ISOLATION OF CRYSTALLINE ALDOSTERONE
FROM THE URINE OF A CHILD WITH THE
NEPHROTIC SYNDROME*

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In 1950, Deming and Luetscher (1, 2) found that the urine of patients with the nephrotic syndrome or with heart failure showed unusual sodium-retaining activity when assayed in adrenalectomized rats. Hyman (3) observed that the biologically active material was more polar than deoxycorticosterone. Luetscher and Johnson (4) described the chromatographic separation of the highly active, sodium-retaining corticoid from the urine of children with nephrosis.

In 1952, Grundy, Simpson, and Tait (5) concentrated a potent mineralocorticoid, which was tentatively called electrocortin, in adrenal cortical extract. Subsequently, Simpson, Tait, Wettstein, Neher, von Euw, Schindler, and Reichstein (6, 7) have crystallized the active material and characterized it as the 11,18-hemiacetal of 18-oxocorticosterone ("aldosterone").

It has been noted (8) that the sodium-retaining corticoid of urine resembled aldosterone. In order to compare the substances more closely, crystalline aldosterone prepared from adrenal cortical extract and purified but amorphous sodium-retaining corticoid from human urine have been exchanged. Certain comparisons of these materials are reported here.

Methods

Urine was collected for 13 days from a 10 year-old boy having the characteristic evidences of the nephrotic syndrome. No steroid or corticotropin had been administered. The whole collection of 14.5 liters contained less than 1 m.eq. of sodium per liter.

Extract A was made by acidifying the urine to pH 1.0 with concentrated

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1 For detection and measurement of aldosterone in urine, see also Neher and Wettstein (9).
hydrochloric acid and then extracting with four aliquots of chloroform, each 15 to 20 per cent by volume. This procedure was completed in 40 minutes.

Extract B was made by allowing the same urine, previously extracted, to stand over chloroform (15 per cent by volume) for 24 hours at 20°, with occasional shaking. The urine was then extracted with four aliquots of chloroform, each 15 to 20 per cent by volume.

### Table I

<table>
<thead>
<tr>
<th>Starting material</th>
<th>Chromatographed in</th>
<th>Fraction assayed</th>
<th>Dose</th>
<th>Bioassay response† estimated from</th>
<th>Na output</th>
<th>K:Na ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extract A</td>
<td>Toluene-propylene glycol</td>
<td>Extract A</td>
<td>20</td>
<td>12.1 ± 4.5</td>
<td>8.4 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>Fraction 10</td>
<td>Bush System B₄</td>
<td>Fraction 10§</td>
<td>20</td>
<td>1.7</td>
<td>6.9 ± 4.2</td>
<td>6.3 ± 2.3</td>
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<tr>
<td></td>
<td>“ 4</td>
<td>“ 4</td>
<td>25</td>
<td>0.80</td>
<td>5.9 ± 4.2</td>
<td>6.0 ± 2.3</td>
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<tr>
<td></td>
<td>“ 5</td>
<td>“ 4</td>
<td>33</td>
<td>0.21</td>
<td>4.2 ± 2.3</td>
<td></td>
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<tr>
<td>Urine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extract B</td>
<td>Toluene-propylene glycol</td>
<td>Extract B</td>
<td>20</td>
<td>11.1 ± 4.2</td>
<td>10.4 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>Fraction 10</td>
<td>Bush System B₅</td>
<td>Fraction 10</td>
<td>20</td>
<td>4.8</td>
<td>10.3 ± 3.0</td>
<td>10.8 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>“ 5</td>
<td>“ 5</td>
<td>25</td>
<td>3.0</td>
<td>12.1 ± 3.9</td>
<td>11.4 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>“ 10</td>
<td>“ 5</td>
<td>20</td>
<td>1.9</td>
<td>10.0 ± 3.9</td>
<td>9.1 ± 2.2</td>
</tr>
<tr>
<td>Fraction 10</td>
<td>Crystalline aldosterone</td>
<td>“ diacetate</td>
<td>2.2</td>
<td>6.9 ± 4.2</td>
<td>5.0 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>Acetylated</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

* The bioassays were performed by B. J. Axelrad, R. H. Curtis, and Way Lew.
† α,β-Unsaturated ketone estimated by ultraviolet absorption at 240 mμ.
‡ Micrograms as equivalent dose of DCA (±95 per cent confidence limits).
§ Only the active fraction is shown; significant activity was not found in other fractions. The position of each active fraction is shown in Figs. 1 and 2.

Each extract was washed with cold sodium hydroxide solution (0.1 n) and then with cold water. After drying over anhydrous sodium sulfate, chloroform was removed under vacuum at a temperature below 40°. The residue was dissolved in ethanol and stored at 5°.

Bioassay was performed as described by Johnson (10). The result is expressed as the equivalent dose of deoxycorticosterone acetate (DCA).

The extracts were chromatographed by the methods of Burton, Zaffaroni, and Keutmann (11) and Bush (12). Photographic prints were made in ultraviolet light (254 mμ), as suggested by Haines (13).
Preparation of Aldosterone from Urine Extract A

Bioassay of Extract A showed marked effects on sodium and potassium excretion (Table I). When the extract was chromatographed in toluene and propylene glycol, the biological activity was found in Fraction 10, which moved at about the same rate as cortisone. This material was then rechromatographed in benzene and aqueous methanol (System B₆, Bush (12)) and in toluene, light petroleum, and aqueous methanol (System B₇). In these two systems, a fraction moving between cortisone and hydrocorti-

<table>
<thead>
<tr>
<th>Method*</th>
<th>Aldosterone (crystalline)</th>
<th>Sodium-retaining corticoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioassay, excretion of Na (10)</td>
<td>Potency 30 times that of DCA (weight for weight)</td>
<td>Potency 20 times that of DCA (weight for weight)</td>
</tr>
<tr>
<td>Ultraviolet absorption</td>
<td>Maximum at 240 μ in 95% ethanol</td>
<td>Maximum at 238 μ in 95% ethanol</td>
</tr>
<tr>
<td>Yellow fluorescence after heating with NaOH (12)</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Blue tetrazolium (15)</td>
<td>Reduced</td>
<td>Reduced</td>
</tr>
<tr>
<td>Chromatography; R_P</td>
<td>Slightly faster than cortisone</td>
<td>Slightly faster than cortisone</td>
</tr>
<tr>
<td>Toluene-propylene glycol (11)</td>
<td>Intermediate between cortisone and hydrocortisone</td>
<td>Intermediate between cortisone and hydrocortisone</td>
</tr>
<tr>
<td>Systems B₂ and B₆ (12)</td>
<td>Near hydrocortisone</td>
<td>Near hydrocortisone</td>
</tr>
<tr>
<td>System C (12)</td>
<td>Like cortisone acetate</td>
<td>Like cortisone acetate</td>
</tr>
<tr>
<td>After acetylation</td>
<td>Like 11-dehydrocorticosterone acetate</td>
<td>Like 11 dehydrocorticosterone acetate</td>
</tr>
<tr>
<td>Monoacetate; R_P in benzene-formamide (11)</td>
<td>Potency greatly reduced</td>
<td>Potency greatly reduced</td>
</tr>
<tr>
<td>Diacetate, R_P in benzene-formamide</td>
<td>Lactone-hemiacetal of 3,18-dioxo-11-hydroxy etilen-4-ic acid</td>
<td>Like neutral steroid</td>
</tr>
<tr>
<td>Bioassay (10)</td>
<td>Peak at 240 μ</td>
<td>Peak at 240 μ</td>
</tr>
<tr>
<td>After oxidation with KIO₄†</td>
<td>Not reduced</td>
<td>Not reduced</td>
</tr>
<tr>
<td>Chief product (8)</td>
<td>Slowly than adrenosterone</td>
<td>Identical</td>
</tr>
<tr>
<td>Ultraviolet absorption</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue tetrazolium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromatography; R_P in benzene-cyclohexane-formamide</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The figures in parentheses refer to the bibliography.
† By a microtechnique kindly suggested by V. Mattox and H. L. Mason.

Table II
Comparison of Aldosterone from Adrenal Cortical Extract and of Sodium-Retaining Corticoid from Urine Extract A
sone was found to contain the active substance. About one-third of the total sodium-retaining activity of the urine extract was recovered in the final preparation. Since the quantity was small, the micromethods suggested by Zaffaroni (14) and Bush (12) were employed.

When this material was compared with aldosterone (Table II, Figs. 1 and 2), the two preparations behaved similarly in all respects. The only differences of possible significance were in the wave-length of maximal ultraviolet absorption and in the bioassay. These differences may have been caused by impurities in the urine preparation.

**Preparation of Crystalline Aldosterone from Urine Extract B**

Extract B showed strong sodium-retaining activity on bioassay (Table I). The active material was chromatographed in toluene and propylene glycol and in Bush’s System B, with little loss of biological activity. Since a preliminary chromatogram in Bush’s System B showed no further purification, this step was omitted. When the material was run again in toluene and propylene glycol, a homogeneous fraction was obtained. This substance behaved like aldosterone in all respects on chromatography, but was different from the preparation from Extract A in several respects. The absorption maximum was higher, appearing regularly at 240 to 241 m\(\mu\).
When the conventional extinction coefficient was employed (14), the ultraviolet absorption indicated the presence of material of lower biological activity. Acetylation yielded two new substances, whose mobilities were

**MONO-ACETATE**

- E
- Eac
- Aac
- DCA

**DI-ACETATE**

- 16α-OH-DOC
- 16α-OH-DOC

**BENZENE : FORMAMIDE**

Fig. 2. Descending chromatogram of aldosterone after acetylation. The material from urine is chromatographed on Strip 1, and that from adrenal cortical extract on Strip 2. The monoacetate moves near cortisone acetate (Eac). The diacetate moves near 11-dehydrocorticosterone acetate (Aac). The acetates of 11-deoxycorticosterone (DCA) and its 16α-hydroxy derivative (16α-OH-DOC) are also shown.

identical with the mono- and diacetates of aldosterone. When acetic anhydride labeled with C14 was used in one experiment, the specific activity of the diacetate was found to be higher than that of the monoacetate, as expected (6). Since the quantity of steroid (1.8 mg.) remaining after these preliminary tests seemed adequate for further analysis, it was sent to Basle.

On chromatography in Bush's System C, the main spot was found to have the same properties as aldosterone in the following respects: $R_F$, re-
duction of blue tetrazolium, absorption of ultraviolet light, yellow fluorescence with NaOH, and only extremely slight color with SbCl₃ and H₃PO₄.

Fig. 3. Infra-red absorption spectrum in chloroform (0.2 mm. micro cell in the Perkin-Elmer spectrophotometer model 21, with NaCl prism; resolution 4, response 1/1, speed 2 minutes per micron, suppression 1). Spectrum I, aldosterone diacetate from beef adrenals, about 500 γ in 4 drops of CHCl₃; Spectrum II, aldosterone diacetate from urine, about 200 γ in 4 drops of CHCl₃.

Fig. 4. Infra-red absorption spectrum in carbon disulfide (conditions as for Fig. 3). Spectrum III, aldosterone diacetate from beef adrenals, about 600 γ in 4 drops of CS₂; Spectrum IV, aldosterone diacetate from urine, about 180 γ in 6 drops of CS₂.

(16), apparently due to some impurities. The remainder of the material was therefore chromatographed in the same way, and the aldosterone band was eluted with 20 per cent methanol. The eluate was extracted with chloroform. This extract, inoculated in moist ether-acetone (3:1),
yielded 1.0 mg. of crystalline aldosterone (final m.p. 150–155°). Recrystallization gave 0.35 mg. of pure aldosterone, with the definitive melting point of 156–165°. Mixed melting point with authentic aldosterone showed no depression. The infra-red spectrum was identical with that of aldosterone in every detail (17). The total yield of aldosterone in this extract was 1.9 ± 0.2 mg.

For an additional confirmation of the identity, the remaining preparation from urine was acetylated in the usual way (6) and chromatographed on paper in Bush’s System B3. The band absorbing ultraviolet light and corresponding to aldosterone diacetate was eluted with methanol. The eluate was used for taking the infra-red spectrum in parallel with that of authentic aldosterone diacetate in chloroform solution (Fig. 3). The two spectra are identical. The same was the case when the substances were regenerated from the chloroform solution, taken up in carbon disulfide, and the infra-red spectra determined with these solutions (Fig. 4).

DISCUSSION

Aldosterone has been found in adrenal cortical extract or mince (6, 18–21), in adrenal venous blood (22, 23), and in peripheral blood (6). The present results demonstrate, in the single case studied, that the sodium-retaining corticoid of human urine is aldosterone. It seems a reasonable assumption that aldosterone is also the material of identical chromatographic and biological behavior which appears in small amounts in normal human urine and in increased quantities during sodium depletion or during the accumulation of edema (24, 25). This highly active, sodium-retaining hormone appears to play a part in the regulation of sodium balance in health and in certain diseases.

SUMMARY

Extracts of urine from a child with the nephrotic syndrome have yielded, successively, (1) a substance which behaves like aldosterone in chromatography, various microchemical tests, and biological activity; (2) crystalline aldosterone, identical in melting point and infra-red spectrum with authentic aldosterone prepared from adrenal cortical extract; and (3) after acetylation, a product identical in infra-red spectrum with authentic aldosterone diacetate.

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