serum inorganic phosphorus, independent from that determined by secondary insulin liberation. Speculatively, it may be thought that this effect depends upon the utilization of phosphate in the disintegration of glycogen by phosphorylase.

Summary. The effect of intravenous glucose upon serum inorganic phosphorus was compared to that of glucagon in 35 diabetic patients taken at random.

The subjects were classified in two groups according to their serum phosphate fall following glucose. In a group of 19 patients, the phosphate fall after glucose was less than 0.30 mg/100 ml, and in another group of 16 patients, more than 0.30 mg/100 ml similar to that found in non-diabetic individuals. As a working hypothesis, it has been assumed that diabetics with a normal phosphate fall are producing insulin. It was found that the group with a normal phosphate fall after glucose showed a greater fall after glucagon than the group with defective phosphate fall. On the other hand, the group with defective or absent phosphate fall after glucose also showed a very definite fall after glucagon. It would seem then that the latter substance exerts a direct effect upon serum inorganic phosphate, independent of the reactive insulin secretion produced by the glucose load. This effect is possibly related to the activation of phosphorylase which acts in the process of disintegration of glycogen through the introduction of phosphate in the bonds existing between the hexose molecules.

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Anti-Inflammatory Activity of Δ^1-9α-Fluorohydrocortisone Acetate. (21956)

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There have been many important advances in the use of steroids as a method of treatment of the inflammatory diseases during the past few years. Since the introduction of hydrocortisone as one of the most efficacious treatments, it has been continually modified with the hopes of increasing its antiphlogistic activity while diminishing its undesirable side effects. It has been found that halogenation at the C-9 position greatly increases the glycogen deposition potency(1,2) and the anti-inflammatory effects(3) but causes a relatively greater increase in mineralocorticoid activity (4,5,6). A second analogue of hydrocortisone, 1-dehydrohydrocortisone, has been reported to possess greater antiarthritic properties in man (7) than the parent compound. It has also been shown that this compound is a more potent glucocorticoid in animals(8,9) and that this increase is not associated with an increase in mineralocorticoid potency(8).

It was, therefore, of interest to study the effects of both of these modifications when introduced into the same molecule. Accordingly, the anti-inflammatory activity of Δ^1-9α-fluorohydrocortisone acetate was compared with that of hydrocortisone. Data are also included showing the relative potencies of Δ^1-hydrocortisone acetate, 9 α-fluorohydrocortisone acetate, and hydrocortisone for comparative purposes.

Materials and methods. Animals. Male rats obtained from the Upjohn colony (Sprague-Dawley ancestry) were used throughout these experiments. The body weights ranged from 150-170 g. All animals

were adrenalectomized and allowed free access to Archer dog pellets and 1% sodium chloride drink. They were housed in small wire cages with 4 animals per cage. *Steroids.* The steroids were injected subcutaneously as a suspension in a vehicle containing 0.5% carboxymethyl cellulose, 0.4% Tween 80, 1.5% benzyl alcohol, and 0.9% sodium chloride. The suspensions were made by grinding the crystalline steroids plus vehicle in a ground glass tissue homogenizer. The compounds* used were: (1) 11 β, 17 α, 21-trihydroxy - 4 - pregnene - 3,20 - dione (hydrocortisone), (2) 9 α-fluoro-11 β, 17 α, 21-trihydroxy-4-pregnene-3,20-dione, 21-acetate (9 α-fluorohydrocortisone acetate), (3) 11 β, 17 α, 21-trihydroxy-1, 4-pregnadiene-3,20-dione, 21-acetate (Δ^1-hydrocortisone acetate), and (4) 9 α-fluoro-11 β, 17 α, 21-trihydroxy-1, 4-pregnadiene-3,20-dione, 21-acetate (Δ^1-9 α-fluorohydrocortisone acetate). *Anti-Inflammatory Test.* The test used was a modification of the cotton pellet test as described by Meyer, *et al.*(11). It consisted essentially of implanting 2 nonsterile cotton dental pellets, weighing 6-8 mg, in the mid ventro-lateral subcutaneous connective tissue through a small incision in the skin which was closed by a metal wound clip. The implanting was done at the time of adrenalectomy while the animals were under anesthesia. Anesthesia was accomplished by intraperitoneal injection of sodium pentobarbital at a dosage of 40 mg per kg. Seven days after implantation, the pellets, with their accumulated granulation tissue, were dissected out, dried at 60°C for 24 hours, and weighed to the nearest 0.1 mg on a Roller-Smith Torsion balance. The original cotton weight was subtracted from the final value in order to get the net amount of granulation tissue. The injection of the compounds was begun at the time of implantation and continued through seven daily injections. The volume of each injection was 0.2 cc. The animals were sacrificed approximately 24 hours following the last injection. The experiments were designed so that each steroid was tested at two dosage levels in parallel with hydrocortisone which was used as the standard. The potency ratios were calculated by the method of Irwin(10) and are expressed in terms of activity of hydrocortisone, which is assigned a value of 1. The data on Δ^1-9 α-fluorohydrocortisone acetate were pooled from 3 separate experiments and the data on Δ^1-hydrocortisone acetate were pooled from 2 experiments.

Results. The comparison of the antiphlogistic potency of Δ^1-hydrocortisone acetate, 9 α-fluorohydrocortisone acetate, and Δ^1-9 α-fluorohydrocortisone acetate with hydrocortisone is summarized in Table I. These data show that the potency ratio for Δ^1-hydrocortisone acetate is 3.1, for 9 α-fluorohydrocortisone acetate 7.3, and for Δ^1-9 α-fluorohydrocortisone 14, whereas hydrocortisone is assigned a value of 1.

Discussion. It has been reported(7) that Δ^1-hydrocortisone is 2 to 3 times as effective as hydrocortisone in the treatment of arthritis. Others(8,12) have found that it is 3.0 to 3.9 times as active as hydrocortisone by the liver glycogen deposition test. The anti-inflammatory test, as described here, shows that Δ^1-hydrocortisone acetate is 3.1 times as active as the parent compound. These results, therefore, are in good agreement with the clinical data and results on glycogen deposition.

The activity of 9 α-fluorohydrocortisone acetate has been found to be 12 times hydrocortisone by the glycogen deposition test(12) and 13 times hydrocortisone acetate by an anti-inflammatory test similar to the one described in this paper(3). It is interesting to note that the data reported here show that this halogenated compound is 7.3 times as active as hydrocortisone.

It is seen by examination of the data in Table I that Δ^1-9 α-fluorohydrocortisone acetate is 14 times as effective as hydrocortisone. It was found by Stafford, *et al.*(12) that it was approximately 50 times as active as hydrocortisone on glycogen deposition. Therefore, an apparent quantitative discrepancy exists between the classical glucocorticoid activity and antiphlogistic effect. This could be due to at least two possible reasons: (1) glycogen deposition and anti-inflammatory ef-

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TABLE I. Anti-Inflammatory Assays of Hydrocortisone, 9α-Fluorohydrocortisone Acetate, Δ^1-Hydrocortisone Acetate and Δ^1-9α-Fluorohydrocortisone Acetate.

Treatment	Dose	No. rats	Mean dry granuloma wt (mg) ± S.E.	Potency (× hydrocortisone)
CMC	0.2 cc	20	14.41 ± .77	
Hydrocortisone	200 μg	17	9.97 ± .46	
	1000 "	16	6.57 ± .34	1
Δ^1-9α-Fluorohydrocortisone acetate	10 "	20	9.91 ± .46	
	50 "	19	7.90 ± .47	14
CMC	0.2 cc	7	13.40 ± .47	
Hydrocortisone	200 μg	7	9.80 ±1.25	
	1000 "	6	6.32 ± .29	1
9α-Fluorohydrocortisone acetate	25 "	7	10.74 ± .34	
	125 "	8	6.05 ± .44	7.3
CMC	0.2 cc	10	12.20 ± .49	
Hydrocortisone	200 μg	8	10.10 ± .65	
	1000 "	9	5.70 ± .42	1
Δ^1-Hydrocortisone acetate	60 "	10	10.00 ± .77	
	300 "	10	6.20 ± .50	3.1

fects are mediated through different mechanisms, or (2) the nature of the tests are such that differenecs in physical properties of the compounds, such as solubilities, could affect the results of the two tests in a quantitatively different manner.

The fact that Δ^1-9 α-fluorohydrocortisone acetate has an anti-inflammatory activity greater than the additive effects of Δ^1-hydrocortisone acetate and 9 α-fluorohydrocortisone acetate is of significance. The mechanism responsible for the increase in activity produced by introducing two important chemical changes into the same molecule is not clear at present but is worthy of further investigation.

Summary. A comparison of anti-inflammatory effects of hydrocortisone with several of its analogues has been made. It was found that Δ^1-hydrocortisone acetate was 3.1 times as active as hydrocortisone; 9 α-fluorohydrocortisone acetate was 7.3 times as active, while Δ^1-9 α-fluorohydrocortisone acetate was 14 times as effective as hydrocortisone, as determined by the cotton pellet implantation method.

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