1931, v25, 367.

2. Engel, M. B., *Arch. Path.*, 1952, v53, 339.

3. Winzler, R. J., In: *Methods of Biochemical Analysis,* Interscience Publishers, New York, 1955, vII, 279.

4. Rehell, B., *Scand. J. Clin. and Lab. Invest*, 1954, v6, 335.

5. Pirani, C. L., and Catchpole, H. R., *A.M.A. Arch. Path.*, 1951, v51, 597.

6. Wolbach, S. B., Maddock, C. L., and Cohen, J., *ibid.*, 1955, v60, 130.

7. Ham, A. W., *ibid.*, 1932, v14, 613.

8. Rubin, P. S., and Howard, J. E., *Tr. Josiah Macy Jr. Foundation, 2nd Conf. on Metabolic Inter., N. Y.*, 1950, 155.

9. Hass, G. M., In: *Biochemistry and Physiology of Bone,* G. H. Bourne, Editor, Academic Press, N. Y., 1956, 767.

10. Hess, A. F., Benjamin, H. R., and Gross, J., *J. Biol. Chem.*, 1931, v94, 1.

11. Barnicot, N. A., *Nature,* 1948, v162, 848.

12. Meyer, K., and Rapport, M. M., *Science,* 1951, v113, 696.

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Sodium Retaining Activity of 19-Nor-Steroids in Adrenalectomized Rats. (22972)

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Small changes in the structure of steroids cause large differences in sodium retaining activity. Aldosterone, for example, possesses about 25 times the potency of desoxycorticosterone(1-3). The 9α-halo-analogs(1,4-6) of cortisone and hydrocortisone and their 6-dehydro-modifications(7) are stronger sodium retainers than the parent compounds. Hydrocortisone is only weakly active, but its 2-methyl- and 2-methyl-9α-halo-derivatives (8,9) are highly potent. Large differences in sodium retention are also seen with removal of the methyl group at carbon 10 (the 19-nor-steroids).

The present report summarizes our results in adrenalectomized rats with a series of these steroids, synthesized by Colton and associates(10,11) of our laboratories.

Materials and methods. The method of bioassay has been described in detail(12). Solutions of steroids in corn oil are injected subcutaneously into adrenalectomized rats with a saline load. A 2-hour sample of urine is collected in the bladder and analyzed for sodium. Amounts of 2 to 12 μg of desoxycorticosterone acetate (DCA) caused by this method a progressive retention of sodium. To minimize the day-to-day variation, one group of rats receiving DCA was used as a standard. An active response was defined arbitrarily as retention of sodium which was at least equal to that of 6 μg DCA. The probability is about 0.05 that an inactive steroid will elicit an equivalent or greater effect. Two steroids, 19-nor-desoxycorticosterone acetate and 19-nor-Reichstein's Compound S acetate, were compared separately with DCA in 4-point bioassays after several preliminary tests. Sodium concentrations were determined on the Beckman flame photometer for calculation of total excretion.

Results. The effects of nine 19-nor-steroids in adrenalectomized rats are summarized in Table I, together with data from simultaneous tests of 6 μg DCA. Sodium excretion with no DCA was 116 μEq/rat in early tests with 111 rats.

With 19-nor-desoxycorticosterone acetate (I), doses of 1.5 to 100 μg elicited a marked retention of sodium. Results of a 4-point bioassay with 0.75 and 1.5 μg of the compound and 2 and 4 μg of DCA indicate that I is approximately 5.1 times as potent as DCA. The results of the test are illustrated in Fig. 1.

The data show that sodium retaining activity is decreased strongly by an hydroxyl group placed at carbon 11 or 17. Fig. 2

TABLE I. Effects of 19-Nor-Steroids on Sodium Excretion in Adrenalectomized Rats.

Chemical name	19-Nor-steroids Dose, μg/rat	No. animals	Sodium excreted, μEq/rat	Desoxycorticosterone acetate (DCA) Dose, μg/rat	No. animals	Sodium excreted, μEq/rat
3,20-Dioxo-19-norpregn-4-ene-21-ol-21-acetate (19-nor-desoxycorticosterone acetate) (I)	100	4	10*	6	3	56
	50	5	10*	6	5	23
	6	5	12*	6	5	23
	1.5	5	11*	6	5	17
	.75	5	56	6	5	48
3,20-Dioxo-19-norpregn-4-ene-17α,21-diol-21-acetate (19-nor-Reichstein's Compound S Acetate) (II)	25	6	26*	6	6	36
	8	5	56	6	5	27
3,20-Dioxo-19-norpregn-4-ene-11β,21-diol (19-nor-corticosterone) (III)	200	6	10*	6	6	60
	50	6	73	6	6	29
3,20-Dioxo-19-norpregn-4-ene-11β, 17α,21-triol (19-nor-hydrocortisone) (IV)	100	4	11*	6	3	56
	60	4	23*	6	3	56
	25	4	10*	6	5	27
	12	6	79	6	6	24
3-Oxo-19-norpregn-4-ene-17α,20Σ, 21-triol-20,21-diacetate (V)	200	6	124	6	6	27
3-Oxo-19-norpregn-4-ene-17β,20Σ, 21-triol (VI)	200	6	113	6	6	50
3-Oxo-19-norpregn-4,16,20-triene (VII)	200	4	136	6	5	28
3-Oxo-17α-ethynyl-19-norandrost-4-ene-17β-ol (17α-ethynyl-19-nortestosterone) (VIII)	200	4	77	6	6	27
3-Oxo-17α-ethyl-19-norandrost-4-ene-17β-ol (17α-ethyl-19-nortestosterone) (IX)	200	8	147	6	8	48
	400	8	107	6	8	48

* Active response (P = 0.05 or less).

Sodium excretion with no DCA (corn oil alone) was 116 μEq/rat in early tests with 111 animals.

summarizes results of bioassay in which 9 and 18 μg doses of 19-nor-Reichstein's Compound S acetate (II) were run simultaneously with 3 and 6 μg DCA. A potency of 0.25 is found for II by this comparison. We find that if II is considered as a struc-

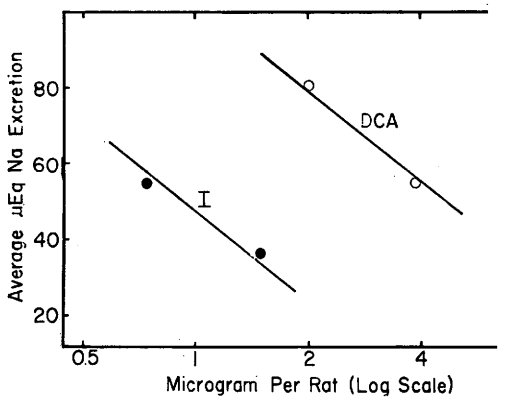

FIG. 1. Relative potencies of DCA and 19-nor-desoxycorticosterone acetate (I) in retention of sodium (DCA = 1, I = 5.1). Each point represents avg response of 5 animals.

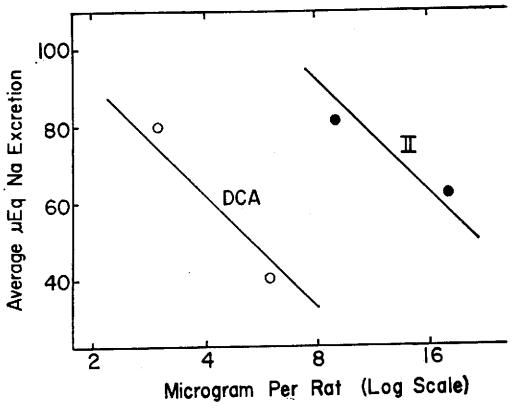

FIG. 2. Relative potencies of DCA and 19-nor-Reichstein's Compound S acetate (II) in the retention of sodium (DCA = 1, II = 0.25). Each point represents avg response of 5 animals.

turally modified 19-nor-desoxycorticosterone the hydroxyl group at carbon 17 reduces activity by 20-fold (5.1/0.25). Similarly, a loss of activity occurs when the same group is placed at carbon 11, as shown for 19-nor-corticosterone (III). Quantitative data for this compound are not available, but the effects with 50 and 200 μg doses suggest a potency of about 0.06 compared with DCA. An oxygen function at carbon 11, therefore, reduces activity of 19-nor-desoxycorticosterone by approximately 85-fold (5.1/0.06).

We find in 19-nor-hydrocortisone (IV) hydroxyl functions at both carbon 11 and 17. Definite salt activity is demonstrated with 25 to 100 μg, but not with a smaller dose. These results suggest a potency of about 0.25 times that of DCA, as discussed earlier for II.

The Table also summarizes data for 5 additional steroids, V, VI, VII, VIII and IX. Except for the 3-oxo-α, β-unsaturated system in ring A, these compounds show less structural resemblance to adrenocortical steroids, than those discussed earlier. Using 200 μg doses, an effect as strong as that of 6 μg DCA could not be seen. Likewise, no activity was found with 400 μg of IX.

Discussion. Our results demonstrate that removal of the methyl group at carbon 10 confers varying degrees of sodium retaining activity, depending upon specific structures of the steroids.

In our experience, 19-nor-desoxycorticosterone acetate shows 5.1 times the potency of DCA in sodium retention. Using the criterion of urinary sodium/potassium(13), this steroid has been reported by Sandoval, Miramontes, Rosenkranz, Djerassi and Sondheimer(14) to be approximately twice as active as desoxycorticosterone. Axelrod, Cates, Johnson and Luetscher(1) measured potencies of 1.9 and 1.5 times DCA using sodium and sodium/potassium responses, respectively. Altogether, these findings suggest increased activity for 19-nor-desoxycorticosterone, and in part do not appear consistent with the lack of survival activity reported by Ehrenstein (15).

The 19-nor-analogs appear to show stronger sodium retaining activity than the corresponding steroids with a methyl group at carbon 10. For example, 200 μg of Reichstein's Compound S produce an effect equivalent to about 8 μg DCA (*i.e.*, a potency of 0.04) (1,16). Its 19-nor-counterpart (II) is 0.25 times as potent as DCA by our measurements. Corticosterone is inactive at 10 to 400 μg doses according to the data of Johnson(16) and Marcus, Romanoff and Pincus(17). Results of our study show a potency of 0.06 (relative to DCA) for 19-nor-corticosterone (III). Likewise, while all observations on activity of hydrocortisone have proved negative(16,17), our studies show 19-nor-hydrocortisone (IV) to be 0.25 times as active as DCA.

The results with the last 5 steroids in Table I suggest that merely removing the methyl group at carbon 10 is not sufficient for increases in sodium retaining activity. Thus, structural and spatial alterations in the ketol side chain (V, VI, VIII and IX) and further changes in ring D (VII) do not give rise to activity. None of these steroids has as much activity as 6 μg DCA. It appears that a 19-nor-modification confers increasing activity only in steroids which show structurally a resemblance to the series of adrenocortical hormones.

Summary. 1. Sodium retaining activity of nine 19-nor-steroids was tested in adrenalectomized rats. 2. The 19-nor-analogs of desoxycorticosterone, Reichstein's Compound S, hydrocortisone and corticosterone showed 5.1, 0.25, 0.25 and 0.06 times the potency of desoxycorticosterone, respectively. 3. Sodium retention equal to that of 6 μg desoxycorticosterone was not detected in 5 other 19-nor-steroids, which showed less structural resemblance to adrenocortical steroids. 4. Our results indicate that varying degrees of activity will be found with removal of the methyl group at carbon 10, depending upon specific structure of the steroid.

1. Axelrad, B. J., Cates, J. E., Johnson, B. B., and Luetscher, J. A., Jr., *Endocrinol.*, 1954, v55, 568.

2. De Saulles, P., Tripod, J., and Schuler, W., *Schweiz. med. Wchnschr.*, 1953, v83, 1088.

3. Simpson, S. A., Tait, J. F., Wettstein, A., Neher, R., v. Euw, J., Schindler, O., and Reichstein, T., *Experientia*, 1954, v10, 132.

4. Borman, A., Singer, F. M., and Numerof, P., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 570.

5. Liddle, G. W., Pechet, M. M., and Bartter, F. C., *Science,* 1954, v120, 496.

6. Stafford, R. O., Barnes, L. E., Bowman, B. J., and Weinzinger, M. M., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 371.

7. Fried, J., Florey, K., Sabo, E. F., Herz, J. E., Restivo, A., Borman, A., and Singer, F. M., *J. Am. Chem. Soc.,* 1955, v77, 4181.

8. Liddle, G. W., Richard, J. E., and Tomkins, G. M., *Metabolism,* 1956, v5, 384.

9. Byrnes, W. W., Barnes, L. E., Bowman, B. J., Dulin, W. E., Morley, E. H., and Stafford, R. O., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 67.

10. Colton, F. B., Chinn, L. C., Ralls, J. W., and Riegel, B., unpublished data, 1956.

11. Colton, F. B., Nysted, L. N., and Riegel, B., *J. Am. Chem. Soc.,* in press, 1957.

12. Kagawa, C. M., Shipley, E. G., and Meyer, R. K., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 281.

13. Simpson, S. A., and Tait, J. F., *Endocrinol.,* 1952, v50, 150.

14. Sandoval, A., Miramontes, L., Rosenkranz, G., Djerassi, C., and Sondheimer, F., *J. Am. Chem. Soc.,* 1953, v75, 4117.

15. Ehrenstein, M., *J. Org. Chem.,* 1944, v9, 435.

16. Johnson, B. B., *Endocrinol.,* 1954, v54, 196.

17. Marcus, F., Romanoff, L. P., and Pincus, G., *ibid.,* 1952, v50, 286.

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Synthesis and Metabolism of Quinolinic Acid Ring Labeled with Tritium.* (22973)

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Since quinolinic acid was identified as a product of tryptophan metabolism in the rat (1), many experiments have been conducted to establish its role in tryptophan metabolism scheme(2). Isotope work has definitely proved that quinolinic acid was a product of tryptophan metabolism(3), and that 3-hydroxyanthranilic acid was converted to quinolinic acid in the rat(4). Various publications have suggested that in the sequence of reactions converting tryptophan to niacin, quinolinic acid functions as a side reaction product(5,6) and that quinolinic acid is not converted to nicotinic acid(7). Synthesis of quinolinic acid labeled in the ring with tritium has made it possible to obtain more dependable information regarding the role of quinolinic acid as an intermediary product in niacin formation. In our experiments labeled quinolinic acid was injected intraperitoneally into rats and labeled N^1-methylnicotinamide was isolated from the urine.

Methods. Preparation of 4,5,6 tritium-labeled quinolinic acid. The tritium ring labeled quinolinic acid was prepared by exposing a mixture of one gram of pure quinolinic acid (recrystallized 12 times) and 110 mg of lithium carbonate to a neutron flux of $1.8 \times 10^{12} n/cm^2/sec$ for 48 hours. This method of tritium-recoil labeling is described by Rowland and Wolfgang(8). After exposure in the nuclear reactor, the quinolinic acid, which had turned from white to tan in color, indicating some decomposition, was dissolved in a small volume of water. This solution was adjusted to pH slightly below 10 with sodium hydroxide to remove the tritium from the carboxyl groups by salt formation. After concentrating the solution to dryness under vacuum and redissolving in water, the quinolinic acid was purified by column chromatography through the following steps: adsorption onto norite at pH of 1.4, elution of norite with 0.1 *N* NH_4OH, concentration of eluate to dryness, extraction of concentrate with acid methanol, and subse-

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