-9α-fluoroprednisolone (which differs from fluorometholone only by presence of 21-hydroxyl). The former compound produced *retention* of sodium at doses from 10 μg to 500 μg, reach-

TABLE I. Typical Glycogen Deposition Assay of Fluorometholone in Comparison with Hydrocortisone.

Compound	Dose* (mg/rat)	Liver glycogen (%)†
Hydrocortisone	.4	.58
	.8	.93
	1.6	1.78
Fluorometholone	.02	.88
	.04	1.21
	.08	1.56

* 5 rats/group. Steroids inj. subcut. as aqueous suspension. Single inj. 7 hr before sacrifice.

† Determined by anthrone method. Values are avg of 5 rats. Estimated potency ratio on this assay shows fluorometholone 24 $\times$ hydrocortisone.

* *Oxylone,* Registered Trade Mark, The Upjohn Co., Kalamazoo, Mich.

TABLE II. Granuloma Pouch Assays of Fluorometholone in Comparison with Hydrocortisone.

Compound	Dose* (mg/rat/day)	Mean vol of exudate in pouch (ml)† Exp. 1	2	3	4
None		6.0	7.1	5.4	6.2
Hydrocortisone	.3				3.6
	.5	4.4	4.8	4.2	
	.6				2.2
	1.0	2.2	3.4	3.0	
	1.2				.3
	2.0	.4	.3	.6	
Fluorometholone	.0025				4.1
	.005		4.8	3.4	1.2
	.0075	2.2			
	.010		1.4	1.1	1.0
	.015	.3			
	.020		.4	.2	
	.030	0			

* No. of rats/group is usually 7. Range, 5-10. Subcut. injections as aqueous suspensions.

† Estimated potency ratios, fluorometholone/hydrocortisone: Exp. 1, 134; Exp. 2, 123; Exp. 3, 153; Exp. 4, 116. Avg of 4 ratios: 131.

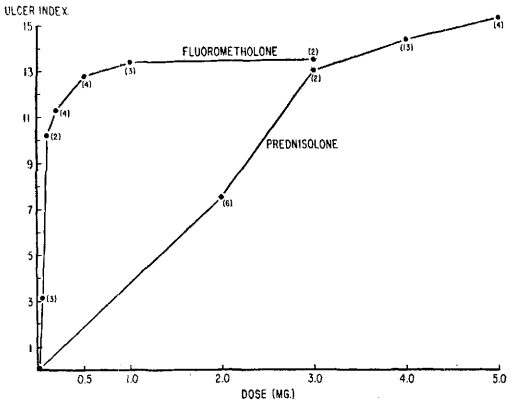

FIG. 1. Comparison of ulcerogenic potencies of fluorometholone and prednisolone. Numbers in parentheses refer to numbers of experiments performed for each dose, each experiment 10 animals. "Ulcer index" combines incidence, severity, and number of lesion/rat(9).

ing a maximal retention (65% of controls) at 200 μg; it caused some increase in urine volume, but only about 30% above controls at maximal dose, 200 μg.

Discussion. There are a number of remarkable things about the data presented. The most striking is the unusual relationship between anti-inflammatory and glucocorticoid potencies. In a large series of active corticoids, glucocorticoid usually exceeds or equals the anti-inflammatory potency. The situation here, *i.e.*, anti-inflammatory outstripping glucocorticoid potency by a ratio of 5:1, is one of the rare exceptions.

A second point of interest is the comparison between the electrolyte-regulating activities of fluorometholone and of 6α-methyl-9α-fluoro-prednisolone. The latter compound exhibits definite sodium-retaining properties (10), while fluorometholone, differing only by absence of 21-hydroxy group, has an opposite effect.

Potency of fluorometholone in producing gastric ulcers is about 30-45 times that of hydrocortisone. This ratio lies between the potency ratio of these 2 compounds in glycogen deposition and anti-inflammatory assays, although somewhat closer to the former. Thus, ulcerogenic potency of corticoids does not necessarily correlate with either of the other major activities.

The disparity between these potencies and those observed in other species by the systemic route is most interesting. Fluorometholone is less potent than prednisolone in producing eosinopenia and hyperglycemia in *man*(12), when given systemically. The fact that when fluorometholone is given *topically* to man, it has anti-inflammatory potency 40 $\times$ that of hydrocortisone(13) indicates that *at the effector site* the compound manifests high potency, but that whether or not it can retain its high activity while passing through the system depends on the species studied. It is possible that man has more efficient systemic mechanisms for destruction of fluorometholone than has the rat.

Summary. Fluorometholone is an unusual synthetic corticoid in the rat because its anti-inflammatory potency (131 $\times$ hydrocortisone) is much greater than its glucocorticoid potency (26 $\times$ hydrocortisone). Its potency in promoting development of ulcers of the pyloric portion of rat stomach is between its glucocorticoid potency and its anti-inflammatory potency. It does not cause sodium retention at doses in which a similar compound (differing only by presence of C-21 hydroxyl) does produce measurable sodium retention.

1. Fried, J., Sabo, E., *J. Am. Chem. Soc.,* 1953, v75, 2273.

2. Perlman, P. L., Tolksdorf, S., *Fed. Proc.,* 1955, v14, 377.

3. Lyster, S. C., Barnes, L. E., Lund, G. H., Meinzinger, M. M., Byrnes, W. W., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 159.

4. Perrine, J. W., Bortle, L., Heyder, E., Partridge, R., Ross, E. K., Ringler, I., *Endocrinology,* 1959, v64, 437.

5. Dulin, W. E., Barnes, L. E., Glenn, E. M., Lyster, S. C., Collins, E. J., *Metabolism,* 1958, v7, 398.

6. Hogg, J. A., *6th Nat. Med. Chem. Symposium, A.C.S.,* Madison, Wis., June 23, 1958.

7. Stafford, R. O., Barnes, L. E., Bowman, B. J., Meinzinger, M. M., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 371.

8. Robert, A., Nezamis, J. E. *Acta Endocrinol.,* 1957, v25, 105.

9. ———, PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 443.

10. Dulin, W. E., *ibid.,* 1955, v90, 115.

12. West, K. M., *Metabolism,* 1958, v7, 441.

13. Schlagel, C. A., Northam, J. I., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 629.

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