Adrenal regeneration hypertension, first described by Skelton, is believed to be due to excessive secretion of a mineralocorticoid that occurs as a consequence of adrenal disruption and subsequent regeneration. Most known mineralocorticoids have been measured and found to be either normal or below normal. One exception is DOC, which investigators have described as elevated in the plasma, even though DOC production is low in the adrenal incubates from ARH animals at the same stage. The acceptance that DOC is the sole causative factor for ARH has not been uniform. Hall and Hall have proposed that the inconsistent finding of saline polydipsia argues against DOC being the cause of ARH. In addition, during the first week of ARH when DOC production and plasma concentrations are low, there is evidence of a salt-retaining factor of adrenal origin of unknown identity.

We have identified a powerful mineralocorticoid, 19-nor-deoxycorticosterone (19-nor-DOC), in the urine of rats with ARH and have recently developed a method to measure the urinary excretion of 19-nor-DOC in rats. We are reporting our results of the measurements of free 19-nor-DOC and DOC in the urine and 19-nor-DOC in the serum of rats with adrenal regeneration hypertension.

Materials and Methods

Male Sprague Dawley rats weighing 100–120 g were obtained from Holtzman Farms (Madison, Wisconsin) and housed in animal quarters at 23° C with a 12-hour light-dark cycle (light, 600–1800). After 1 week of acclimatization, two groups of 12 rats underwent right adrenalectomy and nephrectomy and, one group, left adrenal enucleation. The rats were maintained on standard lab chow and 1% sodium chloride drinking water. Indirect blood pressures and urine output were measured weekly. Urine from the 23rd day was used for the steroid measurements. This urine was collected in beakers containing 10 mg of sodium azide to minimize bacterial contamination. At the end of the experiment, rats were sacrificed by decapitation under quiescent conditions at 1700. Blood was collect-
The following names have been used in this paper:

- 19-hydroxy-deoxycorticosterone = 19,21-dihydroxy-4-pregnene-3,20-dione

ed, allowed to clot, and centrifuged, and the serum was stored at −60°C until assayed. This time of collection was elected because we have shown that it approximates the time of the peak of the adrenal circadian rhythm.12

**Assay Methods**

Free DOC and 19-nor-DOC were measured in the urine using a radioimmunoassay technique (Gomez-Sanchez CE et al., unpublished data, 1983). In short, to one-fifth of a 24-hour urine collection, 3000 dpm of HPLC purified (1,2 3H)-DOC and (1,2 3H) 19-nor-DOC were added for estimation of recoveries. The urine was extracted with dichloromethane. This extract was evaporated, redissolved in heptane: isopropanol, and eluted with a gradient corresponding to DOC and 19-nor-DOC were used for radioimmunoassay determinations using relatively specific antibodies previously described.10-13 The average recovery was 94% ± 4% and 53% ± 4% respectively. Intraassay variability was 9.8% and 10%, and interassay variability was 9.8% and 16.6%, for DOC and 19-nor-DOC respectively. Accuracy was measured by adding 5, 10, and 20 ng to 20 ml of urine of DOC and 19-nor-DOC and subjecting it to the procedure. The average recovery was 94% ± 4% (SD). The blanks were low and indistinguishable from zero. The sensitivity of the standard curve was 3 pg (SD). Serum 19-nor-DOC measured was radioimmunoassay.

**Results**

The fluid intake and systolic blood pressure are shown in table 1. Fluid intake showed a statistically significant increase (p < 0.01) at 9 days, a difference that remained relatively constant throughout the experimental period. Systolic blood pressure became significantly elevated at Day 16 and continued to increase up to the end of the experiment on Day 23. Serum corticosterone and urinary free 19-nor-DOC and DOC are shown in table 2. Serum corticosterone was lower, though not significantly so, in rats with ARH compared to the controls. The excretion of free urinary DOC was clearly increased in rats with ARH at Day 23. Normal rats excreted 0.9 ± 0.1 ng/day (x ± SEM) and ARH rats 2.3 ± 0.6 ng/day (p < 0.01). The excretion of free 19-nor-DOC was 5.0 ± 1.1 ng/day in normal rats and 7.9 ± 1.4 ng/day in rats with ARH (0.05 < p < 0.06). Serum 19-nor-DOC was undetectable in both groups (less than 1 ng/dl).

**Discussion**

The evidence that ARH is caused by increased secretion of a mineralocorticoid is universally accepted. The exact mineralocorticoid(s) responsible for ARH is somewhat controversial. DOC is elevated after the second week of the development of ARH and must play a crucial role in the hypertension, yet the increased sodium reabsorption that occurs during the first week of regeneration precedes the increase in DOC by 1 week. Saline polydipsia, as found in this study and others' during this early period, is also difficult to explain with the DOC hypothesis. The increased 19-nor-DOC excretions, even though small, in the urine of rats with ARH suggest that it may play a role in ARH. This steroid is two to five times more potent than DOC as a mineralocorticoid and can induce hypertension when injected chronically into rats. Previous studies have shown that 19-nor-DOC is not formed in the adrenal gland, but three possible precursors have been identified in rat adrenal incubations, 19-hydroxydeoxycorticosterone, 19-oxo-deoxycorticosterone, and 19-oic-deoxycorticosterone. Previous studies have also shown that these precursors are formed in both normal and regenerating adrenals. It has also been reported that one of these intermediates, 19-hydroxydeoxycorticosterone, is formed in greater quantities in the regenerating adrenals. The finding of

**Table 1. Fluid Intake and Systolic Blood Pressure**

<table>
<thead>
<tr>
<th></th>
<th>Day 9</th>
<th>Day 16</th>
<th>Day 23</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine output (ml/day)</td>
<td>40 ± 6</td>
<td>69 ± 8*</td>
<td>40 ± 7</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>111 ± 3</td>
<td>109 ± 2</td>
<td>124 ± 3</td>
</tr>
<tr>
<td><strong>ARH</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine output (ml/day)</td>
<td>69 ± 8*</td>
<td>83 ± 16*</td>
<td>67 ± 12*</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>109 ± 2</td>
<td>152 ± 7*</td>
<td>172 ± 5*</td>
</tr>
</tbody>
</table>

Values are means ± SEM.
*p < 0.01.
undetectable concentrations of 19-nor-DOC in the serum clearly shows that this is not a circulating steroid. In order for 19-nor-DOC to play a role in the pathogenesis of ARH, the conversion from a precursor (probably 19-oxo-deoxycorticosterone) to 19-nor-DOC would have to occur in the target organ, the kidney. This would explain the presence of undetectable circulating levels of 19-nor-DOC, while clearly detectable levels of this steroid are found in the urine.

The mechanisms and relative quantitative contributions of both steroids to ARH remains to be elucidated. Dale et al. have recently shown that 19-nor-DOC excretions are elevated early in the development of hypertension in the spontaneously hypertensive rat. Further studies will be needed to establish the temporal relationship between 19-nor-DOC formation and ARH. It might be possible that the saline polydipsia and increased sodium reabsorption during the first few days of ARH could be explained by 19-nor-DOC production directly at the target organs.

Acknowledgments
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