in accord with experimentally determined *in ovo* test results. Possible standard procedures that might make possible direct comparison of test results reported by different laboratories are discussed.

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Renal Electrolyte Effects of Synthetic 18-Hydroxylated Steroids in Adrenalectomized Rats. (27398)

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The ability of the adrenal cortex to synthesize steroids with oxygen functions at carbon 18 has been recognized only in the past decade. The first evidence for such a mechanism was found in the structure of aldosterone (1,2), a corticosteroid oxygenated at both carbon 11 and 18(3). Additional evidence was derived from data of various *in vitro* studies using adrenal tissue, which demonstrated biosynthesis of other corticosteroids similarly oxygenated as in aldosterone(4,5) and of others with oxygen at carbon 18, but not at 11(6-13). The data have indicated, therefore, presence of a distinctive adrenal mechanism for oxidation at carbon 18.

Recently, Pappo reported on the synthesis of 18-hydroxylated derivatives of deoxycorticosterone and progesterone, to provide supplies for assay in laboratory animals. The data concerning renal electrolyte properties of 18-hydroxylated steroids, reported herein, are believed to be of interest as only limited biological data have appeared to date for this class of steroids(13).

Materials and methods. Mineralocorticoid and anti-mineralocorticoid activities of the steroids were investigated in acute experiments with adrenalectomized male rats (150-200 g body weight), which were maintained postoperatively for about 24 hours with sucrose cubes and tap water. In the assay for mineralocorticoid activity, groups of animals were injected subcutaneously with 2.0 ml isotonic saline and with deoxycorticosterone acetate (DCA) or test compounds suspended in 0.5 ml saline. The animals were placed in circular metabolism cages (4 rats/cage) after treatment for collection of a 4-hour sample of urine. Voiding was induced by application of pressure to the bladder at the beginning and end of the collection period to insure recovery of a complete urinary specimen. Each sample from a cage, including rinse water, was diluted to 50 ml for analyses of Na and K concentration. In the assay DCA produces Na retention, K loss and a reduction of the ratio of Na/K in the urine. All compounds were tested at several dosage levels in groups

TABLE I. Mineralocorticoid Properties of 18-Hydroxydeoxycorticosterone Acetate in Adrenalectomized Rats.

Compound	Treatment, dose (μg) per animal	No. of measurements	Mean log response Na	K	(Nax10) /K
—	—	7	1.27	.91	1.36
DCA	4	5	1.01*	1.12*	.90*
18-OH-DCA	4	5	1.15*	.89	1.27
	16	5	1.18	.99*	1.19*
	64	5	1.03*	1.08*	.95*
	256	5	.87*	1.16*	.71*
	1024	4	.80*	1.11*	.69*

* $P \leq 0.05$ relative to untreated controls; Na and K = log meq/l after dilution of sample/4 rats/4 hr to 50 ml; value of Na in the Na/K response was multiplied by 10 to eliminate negative logarithms; 4 animals/measurement.

of 8 or more animals apiece and estimates of potency determined by interpolation using the response of animals treated with a standard dose of DCA. The anti-mineralocorticoid properties of the 18-hydroxylated compounds were determined in similar adrenalectomized rats, which were treated in addition with a standard dose of DCA. In this evaluation, blocking activity of the compounds was measured by the degree of reversal of the Na/K response to DCA in a 4-hour specimen of urine(15). The dosage required for 50% block of the standard dose of DCA (median effective dose, or MED) was determined by interpolation using cumulated dose-response data, and estimates on relative potency of the compounds obtained by a comparison of the MED values. No food or water was provided to the animals during the period of the tests. Statistical analyses for significance were done by Student's *t* test, and urinary Na and K analyzed on the Beckman flame photometer.

Compounds receiving tests were: 20,21-dihydroxy-18,20-epoxy-4-pregnen-3-one (18-hydroxydeoxycorticosterone or 18-OH-DOC), its 21-acetate derivative (18-hydroxydeoxycorticosterone acetate or 18-OH-DCA), and 20-hydroxy-18,20-epoxy-4-pregnen-3-one (18-hydroxyprogesterone). Chemical synthesis of the compounds was described by Pappo(14).

Results. Table I demonstrates data for 18-OH-DCA given over a wide range of doses and for DCA*. Relative to untreated controls, DCA at a 4 μg dose induced the expected retention of Na and a loss of K ions in the urine, to alter as a consequence the ratio of Na/K. All changes with DCA were significant compared to the control responses ($P < 0.05$). 18-OH-DCA gave responses which were qualitatively similar to those obtained with DCA; however, as is evident in the Table, distinctly larger doses were required for comparable urinary effects. Quantitative data on relative potencies were obtained from the simultaneous assay of multiple doses of DCA and 18-OH-DCA. Analysis of the data by the method of Irwin as discussed by Pugsley (16) demonstrated potencies of 4.2, 8.0 and 5.1% for the 18-hydroxy derivative relative to DCA using the Na, K and Na/K indices, respectively.† Table II lists the relative potencies of DCA and 18-OH-DCA, and those of 2 additional 18-oxygenated steroids as estimated by interpolation using dose-response data. 18-OH-DOC gave qualitatively and quantitatively similar effects as the acetate form, with available data over the range of 3-300 μg doses for the 2 compounds indistinguishable statistically ($P > 0.05$). The 18-hydroxylated derivative of progesterone at 3, 30, 300 or 3,000 μg was less effective than 6 μg of DCA; thus, its mineralocorticoid potency is believed to be <0.2% that of DCA.

The anti-DCA properties of the 18-hydroxylated compounds are summarized in Table III, in terms of MED values or that dosage for a standard blocking response.‡ 18-OH-Progesterone at a 3.0 mg dose subcutaneously failed to inhibit the Na/K response to DCA (MED > 3.0 mg), and similarly no

* Transformation of the responses to logs provided homogenous variance with the different treatments and thereby a statistically useful index for evaluation of the data; a change by 0.30 log unit involving the comparisons in Table I represents a 2-fold deviation in basic urinary measurements.

† Chi-square analysis for parallelism of the dose-response curves indicated validity of the assays ($\chi^2 < 0.16$ for each comparison); composite slopes were −0.29 for Na, 0.13 for K and −0.42 for Na/K with each 10-fold increment of log doses.

‡ Dosage/rat required for 50% block, or for reduction of the Na/K effect of 12 μg of DCA to that obtained with 6 μg.

TABLE II. Mineralocorticoid Potencies of 18-Hydroxylated Steroids Compared to DCA in Adrenalectomized Rats.

Compound	% Relative potency*		
	Na retention	K loss	Reduction of Na/K
DCA	100	100	100
18-OH-DCA	4.2 (2.9–6.2)	8.0 (2.0–31.0)	5.1 (2.6–9.9)
18-OH-DOC	7.5	6.0	6.7
18-OH-Progesterone	<.2	<.2	<.2

* Values in parentheses = 95% confidence interval.

effects were evident at 3,30 and 300 μg doses. The acetate of 18-OH-DOC was ineffective at 0.3, 3 and 30 μg doses; no further tests were undertaken at larger doses because of its DCA-like properties. Similar data are also shown in the table for progesterone and spironolactone§ for comparative reference.

Discussion. Our laboratory data demonstrate the ability of certain 18-hydroxylated steroids to alter the urinary output of electrolytes. 18-OH-DCA produced Na retention, K loss and a reduction of Na/K in the urine, to show qualitatively similar effects as DCA. However, its mineralocortocoid potency as judged by data of a multiple dose assay was not striking, being < 10% compared to DCA. The 18-hydroxylated form of deoxycorticosterone or 18-OH-DOC showed quantitatively similar urinary effects on Na, K and Na/K as 18-OH-DCA. No DCA-like properties were apparent with the hydroxylated derivative of progesterone over a wide dosage range. Structure-activity considerations with the available data indicate that a loss of mineralocorticoid potency occurred with 18-hydroxylation (cf. DCA *vs* 18-OH-DCA or -DOC) and with removal of the oxygen atom at carbon 21 (cf. 18-OH-DOC *vs* 18-OH-progesterone). Unlike progesterone(17), 18-OH-DCA did not show measurable anti-DCA activity at dosage levels below that required for mineralocorticoid properties. 18-OH-Progesterone was ineffective as a DCA antagonist over a wide range of doses, which observation contrasts thereby with data for progesterone(17).

Excluding the present studies, only limited biological data have been available for 18-hydroxylated steroids. Ward and Birmingham(13) recently commented on results with a purified lipid extract from a paper chromatogram containing the 18-21-cyclic hemiketal form of 18-OH-DOC. In an assay using the urinary Na/K response in adrenalectomized rats, the compound at 2.5, 5 and 5 μg dose/rat gave equivalent effects of 1.9, 2.2 and 0.5 μg dose of DCA in 3 separate assays. Altogether, these data would suggest an average relative potency of about 40%, compared to the potency of about 7% derived from our tests of 18-OH-DOC. Additionally, the compound was reported to be ineffective at a 25 μg dose in producing eosinopenia in adrenalectomized mice, while a similar dose of cortisone proved to be effective. So far, therefore, available data suggest mineralocorticoid activity as the most prominent action for 18-hydroxylated forms of DOC. Its significance in electrolyte metabolism physiologically and in disease is at present unclear, but requires investigation as several *in vitro* studies suggest possibility of its *in vivo* biosynthesis(11-13). With available synthetic means(14,18,19), it should not be too difficult to supplement the present data for better description of biological properties of 18-hydroxylated steroids.

§ 3-(3-Oxo-7α-acetylthio-17β-hydroxy-4-androsten-17α-yl)-propanoic acid lactone; Aldactone is the trademark of G. D. Searle & Co. for brand spironolactone.

TABLE III. Anti-DCA Properties of 18-Hydroxylated Steroids and of Several Reference Steroids in Adrenalectomized Rats.

Compound	MED (95% limits)
18-OH-Progesterone	>3.0
18-OH-DCA	> .03
Spironolactone	.33 (.18– .61)
Progesterone	1.30 (.52–3.24)

MED = dosage in mg/rat for 50% block of urinary Na/K effect of 12 μg of DCA; 18-hydroxy steroids were inj. subcut. as a saline suspension and the 2 remaining compounds as an oil solution.

Summary. Several synthetic 18-hydroxylated steroids lacking oxygen at carbon 11 were tested for mineralocorticoid properties in acute studies using adrenalectomized rats. A multiple dose assay with DCA described relative potencies of 4, 8 and 5% parenterally for 18-OH-DCA by the criteria of Na retention, K loss and reduction of the Na/K ratio in the urine. Approximately the same order of potency was found for the alcohol derivative, 18-OH-DOC. 18-OH-Progesterone was ineffective as a mineralocorticoid. No significant anti-mineralocorticoid activity was found with 18-OH-progesterone or 18-OH-DCA.

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Lipoprotein Lipase in Human Adipose Tissue.* (27399)

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Lipoprotein lipase(LL) catalyzes the hydrolysis of the triglyceride portion of plasma lipoproteins(1). Small amounts of this enzymatic activity are occasionally present in blood plasma in man(2-4) and in animals(1) but do not approach the high levels found after injection of heparin. However, LL activity is readily detected in several tissues of animals, heart and adipose tissue containing the largest amounts(1). There is considerable evidence that triglyceride hydrolysis catalyzed by LL occurs during removal of triglyceride fatty acids from the blood into extrahepatic tissues(5) and it has been shown that uptake of triglyceride fatty acids by adipose tissue *in vitro* is closely related to the local activity of lipoprotein lipase(6). On the other hand, the removal of chylomicrons from blood plasma does not depend to any significant extent on intravascular hydrolysis (7). It is therefore probable that LL is concerned more with the metabolism of triglyceride in tissues than with that in the circulating blood itself. The precise location of LL is uncertain but available evidence suggests that it is closely related to the capillary bed(8,9).

LL activity in human tissue has been difficult to demonstrate. Some lipase activity of a nonspecific nature has been identified in human adipose tissue by histochemical(10)

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