

THE EFFECT OF CORTICOSTERONE AND RELATED
COMPOUNDS ON THE RENAL EXCRETION
OF ELECTROLYTES*

BY GEORGE W. THORN, M.D., LEWIS L. ENGEL,** Ph.D.,
AND HARRY EISENBERG

(From the Chemical Division, Medical Clinic, Johns Hopkins University and
Hospital, Baltimore)

(Received for publication, May 7, 1938)

In earlier studies (1-3) we have observed that subcutaneous injections of suprarenal cortical extract affected the renal excretion of sodium, chloride and potassium in normal subjects and in normal dogs as well as in suprarenalectomized dogs and patients suffering from Addison's disease. With the isolation of steroid compounds from the suprarenal cortex (Kendall, and his coworkers, Reichstein, Wintersteiner and Pfiffner, and Grollman (4)) it has become possible to test crystalline preparations for their effect on the renal excretion of electrolytes and to determine whether this effect parallels their potency in maintaining suprarenalectomized animals. In addition to crystalline compounds¹ derived from the suprarenal cortex we have tested a synthetic compound (desoxy-corticosterone acetate) prepared by Steiger and Reichstein (5).

Methods

Male dogs (approximately 10 kilos) were maintained in metabolism cages under constant dietary conditions. The routine care of the dogs and the conduct of the metabolic studies have been described elsewhere (3). Bilaterally suprarenalectomized dogs were used for the studies on suprarenal insufficient animals.²

* Aided by a grant from the Committee on Research in Endocrinology, National Research Council.

** John D. Archbold Fellow in Medicine.

¹ We are greatly indebted to Professor T. Reichstein of Zurich, who has provided the crystalline compounds used in this study.

² Under spinal anesthesia a bilateral suprarenalectomy was performed by Dr. Warfield M. Firor of the Department of Surgery, Johns Hopkins University and Hospital, who has perfected this operative technique. We wish to acknowledge our appreciation of his assistance and cooperation.

These animals were maintained on exactly the same regimen as the normal dogs, with the single exception that suprarenal cortical extract³ in maintenance doses was injected twice daily during the intervals between experiments. All of the animals were catheterized at the completion of each 24 hour period and the 24 hour urine specimens were collected and preserved with toluene. Specimens of blood were withdrawn under oil from the jugular veins, care being taken to avoid stasis. Urine and blood specimens were analyzed for sodium (6), chloride (7), potassium (8, 9), inorganic phosphate (10), total urine nitrogen and serum protein nitrogen (macro Kjeldahl), non-protein nitrogen (11), blood sugar (Folin-Wu) and CO₂ combining power of the serum (12).

One-half of the total daily quantity of extract or crystalline compound was injected at 10 a.m. and the remainder at 5 p.m. The possible effect of the solvents employed was controlled by experiments in which an equal quantity of either the saline solution or mazola oil was injected.

OBSERVATIONS

Suprarenal Cortical Extracts.—The injection of suprarenal cortical extracts produced a marked retention of sodium and chloride and an increased renal excretion of potassium in normal dogs (Table I). In this experiment 10 cc. of extract contained 10 mg. of solid material. An aliquot of this same preparation was extracted with ethyl acetate according to the method described by Grollman (13).⁴ This procedure yielded an inactive⁵ fraction containing 8 mg. of the original solid material and an active⁵ fraction containing 2 mg. of the original solids. The active fraction produced a marked decrease in the renal excretion of sodium and chloride when injected into a normal dog (Table II), whereas the inactive fraction failed to produce this effect (Table III). The injection of either the active or the inactive fraction produced a potassium diuresis (Tables II and III). It therefore appeared desirable to test possible contaminants of the extract for a potassium diuretic effect. Experiments in which freshly prepared dilute solutions of epinephrin (1:250,000) were injected demonstrate the effectiveness of this substance in producing both a potassium and

³ We are indebted to Dr. David Klein of the Wilson Laboratories, Chicago, Illinois, for the generous supply of suprarenal cortical extract which was provided for this study.

⁴ We are indebted to Dr. Arthur Grollman for carrying out this procedure and also for the gift of dehydro-corticosterone.

⁵ The use of the term active or inactive in this study, refers specifically to the effectiveness of the preparation in maintaining suprarenalectomized dogs.

TABLE I

The Effect of the Subcutaneous Injections of Suprarenal Cortical Extract (Aqueous Solution) on the Renal Excretion of Electrolytes in the Normal Dog (Dog 1)

24 hr. period	Urine volume	Sodium	Chloride	Potassium	Treatment
	cc.	m. eq.	m. eq.	m. eq.	
Control.....	455	51.6	49.8	16.5	10 cc. of suprarenal cortical extract*
Treated.....	545	30.5	34.0	21.3	
Control.....	625	86.6	79.2	10.3	

* 10 cc. of suprarenal cortical extract which contained a total of 10 mg. of solid material.

TABLE II

The Effect of the Subcutaneous Injections of the Active Fraction of Suprarenal Cortical Extract (Aqueous Solution) on the Renal Excretion of Electrolytes in the Normal Dog (Dog 2)

24 hr. period	Urine volume	Sodium	Chloride	Potassium	Treatment
	cc.	m. eq.	m. eq.	m. eq.	
Control.....	735	62.7	58.2	21.4	Active fraction of original extract*
Treated.....	490	49.2	50.8	26.1	
Control.....	640	64.2	62.0	17.6	

* This fraction represents a total of 2 mg. or 20 per cent of the solid material which the original extract contained.

TABLE III

The Effect of the Subcutaneous Injections of the Inactive Fraction of Suprarenal Cortical Extract (Aqueous Solution) on the Renal Excretion of Electrolytes in the Normal Dog (Dog 3)

24 hr. period	Urine volume	Sodium	Chloride	Potassium	Treatment
	cc.	m. eq.	m. eq.	m. eq.	
Control.....	540	51.1	56.0	15.8	Inactive fraction of original extract*
Treated.....	585	54.4	58.8	18.7	
Control.....	585	55.0	59.0	13.9	

* This fraction represents a total of 8 mg. or 80 per cent of the solid material which the original extract contained.

a sodium diuresis (Table IV). It appears that in suprarenal cortical extracts the sodium and chloride retaining effect follows the cortical hormone activity, whereas the potassium diuresis may be due to the effect of at least two substances, *i.e.* suprarenal cortical hormone and traces of epinephrin.

Crystalline Compounds Derived from the Suprarenal Cortex.—It has been reported by Reichstein *et al.* (14) and Kendall *et al.* (15) that the

TABLE IV

The Effect of the Subcutaneous Injections of a Dilute Solution of Epinephrin (Aqueous Solution) on the Renal Excretion of Electrolytes in the Normal Dog (Dog 4)

24 hr. period	Urine volume	Sodium	Chloride	Potassium	Treatment
	<i>cc.</i>	<i>m. eq.</i>	<i>m. eq.</i>	<i>m. eq.</i>	
Control.....	570	54.2	55.6	7.5	Epinephrin*
Treated.....	555	66.3	57.8	15.6	
Control.....	530	43.9	46.9	8.2	

* 12.5 cc. of a 1:250,000 solution of epinephrin, freshly prepared, were injected twice daily.

TABLE V

The Effect of the Subcutaneous Injections of Corticosterone in Oil on the Renal Excretion of Electrolytes in the Normal Dog (Dog 1)

24 hr. period	Urine volume	Sodium	Chloride	Potassium	Treatment
	<i>cc.</i>	<i>m. eq.</i>	<i>m. eq.</i>	<i>m. eq.</i>	
Control.....	450	54.3	55.2	19.0	4 mg. corticosterone*
Treated.....	515	45.8	48.9	22.7	
Control.....	635	64.9	63.3	16.0	

* In 3 cc. of mazola oil.

crystalline compounds, corticosterone (Δ^4 -pregnene-11, 21-diol-3, 20-dione) and dehydro-corticosterone (Δ^4 -pregnene-21-ol-3, 11, 20-trione) maintain suprarenalectomized animals in good condition. The injection of corticosterone (4 mg.) into a normal male dog resulted in a marked decrease in the renal excretion of sodium and chloride and an increased potassium excretion (Table V). Dehydro-corticosterone was found to produce a similar effect in approximately the same dosage (Chart 1). The effect of these two compounds on the renal

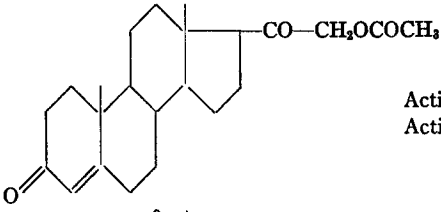
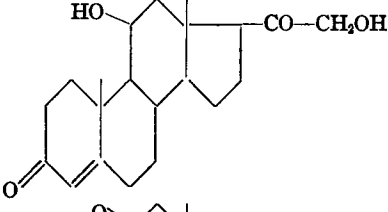
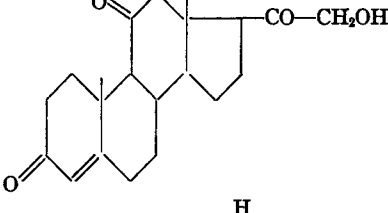
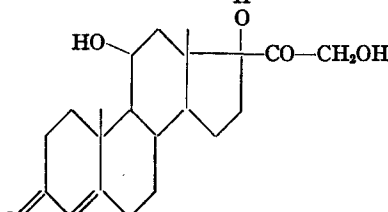
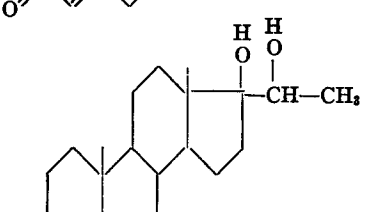
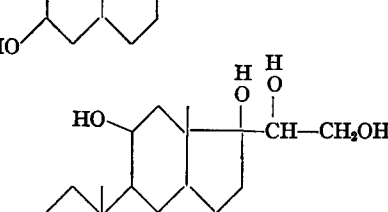
<i>Compound</i>	<i>Structure</i>	<i>Activity</i>	<i>Sodium Retaining Effect in Normal Dog</i>
Desoxy-corticosterone acetate		Active (A) Active (B) 0.3 mg.	Present (0.5-1 mg.)
Corticosterone		Active (A) Active (B) 0.8 mg.	Present (4 mg.)
Dehydro-corticosterone		Active (A) Active (B) > 0.8 mg.	Present (4 mg.)
Compound M		Active (B) 1.5 mg.	Absent (8 mg.)
Compound J		Inactive (B) 2.0 mg.	Absent (10 mg.)
Compound A		Inactive (A) Inactive (B) 2.0 mg.	Absent (10 mg.)

CHART 1. The correlation between suprarenal cortical hormone activity and sodium and chloride retaining effect.

A, maintenance of suprarenalectomized dog.

B, Everse-de Fremery test.

excretion of sodium, chloride and potassium was similar to that noted when suprarenal cortical extract was injected.

Three other crystalline compounds derived from the suprarenal cortex have been tested on normal dogs (Chart 1). Allopregnane-3, 11, 17, 20, 21-pentol (compound A, Reichstein and Wintersteiner, compound D, Kendall), which has been shown previously to be inactive in both the Everse-de Fremery test in doses of 2 mg. per day (16, 17) and the suprarenalectomized dog (18), was found to have no sodium and chloride retaining effect when injected in doses up to 10 mg. Allopregnane-3, 17, 20-triol (compound J, Reichstein) (19), which is also inactive in the Everse-de Fremery test in doses of 2 mg. daily (17), had no sodium and chloride retaining effect when injected in doses up to 10 mg. The injection of Δ^4 -pregnene-11, 17, 21-triol-3, 20-dione (compound M, Reichstein) did not produce a sodium and chloride retention in doses up to 8 mg. although it appears to be active in the Everse-de Fremery test in doses of 1.5 mg. daily (17). No reports are available as to the potency of this compound in maintaining suprarenalectomized dogs. In the normal dog the injection of this compound consistently increased the renal excretion of potassium in the higher doses (5 and 8 mg.).

A Synthetic Compound, Desoxy-Corticosterone Acetate.—Desoxycorticosterone acetate (Δ^4 -pregnene-21-ol-3, 20-dione acetate, 21-acetoxy-progesterone) has been prepared by Steiger and Reichstein (5) from stigmaterol. This substance has been shown by Reichstein to be capable of maintaining suprarenalectomized dogs, and in a dosage of 0.3 mg. daily gives a positive Everse-de Fremery test (17). A single injection of 1 mg. of desoxy-corticosterone acetate produced a very marked retention of sodium and chloride and an increased renal excretion of potassium in a normal dog (Table VI). In this respect desoxy-corticosterone acetate appeared to be much more active than corticosterone or dehydro-corticosterone (Chart 1).

Further studies with desoxy-corticosterone acetate were carried out on a suprarenalectomized dog (Chart 2).

When a quantity of suprarenal cortical extract equivalent to 600 gm. of fresh cortex per day was injected, the 24 hour renal excretion of sodium and chloride amounted to 40 and 45 m.eq. respectively (intake 62 m.eq. of sodium and 63 m.eq. of chloride) and the animal gained weight rapidly. Desoxy-corticosterone acetate

(1 mg. daily) was then substituted for the suprarenal cortical extract. The injection of this quantity of synthetic substance was associated with a renal excretion of 48 m.eq. of sodium and 52 m.eq. of chloride, the animal apparently being in positive sodium and chloride balance and the weight increase continuing. During the 7 days of treatment with desoxy-corticosterone acetate, the animal ate well, appeared well, gained weight (0.35 kilo), maintained normal blood levels of sugar, non-protein nitrogen, serum sodium and chloride, CO₂ combining power and plasma volume (hematocrit and serum protein). On the 8th day the treatment was discontinued; diuresis was noted during the first 24 hours and the renal excretion of sodium and chloride rose from an average daily level of 48 and 52 m.eq. respectively to 70 and 85 m.eq. respectively. The excretion of potassium and phosphate was decreased. The animal lost weight (0.25 kilo) and on the morning of the 3rd day appeared to be weak and refused food. At this time it was

TABLE VI

The Effect of the Subcutaneous Injection of Desoxy-Corticosterone Acetate in Oil (1 Mg. Given in a Single Injection) on the Renal Excretion of Electrolytes in the Normal Dog (Dog 1)

24 hr. period	Urine volume	Sodium	Chloride	Potassium	Treatment
	cc.	m. eq.	m. eq.	m. eq.	
Control.....	500	60.0	58.8	16.8	1 mg. of desoxy-corticosterone acetate*
Treated.....	525	41.4	49.1	21.4	
Control.....	715	71.6	66.8	16.9	

* In 2 cc. of mazola oil.

found that the non-protein nitrogen of the blood had risen from its original level of 30 mg. per cent to 60 mg. per cent, the cell volume (hematocrit) had increased from 30 per cent to 40 per cent and the serum concentration of sodium, chloride and bicarbonate were decreased, the serum sodium falling from a level of 150 m.eq. to 130 m.eq. per liter of serum (20, 21). The fasting blood sugar level was maintained during this period. Since a crisis appeared to be impending, the animal was given 1 mg. of desoxy-corticosterone acetate daily for 3 days. During this period a marked decrease in urine volume was observed, associated with a retention of sodium and chloride. The excretion of potassium and phosphate was increased. No further weight loss was observed and the animal's appetite and strength improved markedly. The non-protein nitrogen of the blood, the plasma volume (hematocrit and serum protein) and the serum concentration of sodium and chloride returned to normal. Further experiments of this nature were impossible at this time because of the very limited supply of desoxy-corticosterone acetate. The animal was then maintained on suprarenal cortical extract. After a period of

10 days on extract during which the animal appeared to be in excellent condition, extract was discontinued and the animal died 3 days later, thus establishing the fact that suprarenalectomy had been complete (as shown by autopsy) and that it was not possible to maintain the animal on the routine metabolic regimen without the addition of cortical extract.

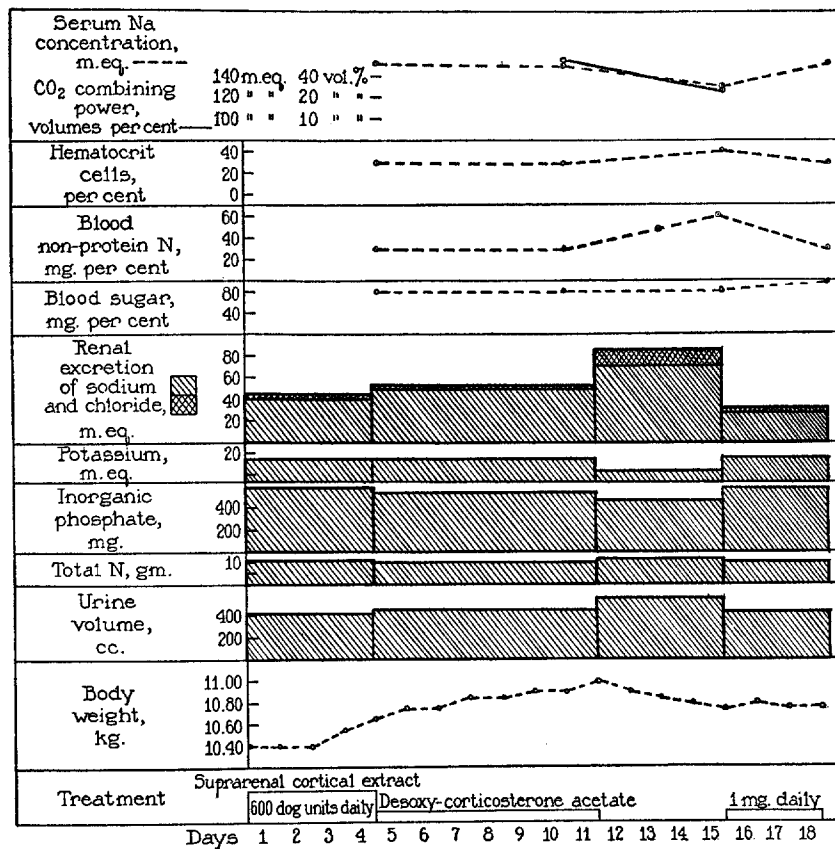


CHART 2. The effect of the subcutaneous injection of desoxy-corticosterone acetate (1 mg. daily as a single injection in oil) on the concentration of blood constituents and on the renal excretion of electrolytes in the bilaterally suprarenalectomized dog.

Dog 5 was maintained on a constant mineral and fluid intake throughout the experiment. The diet contained 62 m.eq. of sodium, 63 m.eq. of chloride, and 30 m.eq. of potassium. 1 mg. of desoxy-corticosterone acetate was taken up in 1 cc. of mazola oil.

Following this experiment the animal was maintained on suprarenal cortical extract for a period of 10 days. Extract was then discontinued and the death of the animal occurred 3 days later.

It would appear from this experiment that replacement of cortical extract with the synthetic compound was complete during the short period of observation.

In studying the possible activity of compounds derived from the suprarenal cortex it is of interest to note that in the suprarenalectomized dog maintained under constant metabolic conditions the renal excretion of electrolytes furnishes an extremely sensitive index of adequate therapy (22). Within 24 hours after the reduction or withdrawal of an active preparation a marked diuresis occurs associated with a considerable loss of sodium and chloride. This change usually precedes the rise in non-protein nitrogen, and occurs while the animal still appears to be in excellent condition.

DISCUSSION

The sodium and chloride retaining effect which has been observed upon injecting suprarenal cortical extract into normal human subjects (1, 2) and normal dogs (3) appears to be due to the cortical hormone present in the extract. Of the crystalline compounds derived from the suprarenal cortex only those which have been shown to be capable of maintaining the life of suprarenalectomized animals have produced a sodium and chloride retention when injected into normal dogs in the dosage described. Compound M, which is active as measured by the Everse-de Fremery test failed to give a sodium and chloride retention when injected in quantities up to 8 mg. Desoxy-corticosterone acetate, a synthetic compound, which has been shown to be capable of maintaining suprarenalectomized dogs and rats gave a very striking sodium and chloride retaining effect when 1 mg. was injected into a normal dog. The sodium and chloride retention induced by both the extract and crystalline compounds is uniformly characteristic and does not appear to depend upon the medium used for injection, as aqueous and oil solutions have given similar responses. The striking characteristics of this response are the marked retention of sodium and chloride and the increased excretion of potassium which take place on the day of injection; and the rebound (Tables I, II, V, and VI) which occurs during the second 24 hour period. This response is not modified qualitatively by increasing the dosage of the substance injected.

Crystalline compounds which are active uniformly induce a potassium diuresis when injected into normal dogs. It appears that this

may constitute a property of the suprarenal cortical hormone although the potassium diuresis induced by the injection of suprarenal cortical extracts may be due in part to traces of epinephrin.

SUMMARY

The sodium and chloride retaining effect of suprarenal cortical extracts and of crystalline compounds derived from the suprarenal cortex parallels their effectiveness in maintaining suprarenalectomized dogs. All of the active compounds thus far studied produce a potassium diuresis when injected into normal dogs. The injection of a synthetic compound, desoxy-corticosterone acetate, produced in normal dogs a very marked sodium and chloride retention and a potassium diuresis. In a suprarenalectomized dog desoxy-corticosterone acetate was substituted successfully for suprarenal cortical extract.

BIBLIOGRAPHY

1. Thorn, G. W., Garbutt, H. R., Hitchcock, F. A. and Hartman, F. A., *Endocrinology*, 1937, **21**, 202.
2. Thorn, G. W., *Proc. Soc. Exp. Biol. and Med.*, 1937, **36**, 361.
3. Harrop, G. A., and Thorn, G. W., *J. Exp. Med.*, 1937, **65**, 757.
4. Reichstein, T., *Ergebnisse der Vitamin-und Hormonforschung*, Leipsic, Akademische Verlagsgesellschaft, 1938, **1**, 335. [Review.]
5. Steiger, M., and Reichstein, T., *Nature*, 1937, **139**, 925; *Helv. Chim. Acta*, 1937, **20**, 1164.
6. Butler, A. M., and Tuthill, T., *J. Biol. Chem.*, 1931, **93**, 171.
7. Peters, J. P., and Van Slyke, D. D., *Quantitative clinical chemistry*. Volume II, Methods, Baltimore, The Williams & Wilkins Co., 1932, 829.
8. Shohl, A. T., and Bennett, H. B., *J. Biol. Chem.*, 1928, **78**, 643.
9. Strauss, M. B., *J. Biol. Chem.*, 1937, **118**, 331.
10. Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1925, **66**, 375.
11. Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, **38**, 81.
12. Van Slyke, D. D., and Cullen, G. E., *J. Biol. Chem.*, 1917, **30**, 289. Van Slyke, D. D., *J. Biol. Chem.*, 1917, **30**, 347. Van Slyke, D. D., and Stadie, W. C., *J. Biol. Chem.*, 1921, **49**, 1.
13. Grollman, A., in *Cold Spring Harbor symposia on quantitative biology*, Cold Spring Harbor, Long Island Biological Association, 1937, **5**, 317.
14. de Fremery, P., Laqueur, E., Reichstein, T., Spanhoff, R. W., and Uyldert, I. E., *Nature*, 1937, **139**, 26. Reichstein, T., *Helv. Chim. Acta*, 1937, **20**, 953.

15. Mason, H. L., Myers, C. S., and Kendall, E. C., *J. Biol. Chem.*, 1936, **114**, 613.
Kendall, E. C., Mason, H. L., Hoehn, W. M., and McKenzie, B. F., *Proc. Staff Meetings Mayo Clinic*, 1937, **12**, 136.
16. Reichstein, T., *Helv. Chim. Acta*, 1936, **19**, 29.
17. Reichstein, T., 1938, personal communication.
18. Wintersteiner, O., and Pffinner, J. J., *J. Biol. Chem.*, 1935, **111**, 599.
19. Steiger, M., and Reichstein, T., *Helv. Chim. Acta*, 1938, **21**, 546.
20. Loeb, R. F., Atchley, D. W., Benedict, E. M., and Leland, J., *J. Exp. Med.*, 1933, **57**, 775.
21. Harrop, G. A., Soffer, L. J., Ellsworth, R., and Trescher, J. R., *J. Exp. Med.*, 1933, **58**, 17.
22. Thorn, G. W., Emerson, K., Jr., and Eisenberg, H., *Endocrinology*, in press.