inhibitor, suggests that the collagenolytic activity contained in this extract was not due to cathepsins(12).

Therefore the ability of alkaline extracts of porcine pancreas to hydrolyze the peptide backbone of collagen would *not* appear to derive from: 1) contamination with bacterial collagenase; 2) "collagen mucoproteinase" activity; 3) general proteolysis of denatured collagen or; 4) non-specific cathepsin activity. Whether pancreatic collagenolytic activity is produced by one enzyme or a system of enzymes (*i.e.*, one solubilizing, another proteolytically acting upon the solubilized product) such as has been found by Franklin and Wynn(13,14) in the liver lysosome is not yet known. Like "collagen mucoproteinase"(4), this latter liver enzyme system was found not to release either dialyzable hydroxyproline or ninhydrin reacting materials from collagen (14).

Summary. Alkaline extracts of the whole pancreas were found to be capable of rendering about one-half the hydroxyproline content of native soluble collagen into a dialyzable form. These extracts could also solubilize small amounts of dialyzable hydroxyproline from washed insoluble collagen. This apparent collagenolytic activity of pancreas extracts was not inhibited by EDTA, soybean trypsin inhibitor or mercurobenzoate. These fractions did *not* demonstrate any proteolytic activity upon denatured hemoglobin.

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Comparison of Anti-Granuloma, Thymolytic and Glucocorticoid Activities of Anti-Inflammatory Steroids. (29255)

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Since the demonstration of anti-rheumatic (1) and anti-inflammatory activities(2) of adrenocortical steroids, there have been numerous reports of the effectiveness of synthetic corticoids in suppressing inflammation in laboratory animals. These compounds were generally compared with cortisone or hydrocortisone for their relative activity in inhibiting artificially induced inflammation of several varieties and for such other corticoid effects as thymolysis, liver glycogen concentration and alterations in the numerical count of various white blood cells. The methods employed for assay of the steroids varied in each of the reporting laboratories, and the strain and species of animal, as well as the laboratory conditions, have not been uniform. Through necessity, comparison of relative activities of commercially available and experimental corticoids have been made using data or reports from several laboratories(3,4,5,6, 7). The present investigation was undertaken to establish the relative activities of most of the commercially available corticoids in well

established assays conducted under identical conditions in the same laboratory and with the same technical personnel. Included in the list of steroids are several new and potent 6α-halogenated triamcinolone and triamcinolone acetonide derivatives which have recently been reported(8,9).

Materials and methods. Anti-granuloma and thymolytic activities. A modification of the method of Meier *et al*(10) was employed. Male Sprague-Dawley Caesarean-derived rats weighing 140-150 g were adrenalectomized and a cotton pellet (5-7 mg) was implanted subcutaneously on each side of the thorax of each animal on the first day of the study. All animals were pair-fed (Purina laboratory chow) to equalize food intake and maintained on a 0.9% saline water supply *ad libitum*. Compounds were administered at 4-7 dose levels and 8-12 animals were used for each dose. Steroids suspended or dissolved in an aqueous menstruum were administered subcutaneously once daily for 6 days. The animals were sacrificed on the eighth day and the thymi and cotton pellets with adhering granulation tissue were removed and weighed. The pellets were dried at 50-60°C for 48-72 hours, equilibrated at room temperature and weighed. Final dry granuloma weight was calculated by subtracting the weight of the cotton pellet. *Glucocorticoid activity.* A slight modification of the liver glycogen deposition assay of Pabst *et al*(11) employing 100-120 g adrenalectomized male rats of the Sprague-Dawley Caesarean-derived strain was used. Steroids were administered subcutaneously at 4 dose levels per compound and 8-10 animals were employed for each dose. Liver glycogen was determined by a method described by Singer *et al*(12).

All assays were of a factorial design employing randomized blocks. Statistical analyses were performed according to methods described by Bliss(13).

Results and discussion. Relative anti-granuloma, thymolytic and glucocorticoid potencies of selected corticoids are recorded in Table I. The anti-granuloma and thymolytic potencies were calculated in relation to hydrocortisone acetate, and the liver glycogen deposition potencies were computed relative to cortisone acetate. Results of these assays confirm the findings of others(3,14,15) that corticoid activity can be increased by introduction of a double bond in the 1,2 position of the steroid or of a chlorine, fluorine or methyl group in the 6α position or of a fluorine atom in the 9α position; by α or β methylation at the 16 carbon position; by 16α,17α-ketolization or by utilizing several of these molecular alterations. Also in confirmation of many other reports, these results demonstrate a decrease in activity by 16α-hydroxylation. The majority of the steroids maintained similar orders of relative potencies regardless of the assay end points studied (Table II). The most potent and the least potent compounds in all assays were 6α,9α-difluoro-16α-hydroxyprednisolone acetonide and cortisone acetate, respectively. Two compounds, 6α-chloro-9α-fluoro-16α-hydroxyprednisolone acetonide, acetate and 6α-fluoro-16α-methylprednisolone, were notably more potent by the anti-granuloma and thymolytic criteria than by the glycogenic criterion. 9α-Fluoro-16α-hydroxyprednisolone acetonide was more potent in the deposition of liver glycogen than in the other 2 assay end points studied.

Seven of the compounds included here have also been studied by Ringler and Dulin(16). The order of activity in the liver glycogen deposition assay reported here for these corticoids is quite similar to that found by these workers. Our assay procedures more closely resemble those utilized by Dulin and the Upjohn Laboratories than those followed by Ringler and the Lederle Laboratories. It is thus not surprising that the relative potencies which we obtained are somewhat closer to those found by the former group. The absolute potency values reported here fall in the range reported by these two laboratories.

Summary. A series of 15 corticoids was assayed for anti-granuloma, thymolytic and liver glycogen deposition activities in adrenalectomized male rats. The order of relative potency for the majority of the steroids was similar in the three bioassays. Exceptions were 6α-chloro-9α-fluoro-16α-hydroxyprednisolone acetonide acetate and 6α-fluoro-16α-methylprednisolone which had greater rela-

TABLE I. Relative Potencies of Corticoids as Determined by Anti-Granuloma, Thymolytic and Liver Glycogen Deposition Assays in the Adrenalectomized Male Rat.

	Compounds	Anti-granuloma-thymolytic assay dose range (μg)	Anti-granuloma activity	Thymolytic activity	Liver glycogen assay dose range (μg)	Liver glycogen deposition activity
I.	Hydrocortisone acetate	100 –3200	1.0	1.0	100 –800	1.9 (1.5– 2.5)*
II.	Cortisone acetate	400 –6400	.5 (.3– .8)*	.5 (.4– .5)*	100 –800	1.0
III.	Prednisone acetate	100 –1600	1.0 (.6– 1.5)	.9 (.8– 1.1)	50 –400	5.2 (2.8– 9.6)
IV.	Prednisolone acetate	50 – 800	2.7 (1.7– 4.2)	4.0 (3.4– 4.7)	10 – 80	9.9 (7.9– 12.4)
V.	6α-Methylprednisolone	100 –1600	6.0 (3.7– 9.5)	3.0 (2.6– 3.4)	10 – 80	26.0 (13.7– 49.4)
VI.	9α-Fluoroprednisolone	100 – 800	17.7 (9.4– 34.6)	4.4 (3.8– 5.2)	10 – 80	53.9 (29.0–100)
VII.	9α-Fluoro-16α-hydroxyprednisolone	50 – 800	2.6 (1.6– 4.0)	3.6 (3.0– 4.2)	5 – 40	34.0 (25.5– 45.4)
VIII.	9α-Fluoro-16α-hydroxyprednisolone, 16α, 17α-acetonide	5 – 80	48.5 (31.2– 75.7)	37.7 (32.0– 44.4)	1.25– 10	216 (165 –281)
IX.	6α, 9α-Difluoro-16α-hydroxy-1,2-dihydro-prednisolone, 16α, 17α-acetonide	.5– 32	103 (62.5–170)	22.0 (14.5– 33.0)	1.25– 10	143 (113 –181)
X.	6α, 9α-Difluoro-16α-hydroxyprednisolone	20 – 160	19.7 (14.4– 26.9)	8.5 (6.2– 11.6)	1.25– 10	88.3 (47.0–166)
XI.	6α, 9α-Difluoro-16α-hydroxyprednisolone, 16α, 17α-acetonide	.5– 32	446 (273 –730)	263 (209 –331)	1.25– 10	276 (120 –638)
XII.	6α-Chloro-9α-fluoro-16α-hydroxyprednisolone, 16α, 17α-acetonide, 21-acetate	5 – 80	123 (100 –151)	188 (162 –219)	1.25– 10	114 (91.1–143)
XIII.	6α-Fluoro-16α-methylprednisolone	2.5– 40	63.6 (34.3–118)	45.1 (37.0– 54.9)	1.25– 10	46.2 (36.8– 57.8)
XIV.	9α-Fluoro-16α-methylprednisolone	5 – 80	104 (72.1–151)	47.0 (41.4– 53.9)	1.25– 10	181 (141 –232)
XV.	9α-Fluoro-16β-methylprednisolone	2.5– 40	35.8 (19.3– 66.2)	11.7 (8.4– 16.2)	1.25– 10	118 (63.3–221)

* 95% confidence limits.

TABLE II. Order of Relative Potencies of Corticoids: Roman Numerals Refer to Compounds in Table I.

Decreasing order of potency	Activity		
	Anti-granuloma	Thymus involution	Liver glycogen deposition
1	XI	XI	XI
2	XII	XII	VIII
3	XIV	XIV	XIV
4	IX	XIII	IX
5	XIII	VIII	XV
6	VIII	IX	XII
7	XV	XV	X
8	X	X	VI
9	VI	VI	XIII
10	V	IV	VII
11	IV	VII	V
12	VII	V	IV
13	III	I	III
14	I	III	I
15	II	II	II

tive potencies in the anti-granuloma and thymolytic assays, and 9α-fluoro-16α-hydroxyprednisolone acetonide which had a greater relative potency in the liver glycogen deposition assay.

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In vitro Activation of Lipolytic Activity in the Serum of Dogs by Bile Salts.* (29256)

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Bile salts have been reported to inhibit the lipolytic activity of heparin-induced clearing factor lipase (CFL)(1). However, in a series of characterization studies of CFL we found that sodium cholate and its conjugates increased the lipolytic activity of dog postheparin plasma. These compounds were found to initiate *in vitro* lipolytic activity in serum from normal, untreated dogs. The present report extends these observations and relates the characteristics of this cholate activated lipase (CAL).

Methods. Lipolytic activity was assayed in citrated plasma or serum from dogs, cats, rabbits, rats, goats and humans. All samples were centrifuged in the cold at 4000 *g* for 10 minutes and maintained at 0°C until assayed for lipolytic activity.

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