almost completely excreted within a few days.

Summary. After intravenous administration of heparin-S^{35} to dogs, it is rapidly cleared from the bloodstream. The highest radioactivity is found in liver and lung. However, no measurable radioactivity is detected in any organ after 48 hours.

1. Jorpes, J. E., *Heparin,* Oxford Univ. Press, 1946.
2. Eiber, H. B., Danishefsky, I., *J. Biol. Chem.,* 1957, v226, 721.
3. ———, *Nature,* 1957, v180, 1359.
4. Cho, M. H., Jacques, L. B., *Can. J. Biochem. and Physiol.,* 1956, v34, 799.

Received May 28, 1958. P.S.E.B.M., 1958, v98.

Evidence for Conversion of Corticosterone to Aldosterone in Man.* (24148)

HOLBROOKE S. SELTZER AND DALE A. CLARK (Introduced by Donald W. Seldin)
Department of Internal Medicine, University of Texas Southwestern Medical School, and Vet. Admin. Hospital, Dallas

Although corticosterone (compound B) might reasonably be considered a biosynthetic precursor of aldosterone (18-aldehydo-corticosterone), conversion of the former steroid to the latter has not previously been demonstrated in man. Moreover, studies using animal tissues have yielded conflicting results. Rosemberg and Dorfman(1) found only inconsequentially increased secretion of aldosterone-like material by calf adrenals perfused with corticosterone. Wettstein(2) reported actual suppression of aldosterone synthesis when beef adrenal homogenates were incubated with compound B. In contrast, upon incubating beef adrenal capsule strippings with corticosterone-4-C^{14}, Travis and Farrell recently isolated aldosterone having a molar specific activity 54% that of the labeled corticosterone(3). The present study revealed that prolonged, continuous infusion of corticosterone† to normal man simultaneously caused sharply increased urinary aldosterone and profound depression of 17-hydroxycorticosteroids. The alterations in steroid excretion were accompanied by marked shifts in urinary electrolytes, but no metabolic evidence of enhanced glucocorticoid activity.

Material and methods. Three male patients, 33 to 43 years of age, having no organic disease were studied. During a 3-day control period on hospital diet, daily sodium and potassium excretions remained essentially constant. On days 4 and 5 corticosterone was administered continuously by vein, patient A receiving 200 mg daily and patients B and C receiving 300 mg/24 hours. Daily steroid was infused in 2000 ml of 5% glucose in water, except that patient C inadvertently received his first 300 mg of compound B in 2000 ml of 0.9% NaCl. Aliquots of 24-hour collections of urine were analyzed by flame photometry for sodium and potassium, and by standard methods for glucose,‡ creatinine(4) and uric acid(5). Urinary aldosterone was assayed by the physico-chemical method of Neher and Wettstein(6), and 17-hydroxycorticosteroids by the Porter-Silber procedure(7).

Results. Qualitatively similar changes were shown by all 3 patients during and after perfusion with corticosterone (Table I). Excretion of aldosterone at least doubled during steroid administration, then fell to sub-baseline values in the recovery period before regaining normal levels. Mean daily aldosterone titers for the group were 8.6 μg in the control period, 20.0 μg at peak excretion during corticosterone infusion, and 6.1 μg on the second day of recovery. Simultaneous mineralocorticoid effects were evidenced by a sharp fall in Na/K ratio during the infusion period (from mean control value of 1.2 to minimum of 0.4), the decrease reflecting

* This study was supported in part by research grant from U.S.P.H.S.

† Corticosterone was generously supplied by Upjohn Co.

‡ "Glucostat," Worthington Biochemical Corp., Freehold, N. J.

TABLE I. Daily Urinary Excretion of Aldosterone (μg), 17-Hydroxycorticosteroids (mg), Sodium and Potassium (Na/K) during and after Administration of Corticosterone Intravenously.

		Patient A (200)*			Patient B (300)			Patient C (300)			Mean		
Day	Period	Aldo	Na/K	17-OH	Aldo	Na/K	17-OH	Aldo	Na/K	17-OH	Aldo	Na/K	17-OH
1–3	Control	5.0	1.0	10.7	11.8	1.0	6.2	8.9	1.6	10.5	8.6	1.2	9.1
4	Corticos-terone	7.6	.6	4.2	21.3	.3	2.6	31.2	.3	3.5	20.0	.4	3.4
5	"	12.8	.9	1.4	15.6	.4	.4	15.7	1.5†	1.4	14.7	.9	1.1
6	Recovery	6.6	3.5	7.1	10.2	2.0	7.1	14.1	5.0	11.6	10.3	3.5	8.6
7	"		2.8	8.8	7.2	6.2	6.6	5.0	5.4	18.0	6.1	4.8	11.1
8	"				6.7	6.7	6.6	9.5	4.8	13.0	8.1	5.8	9.8

* Parenthesized figures designate mg corticosterone administered/24 hr.
† This high value followed inadvertent infusion 2000 ml 0.9% NaCl on previous day.

marked sodium retention coupled with moderate potassium diuresis. Reciprocal and actually excessive shifts in sodium and potassium excretion, appearing immediately after stopping corticosterone and persisting throughout the recovery period, increased mean Na/K to 3.5 on the first post-infusion day. In contrast to its striking enhancement of mineralocorticoid activity, exogenous corticosterone appeared to suppress adrenal secretion of glucocorticoids, since average daily output of 17-hydroxysteroids plummeted from the control level of 9.1 mg to a minimum of 1.1 mg on the second day of infusion. However, prompt resumption of normal 17-hydroxycorticoid excretion (8.6 mg) appeared on the first day of recovery. Daily urinary glucose and uric acid/creatinine ratio showed essentially no changes from control values either during or after administration of corticosterone (data not shown).

The patterns of aldosterone excretion by both patients who received 300 mg of corticosterone daily exhibited identical features of special interest (Table I). Maximal increases of urinary aldosterone were attained on the first day of infusion (from control values of 11.8 and 8.9 to 21.3 and 31.2 μg per day, respectively). During the second day of sustained high-dosage perfusion with corticosterone, respective excretions unexpectedly fell to 15.6 and 15.7 μg. Aldosterone titers then continued to decline progressively after the infusion was stopped, and reached sub-baseline values of 7.2 and 5.0 μg on the second day of recovery. Despite seemingly premature decrease in aldosterone excretion, profound reduction of both urinary Na/K and 17-hydroxycorticoids persisted until administration of corticosterone was discontinued, whereupon they rebounded vertically within 24 hours to supra-normal and normal values, respectively.

Discussion. The present data strongly suggest that normal man is capable of oxygenating corticosterone to aldosterone. Considerable fortifying evidence is required, however, before corticosterone commands serious attention as a physiological precursor of aldosterone. It must first be shown that the material behaving like aldosterone in the Neher-Wettstein chromatographic systems is in fact aldosterone. Comparison with authentic aldosterone by infra-red spectrophotometry awaits satisfactory purification of representative eluates. Secondly, the infused corticosterone must be certified as the actual source of the accompanying increment in urinary aldosterone, in order to exclude indirect cause-and-effect relationships. The *in vitro* studies of Travis(3) suggest that direct transformation of exogenous compound B to aldosterone should be demonstrable by isolation of labeled aldosterone from urine following administration of tracer corticosterone-4-C^{14}. Lastly, with respect to the postulated oxygenation of compound B to aldosterone, present data do not elucidate the anatomical site of conversion. Although the adrenal cortex attracts primary attention, an extra-adrenal locus (*e.g.*, the liver) remains a possibility. Studies in the adrenalectomized patient are clearly necessary.

Administration of exogenous corticosterone

may have directly suppressed endogenous synthesis of aldosterone. This is one interpretation of the fall in urinary aldosterone during the second day of infusion with compound B, followed by sustained depression of aldosterone output for 2 days or more after corticosterone was discontinued. An alternative explanation is that decreased endogenous aldosterone secretion merely represented a compensatory response to marked sodium retention induced by corticosterone itself. Final judgment will depend upon the pattern of aldosterone excretion during administration of corticosterone to a subject on very low sodium intake.

The essentially constant excretion rates of glucose and uric acid during and after administration of corticosterone imply that the latter was devoid of glucocorticoid activity. This negative finding was surprising in view of Conn's detailed studies showing that compound B not only exerted mild effects upon organic metabolism in normal subjects, but was able to maintain patients with Addison's disease in electrolyte, carbohydrate and nitrogen balance(8).

Finally, the present data indicate that continuous high-dosage infusion of corticosterone suppressed endogenous corticoid secretion, either by inhibiting pituitary release of corticotrophin or by blocking formation of 17-hydroxysteroids within the adrenal cortex at a biosynthetic step proximal to 21-hydroxylation of 17-hydroxyprogesterone. In any event, corticosterone was not transformed to hydrocortisone (*i.e.*, Porter-Silber chromogens), a finding which supports the thesis that existence of 11-β-hydroxylation blocks subsequent hydroxylation at the C-17 position(9).

Summary. Continuous administration by vein of 300 mg of corticosterone daily for 48 hours to 2 normal men caused sharp rise in urinary aldosterone, retention of sodium, and depression of urinary 17-hydroxycorticosteroids the first day. On the second day aldosterone values began to fall, while sodium retention persisted and 17-hydroxysteroid output was negligible. During the recovery period aldosterone excretion remained below baseline levels for 2 or 3 days, whereas both profuse sodium diuresis and normal 17-hydroxycorticoid excretion appeared promptly on the first day. Essentially similar but less intense effects were exhibited by 1 patient who received 200 mg of corticosterone daily for 2 days. The data suggest that normal man is capable of performing 18-oxygenation of corticosterone to aldosterone. Should further study establish that this conversion occurs within the adrenal cortex, the tentative thesis will be fortified that corticosterone is a physiological precursor in the biosynthesis of aldosterone.

The technical assistance of Melba Loveall, Mildred Brennan, Anton Boer, and Virgil Doty is gratefully acknowledged.

1. Rosemberg, E., Rosenfeld, G., Ungar, F., Dorfman, R. I., *Endocrinol.*, 1956, v58, 708.

2. Wettstein, A., Anner, G., *Experientia,* 1954, v10, 397.

3. Travis, R. H., Farrell, G. L., *Fed. Proc.,* 1958, v17, 324.

4. Bonsnes, R. W., Taussky, H. H., *J. Biol. Chem.,* 1945, v158, 581.

5. Forsham, P. H., Thorn, G. W., Prunty, F. T. G., Hills, A. G., *J. Clin. Endocrinol.,* 1948, v8, 15.

6. Neher, R., Wettstein, A., *J. Clin. Invest.,* 1956, v35, 800.

7. Silber, R. H., Porter, C. C., *J. Biol. Chem.,* 1954, v210, 923.

8. Conn, J. W., Fajans, S. S., Louis, L. H., Johnson, B., *Proc. 2nd Clin. ACTH Conference,* vI, 1951, Blakiston Co., Philadelphia, 221.

9. Hechter, O., Pincus, G., *Physiol. Revs.,* 1954, v34, 459.

Received June 2, 1958. P.S.E.B.M., 1958, v98.