seminated necrosis or arterial hyalinization, characteristic of isoproterenol overdosage, was not observed anywhere in the surviving myocardium, except in 2 of the 20 animals in Group 2, and even here, the lesion was barely detectable. Mortality due to isoproterenol intoxication occurred only on 7th day; it was not markedly altered by coronary ligature, perhaps because the detrimental effect of the large infarct nullifies protection against focal necrosis.

Discussion. Evidently, ligature of the left coronary artery near its origin offers considerable protection against subsequent induction of diffuse myocardial lesions by isoproterenol. This protection dose not appear to be due to handling the heart at time of operation, since sham coronary ligatures are ineffective in this respect. Systemic stress may also be excluded as the protective agent. Rats recover from such coronary ligatures almost completely within one or 2 days, as judged by their appetite and general clinical condition; besides, neither forced restraint nor motor denervation —both of which elicited much more pronounced manifestations of stress (adrenal enlargement, thymic involution, loss of body weight)—offers protection against isoproterenol-induced disseminated cardiac necroses. After coronary ligature, systemic blood pressure remained essentially within normal limits; hence, a pre-existent hypotension likewise could not be held responsible for the isoproterenol resistance of surviving myocardium.

The mechanism of this protection remains obscure. Perhaps, when in the state of compensatory hypertrophy (such as occurs after infarction), cardiac muscle becomes insensitive to toxic action of isoproterenol. The resistance may also be due to hemodynamic changes within the myocardium and its vessels, or to absorption of chemical compounds from the infarcted muscle. It remains to be seen, furthermore, whether this protection is specific for isoproterenol and related catecholamines. Additional work will be necessary to clarify these points, but the protective effect of coronary ligature appears to be well established.

Summary. Experiments on rats indicate that, following occlusion of the left coronary artery near its origin, the myocardium outside resulting infarct becomes unusually resistant to production of diffuse cardiac necroses by isoproterenol.

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Biological Activity of Some 6α-Fluoro and 16α-Methyl C-21 Steroids.* (25831)

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Discovery that cortisone was effective as treatment for inflammatory conditions(1) has led to attempts to increase efficacy by chemical modifications. Notable among these have been Δ^1, 2α-methyl, 6α-methyl, 9α-halo, 16α-hydroxy and, recently, 16α-methyl(2,3,4,5,6,7,8,9,10,11). These changes have also been used in combination in attempts to alter some of the undesirable biological effects resulting from some of the single modifications. The present communication describes the influence of the 6α-fluoro and 16α-methyl groups, alone

* Steroids kindly supplied by Chemistry Dept., Upjohn Co.

TABLE I. Anti-Inflammatory, Liver Glycogenic and Sodium Retaining Activities of Some 6-Fluoro and 16-Methyl C-21 Steroids.

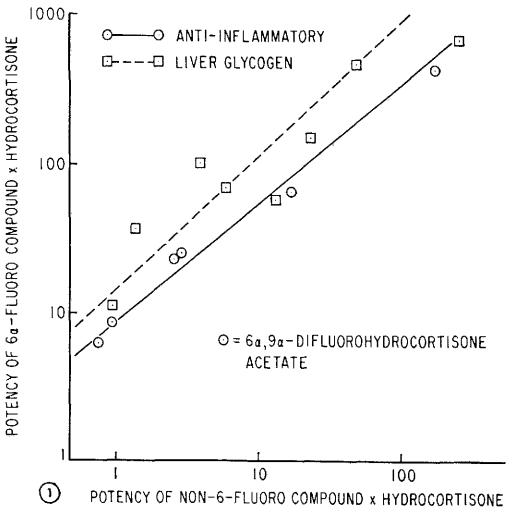
Parent compound	Activity		
	Anti-inflammatory × hydrocortisone	Liver glycogen × hydrocortisone	Sodium retention × DCA
Hydrocortisone	1.0	1.0	<.02
16α-methylhydrocortisone	.8	1.4	"
Prednisolone	3.1(6)	3.0(6)	"
16α-methylprednisolone acetate	8.7	24.0	"
9α-fluorohydrocortisone acetate	8.0	12.6	5.0
9α-fluoro-16α-methylprednisolone	164.0	251.0	<.02
2α-methylhydrocortisone acetate	2.8	5.8(3)	2.7
9α-fluoroprednisolone acetate	16.5	50.0	20.
6-Fluoro compound			
6α-fluorohydrocortisone acetate	8.7	10.9	Slight retention
6α-fluoro-16α-methylhydrocortisone	6.1	36.0	<.02
6α-fluoroprednisolone acetate	25.0	100.0	Slight retention
6α-fluoro-16α-methylprednisolone acetate	50.0	150.0	<.02
6α,9α-difluorohydrocortisone acetate	8.0	63.0	4.0
6α,9α-difluoro-16α-methylprednisolone acetate	424.0	677.0	<.02
6α-fluoro-2α-methylhydrocortisone acetate	23.0	60.0	3.0
6α,9α-difluoroprednisolone acetate	66.0	443.0	2.5

and in combination with other potentiating groups on the anti-inflammatory, liver glycogenic, and mineralocorticoid activities of hydrocortisone.

Materials and methods. Anti-inflammatory test. Granuloma pouch method(12) was used to determine anti-inflammatory potency of the various steroids. In all cases, unknowns were tested in parallel with hydrocortisone on a 3-dose basis with a minimum of 14 animals per dosage group. Potency calculations were carried out by a standard U.S.P. method(13). Steroids were injected subcutaneously in CMC vehicle.† Animals were intact Sprague-Dawley females, weighing 150-160 g. *Liver glycogen deposition test.* Liver glycogens were determined by the anthrone method(14) on tissues removed 7 hr following subcutaneous injection in CMC vehicle. Three doses of unknowns were assayed in parallel with 3 doses of standard with at least 10 rats per group. Potency ratios were calculated by the method of Irwin(15). *Sodium retention test.* This test(16) measures the influence of compounds on sodium and water excretion in salt and water loaded, adrenalectomized rats over a 4 hr period, following a single subcutaneous injection of the compound. Compound is administered as an oil solution or suspension, depending upon concentration and solubility. Compounds possessing little or no sodium retaining activity were tested at a dose of 500 μg and compared to a dose response curve obtained with desoxycorticosterone. When compounds possessed a significant degree of sodium retaining activity, they were tested in parallel on a multiple dose basis with the standard. Potencies in the cases of active compounds were calculated by the Irwin method(15).

Results. Anti-inflammatory potency of the steroids included in this study was increased by 6α-fluoro in all cases except 9α-fluorohydrocortisone (Table I, Fig. 1). 6α-Fluoro increased the glycogenic potency of all compounds in this series. Variable results were obtained on sodium excretion in presence of 6-fluoro since, in some cases there was an increase, in others no change, while sodium retention of one compound (9α-fluoro-prednisolone) was markedly depressed when 6α-fluoro was present (Table I). Influence of the 6α-fluoro on sodium retention is different than that obtained with 6α-methyl which consistently decreased the activity of sodium retainers or in the case of 2α-methyl or 9α-fluoro which generally increased sodium retention(6). Reason for the variable results of 6α-fluoro on anti-inflammatory and sodium retaining activity is difficult to interpret but

† 0.5% carboxymethylcellulose, 0.5% Tween 80, 1.5% benzyl alcohol, 0.9% sodium chloride.



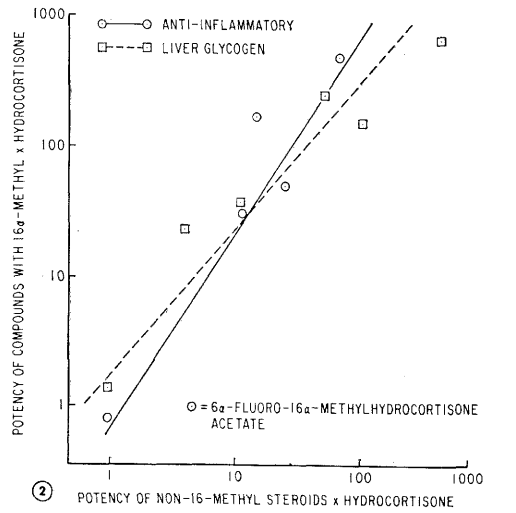


FIG. 1. Graphic representation of data in Table I showing relationship of 6-fluoro and non-6-fluoro steroids on anti-inflammatory and liver glycogenic potencies.

FIG. 2. Summary of relationship of 16-methyl and non-16-methyl compounds on anti-inflammatory and liver glycogenic activities.

may be due to 1) differences in physical properties of compounds which produce different results due to characteristics of a given test, or 2) actual changes in inherent activity which is manifested by different effects at the target organs.

16α-methyl is also not a universal potentiator of glycogenic or anti-inflammatory activity (Table I, Fig. 2). This is in contrast to other published reports(5,7). When the parent compound was a sodium retainer, the compound with the 16α-methyl generally possesses less sodium retaining activity. In one case where the parent compound was inactive, the presence of 16α-methyl did not cause any change in sodium retaining activity. It is therefore concluded from the series studied here that 16α-methyl does not cause sodium retention but actually depresses sodium retaining activity of sodium retainers.

Results reported here show that it is not yet possible to predict accurately the effects of chemical modifications on biological activities of steroids. Chemical changes in certain combinations can be expected to produce variable alterations in quantitative activities. It has been our experience in certain instances, however, when a single change increases the anti-inflammatory or glycogenic potency of a compound, it does not decrease potency when in combination with other potentiators.

One compound (6,9-difluoro-16α-methylprednisolone) is one of the most potent anti-inflammatory or glycogenic steroids tested. When this is considered along with the fact that the compound does not possess significant sodium retention at doses which are anti-inflammatory, it shows that one biological activity can be altered independently of another. This compound is therefore potentially useful clinically in inflammatory conditions.

Observations of marked increase in anti-inflammatory activity with no change or decrease in sodium retaining activity lead to optimism in testing new steroids for differential activities. Since it is possible to reduce sodium retention activity while retaining glycogenic or anti-inflammatory potency, it may also be possible to dissociate, by chemical modification, protein catabolic and ulcerogenic activities from anti-inflammatory effects.

Summary. The anti-inflammatory, liver glycogenic, and sodium retaining activities of a series of 6α-fluoro and 16α-methyl steroids and their parent compounds were determined. The 6α-fluoro substitution increased liver glycogenic activity in all compounds studied, and anti-inflammatory potency in all but one

of compounds. This modification also produced variable effects on sodium retention. The 16α-methyl group did not consistently increase liver glycogenic or anti-inflammatory activities. Sodium retention effect of the corticoids was not increased by 16-methylation if parent compound did not possess mineralocorticoid activity. In examples where parent steroid was a sodium retainer, addition of 16α-methyl group caused reduction of this activity.

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Zn^{65} Uptake by Rat Dorsolateral Prostate as Indicator of I.C.S.H. Activity.* (25832)

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Previous investigations have shown that Zn^{65} uptake by dorsolateral prostate (DLP) of the mature rat reflects high natural zinc content of the gland(1). In contrast the ventral lobes of prostate, which are low in natural zinc content(2), do not take up Zn^{65} appreciably(1). It was also demonstrated that amount of Zn^{65} concentrated by DLP depended upon androgen level. Zn^{65} uptake was low in DLP of immature rats and did not reach maximum levels until animals were 14-16 weeks of age (3). This also paralleled other reports on increase in natural zinc content of the rat DLP with age(4). Further experimentation showed that Zn^{65} uptake in DLP of the mature rat was lowered by castration, but could be maintained at control levels by administration of suitable doses of testosterone propionate(3,5). Even more striking fall in Zn^{65} uptake was noted following hypophysectomy, but Zn^{65} uptake levels could be maintained by administration of appropriate doses of either chorionic gonadotrophin or testosterone propionate(5,6). One biological indicator of I.C.S.H. activity frequently used is based on enlargement of the ventral prostate in hypophysectomized rats(7). In view of availability of purified I.C.S.H.† experiments

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