In comparable investigations, to be reported in detail subsequently, we have found inhibition of insulin degradation by the following compounds: thiamine, thioctic acid, indole, skatole, phenylacetic acid, phenylpropionic acid and indole-3-propionic acid.

Studies are being conducted in mice and man to determine to what extent the effective inhibitors augment the hypoglycemic action of insulin.

Summary. In an *in vitro* system using insulin-I^{131} and a special liver preparation which degrades insulin, it has been shown that certain amino acids and related compounds inhibit insulin-I^{131} degradation, as measured by the accumulation of trichloracetic acid soluble radioactivity. Marked inhibition was demonstrated using cystine, ergothioneine, 3, 4-dihydroxyphenylalanine, isoleucine, tryptophane, indole-3-acetic acid, and casein hydrolysate (Amigen). In the concentrations tested no significant inhibition was exerted by glutamine, glycine, β-alanine, asparagine, arginine, serine, threonine, leucine, methionine, histidine, hydroxyproline or tyrosine.

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Anti-Inflammatory Activities of Several 9 α-Halo Derivatives of Adrenal Steroids.* (22372)

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The synthesis of a series of 9 α-halo derivatives of adrenal steroids has been reported by Fried and co-workers(1-3). These compounds have been shown to possess unusually high adrenocortical activity in the rat(3-5), in the dog(6,7), and in man(7,8). Several of these compounds are part of a series prepared to study the possible influence of variations in side chain upon adrenocortical action. In the present paper the anti-inflammatory activities of these compounds are reported, using as the anti-inflammatory index the inhibition of formation of granulation tissue around a cotton pellet.

The use of cotton as an irritant in anti-inflammatory studies has been previously reported(9,10). Inhibition of the inflammatory reaction to cotton by either systemic or local administration of cortisone has been demonstrated by Meier, Schuler and Desaulles(9).

Materials and methods. A 5 to 7 mg cotton pellet is inserted high into the upper dorsal area of a male rat (weight range 120-150 g) at the time of bilateral adrenalectomy. The pellet is prepared from non-sterile unbleached cotton, which is rolled into a ball with the fingers(11). The pellet is inserted with long thin forceps, subcutaneously,

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TABLE I. Granuloma Formation in Adrenalectomized Male Rats Treated with Adrenocortical Steroids and Some of Their 9α-Halo Derivatives.

Compounds	Total dose (mg), 4-day period	No. of animals	Mean granuloma dry wt (mg)	Potency (12) (E or F acetate = 1)	95% confidence limits (12)
Controls	—	20	25.5		
E acetate	1.2	19	17.0	1.0	
	3.6	20	12.5		
	10.8	18	10.2		
F "	1.2	20	20.4	1.0	
	3.6	20	13.5		
	18.8	19	10.0		
Controls	—	29	25.1		
Fluoro F acetate	.13	19	19.8		
	.4	29	15.1	13.2	8.7–20.0
	1.2	20	7.8		
	3.6	10	5.0		
E acetate	1.2	19	19.8		
	3.6	18	11.1		
	10.8	10	10.9		
F "	1.2	10	30.9		
	3.6	10	19.9		
	10.8	10	14.1		
Controls	—	20	19.7		
Fluoro E acetate	.13	20	12.0	10.0	7.1–14.2
	.4	10	7.9		
	1.2	19	4.3		
F acetate	1.2	19	12.9		
	3.6	10	8.1		
	10.8	19	4.7		
Controls	—	20	25.5		
Chloro F acetate	.8	20	14.5	3.4	2.0– 5.9
	2.4	20	6.9		
	7.2	8	4.1		
F acetate	1.2	20	20.4		
	3.6	20	13.5		
	10.8	9	5.4		
Controls	—	19	17.5		
Corticosterone	3.6	8	16.4	.32	.24– .44
	5.4	9	12.0		
	10.8	19	7.1		
F acetate	1.2	19	13.6		
	3.6	20	7.5		
	10.8	20	5.4		
Controls	—	19	15.0		
9α-Fluorocorticosterone acetate	.4	10	14.0		
	.8	8	8.8	2.7	1.8 –4.0
	1.2	10	8.4		
	2.4	9	3.9		
	3.6	10	4.9		
F acetate	1.2	18	12.3		
	3.6	20	7.8		
	10.8	9	4.1		
Controls	—	18	17.0		
9α-Fluoro-11β, 17α-dihydroxyprogesterone	1.2	18	17.8		
	3.6	16	15.3	.37	.16– .85
	10.8	19	9.9		
F acetate	1.2	17	13.6		
	3.6	20	9.8		
	10.8	15	8.7		
Controls	—	18	17.1		
9α-Fluoro-11β, hydroxyprogesterone	1.2	17	12.4		
	3.6	16	14.4	<.1	
	10.8	16	12.7		
F acetate	1.2	17	13.8		
	3.6	20	9.8		
	10.8	15	9.4		
Control	—	10	23.5		
Progesterone	32.4	10	22.5	<.04	
DCA	32.4	10	22.8	"	
F acetate	1.2	10	17.2		
	3.6	8	12.1		
	10.8	10	10.7		

through the incision used for adrenalectomy. Care is taken neither to separate the skin from the connective tissue nor to form a pocket into which the pellet fits. The animal is then injected subcutaneously with an aqueous suspension(4) of the steroid under examination or with the aqueous menstruum (control) and placed on a normal stock diet, with 1% NaCl in the drinking water. On the following 3 days the animal receives once daily an identical injection of the steroid or menstruum. Twenty-four hours after the last injection, corresponding to 96 hours after pellet implantation, the animal is sacrificed and the pellet, along with the granulation tissue that surrounds it, is carefully dissected from the animal and weighed. The dry weight of the granuloma and pellet is obtained after heating at 80°C overnight. By subtracting the initial weight of the pellet, the dry weight of the granuloma is determined. The animals treated with high doses of steroids presented a pellet that appeared by gross inspection in most instances to be almost entirely devoid of granulation tissue. Probably the increase in the dry weight of these pellets is due to an accumulation of adsorbed body fluids. It is reasonable to assume that the solids in these fluids contribute to the weight of the granulomas in the animals treated with the lower doses of steroids or the control menstruum. No attempt has been made to differentiate the weight increments of the granulation tissue from the tissue fluid solids. In the assay results discussed subsequently, the term granuloma applies to the total increase in dry weight of the implanted pellet. Decreasing granuloma weights were observed with increasing doses of active steroids. A linear log dose response has been obtained, using daily levels of 300, 900 and 2700 μg of cortisone acetate (E acetate) or hydrocortisone acetate (F acetate). Occasionally only a minimal response was obtained at the low dose level. Aqueous suspensions of 9 α-fluorohydrocortisone acetate (fluoro F acetate), 9 α-fluorocortisone acetate (fluoro E acetate), 9 α-chlorohydrocortisone acetate (chloro F acetate), desoxycorticosterone acetate (DCA), corticosterone, 9 α-fluorocorticosterone acetate, progesterone, 9 α-fluoro-11 β-hydroxyprogesterone, and 9 α-fluoro-11 β, 17 α-dihydroxyprogesterone have been studied in this manner.

Results. There is no significant difference (12) in the response to E acetate and F acetate in this test. As a result these compounds were assigned a potency of 1 for comparative studies with the other steroids. The results obtained with all of the steroids mentioned above are presented in Table I. It can be seen from the data that there is a decrease in granuloma formation as the dosage of the steroid is increased. The greatest response was obtained with the fluoro derivatives, fluoro F acetate and fluoro E acetate. An intermediate response was obtained with chloro F acetate and 9 α-fluorocorticosterone acetate. The comparative potencies along with 95% confidence limits as calculated by the method of Bliss(12) are listed in Table I. It can be seen that fluoro F acetate is the most active steroid tested having a potency of 13.2 times that of E or F acetate.

In Table II are summarized the data obtained for all of the 9 α-fluoro-11 β-hydroxy steroids containing various combinations of 17 α- and 21-hydroxyl groups. Corticosterone and F acetate are also included in order to compare the activity of these compounds

TABLE II. The Effect of 9α-Halogenation and Hydroxyl Substitution on the Anti-Inflammatory Activity of Adrenal Steroids.

Compound	Hydroxyl at position	Potency	95% confidence limits(12)
F acetate	11β, 17α, 21	1.0	—
Fluoro-F-acetate	11β, 17α, 21	13.2	8.7 –20.0
Corticosterone	11β, 21	.32	.24– .44
9α-Fluorocorticosterone acetate	11β, 21	2.7	1.8 – 4.0
9α-Fluorodihydroxyprogesterone	11β, 17α	.37	.16– .85
9α-Fluorohydroxyprogesterone	11β	<.10*	—
Progesterone	—	<.04†	—
Desoxycorticosterone acetate	21	" †	—

* No activity observed at doses as high as 2.7 mg/day.

† No activity observed at doses as high as 8.1 mg/day.

with their 9 α-fluoro analogues. It can be seen that introduction of the fluorine atom into the 9 α-position of either F acetate or corticosterone increases the anti-inflammatory activity of these steroids approximately 13 and 9 times, respectively. It is also noted that the 17 α- and 21-hydroxyl groups are required for maximum activity. From a comparison of the data obtained with 9 α-fluoro-11 β, 17 α-dihydroxyprogesterone with 9 α-fluorocorticosterone acetate, it appears that the 21-hydroxyl group has a much greater influence on activity than the 17 α-hydroxyl group. The importance of the 17 α-hydroxyl group, however, is illustrated by the lack of activity of 9 α-fluoro-11 β-hydroxyprogesterone, in which this group is absent. It should be noted that both mono- and dihydroxy derivatives of 9 α-fluoroprogesterone are very insoluble, and the possibility exists that the crystalline suspensions used in this study may not be the optimum formulations for properly evaluating these compounds.

Progesterone, which contains none of the substituents described above is inactive at levels of 8.1 mg per day. DCA, containing only the 21-hydroxyl group, is inactive at 8.1 mg per day. This latter observation adds further confirmation to the concept that 11 β-oxygen is required for the anti-inflammatory activity of corticoids.

Summary. The anti-inflammatory properties of a number of 9 α-halo-11 β-hydroxy steroid derivatives have been compared with some of their halogen-free analogues. The substitution of a fluorine atom at the 9 α-position enhances activity. The presence of various hydroxyl groups in the steroid molecule is essential for maximum anti-inflammatory effect. Hydroxylation at the 17 α-position results in a steroid with increased anti-inflammatory activity. A considerably greater response is demonstrated by the introduction of a 21-hydroxyl group, while maximum activity is achieved by the presence of both 17 α- and 21-hydroxyl groups. The most active compound tested, containing a 9 α-fluorine atom and hydroxyl groups at carbon positions 11 β, 17 α, and 21, was fluoro F acetate. This steroid is approximately 13 times as potent an anti-inflammatory agent as E or F acetate.

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