SODIUM AND POTASSIUM BALANCE IN THE BRATTLEBORO RAT

Susan Opava-Stitzer, Emma Fernández-Repollet, and Paul Stern*

Department of Physiology
University of Puerto Rico School of Medicine
San Juan, Puerto Rico 00936

*Departments of Maternal and Child Health, and Physiology
Dartmouth Medical School
Hanover, New Hampshire 03755

Since its discovery in 1961, it has become apparent that the Brattleboro (DI) rat is a useful model for the study of a variety of physiological problems in addition to the obvious one of the role of antidiuretic hormone (vasopressin, ADH) in urine concentration and water balance. In the study of the control of extracellular fluid volume and electrolyte balance, in particular, the DI rat offers a unique opportunity to observe the spontaneous interaction of homeostatic mechanisms when a single disturbance, namely the absence of ADH, has been introduced.

This review will attempt to present a comprehensive description of the state of sodium and potassium balance in the DI rat. Although some data exist on the handling of other electrolytes, these have not been studied extensively. Mention will also be made of the multiple factors that may influence electrolyte balance in the Brattleboro rat. New data, obtained by Opava-Stitzer and Fernández-Repollet, will be included when relevant.

SODIUM BALANCE

Among the early observations of Valtin was the finding that, as a result of their inability to conserve water, DI rats were in a fairly continuous state of mild dehydration interrupted by periods of hydration or even overhydration. This was evidenced by an elevated plasma sodium concentration and plasma osmolality compared to normal or heterozygous rats. As shown in Table 1, the plasma sodium concentration of DI rats averaged 152 mEq/L as compared to 145 mEq/L in normal Long-Evans and in heterozygous rats. The plasma osmolality of DI rats was 320 mOsm, significantly higher than the values found in normals and heterozygotes.

In addition, as a result of the absence of ADH, electrolyte concentrations in renal tissue were dramatically affected. The greatest effect, as shown in Table 2, was on interstitial sodium concentration. The concentration of sodium in the papilla of DI rats was significantly reduced compared to Long-Evans rats, a difference that was not eliminated by treatment with one unit of vasopressin per day for three days, despite a significant increase in the papillary sodium concentration. Since there were no differences in the papillary sodium content (mM/g dry solids) of DI and Long-Evans rats, the reduced papillary
<p>| TABLE 1 |
| Plasma Sodium Concentration ($P_{Na}$) and Osmolality ($P_{osm}$) in Normal, Heterozygous, and DI Rats |</p>
<table>
<thead>
<tr>
<th>Long-Evans</th>
<th>Heterozygotes</th>
<th>DI</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{Na}$ (mEq/L)</td>
<td>145</td>
<td>145</td>
</tr>
<tr>
<td>$P_{osm}$ (mOsm/Kg H$_2$O)</td>
<td>307</td>
<td>301</td>
</tr>
</tbody>
</table>

* Significantly different from normal, Long-Evans rats. Data from Valtin and Schroeder and Valtin et al.

Concentration could be attributed to increased water reabsorption by the papillary collecting duct in the absence of ADH, and thus a dilution of papillary interstitial solute. Indeed, Valtin observed a greater papillary water content in untreated DI rats than in Long-Evans controls, and a significant decrease in papillary water content after ADH treatment. It was later shown by Jamison et al. in micropuncture studies in the DI rat, that a greater fraction of the glomerular filtrate is reabsorbed by the medullary collecting duct in the absence of ADH than during ADH-induced antidiuresis.

In contrast to the variations noted in the renal papilla, the medullary sodium concentrations of DI and Long-Evans rats were not different and ADH treatment of DI rats resulted in a paradoxical reduction in medullary sodium content reflected here in a reduced sodium concentration in this zone. In cortex, both sodium concentration and content were elevated in untreated DI rats, no doubt reflecting the augmented plasma sodium concentration and the reduced water reabsorption in the cortex in the absence of ADH.

Valtin's observation that ADH administration to the DI rat did not result in enhanced sequestration of sodium in the medullary interstitium was surprising in view of prior reports, which had shown that the infusion of ADH in dogs undergoing water diuresis significantly raised both the sodium concentration

<p>| TABLE 2 |
| Renal Sodium Concentration in Normal (LE), Untreated (DI), and ADH-Treated (DI-ADH) Rats |
| [Na] (mMoles/Kg H$_2$O) |</p>
<table>
<thead>
<tr>
<th>Papilla</th>
<th>Medulla</th>
<th>Cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>LE</td>
<td>358.4 ± 33.2</td>
<td>223.8 ± 19.0</td>
</tr>
<tr>
<td>DI</td>
<td>201.5 * ± 31.0</td>
<td>193.5 ± 23.5</td>
</tr>
<tr>
<td>DI-ADH</td>
<td>236.8 * ± 36.1</td>
<td>158.0 * ± 21.0</td>
</tr>
</tbody>
</table>

Data from Valtin. * Significantly different from normal rats.
and content of papillary and medullary tissue. In addition, in 1971, Atherton et al. demonstrated a dose-dependent increase in sodium content of renal tissue in response to the infusion of small amounts of ADH in water-loaded normal rats. Using a dose of ADH of 100 mU/100 g/day, considerably lower than that used by Valtin, we have consistently observed the accumulation of sodium in the kidneys of DI rats. As shown in Table 3, the administration of ADH to DI rats for three weeks resulted in a significant increase in both sodium content and concentration in renal medulla and papilla compared to values in untreated DI rats.

Interestingly, the state of potassium balance had a profound effect on this sodium-sequestering activity of ADH. We studied the effects of administration of ADH for three weeks on the sodium content of kidneys of DI rats maintained for the same period of time on either high potassium or potassium-free diets. As shown in Table 4, ADH caused increased tissue sodium deposition in both groups of rats. In the rats on a potassium-free diet, the increase in sodium content was significant only in papilla and was markedly less than that observed in rats on a high potassium diet, or in rats on a normal diet, shown earlier. On a high potassium diet, the increase in sodium content occurred in medulla as well as papilla and the magnitude of the changes was also greater than that seen on a normal diet. It thus appears that during potassium loading the sodium-retaining effects of ADH are exaggerated. This finding may be related to the natriuresis which accompanies potassium loading particularly in the presence of aldosterone deficiency. An increased delivery of sodium to the loops of Henle during potassium loading would provide more sodium for the medullary pool and thus make more apparent any effect of ADH on the interstitial deposition of sodium. Furthermore such a mechanism could be exaggerated in the aldosterone-deficient DI rat.

Thus, despite some discrepancies in the literature, there is ample evidence to indicate that ADH can enhance the sequestration of sodium in the renal medulla and papilla, and that the cortico-papillary solute gradient of the DI rat may be further compromised by the lack of this action of ADH as well as its effects on water permeability. Whether or not the sodium-retaining effects of ADH are apparent in any given situation, no doubt depends on a variety of factors which may influence this particular action of ADH. Dosage may be critical. In early studies in particular, in which larger doses of ADH were

### Table 3

**Renal Sodium Content and Concentration and Plasma Sodium Concentration in Untreated (DI) and ADH-Treated (DI-ADH) DI Rats**

<table>
<thead>
<tr>
<th></th>
<th>Na$_c$</th>
<th>[Na$^+$]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mMoles/Kg FFDW)</td>
<td>(mM)</td>
</tr>
<tr>
<td>Medulla</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DI</td>
<td>634 ± 64 *</td>
<td>152 ± 13 *</td>
</tr>
<tr>
<td>DI-ADH</td>
<td>1271 ± 80</td>
<td>280 ± 19</td>
</tr>
<tr>
<td>Papilla</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DI</td>
<td>1158 ± 141 *</td>
<td>402 ± 19 *</td>
</tr>
<tr>
<td>DI-ADH</td>
<td>2916 ± 205</td>
<td>640 ± 45</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DI</td>
<td>—</td>
<td>137 ± 3</td>
</tr>
<tr>
<td>DI-ADH</td>
<td>—</td>
<td>140 ± 1</td>
</tr>
</tbody>
</table>

* Significantly different from ADH-treated DI rats, p < 0.05.
Table 4

RENAL SODIUM CONTENT (Na\textsubscript{o}) AND CONCENTRATION AND PLASMA SODIUM CONCENTRATION IN UNTREATED (DI) AND ADH-TREATED (DI-ADH) DI RATS ON DIFFERENT POTASSIUM DIETS

<table>
<thead>
<tr>
<th></th>
<th>Na\textsubscript{o} (mMoles/Kg FFDW)</th>
<th>[Na\textsuperscript{+}] (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High K</td>
<td>K-free</td>
</tr>
<tr>
<td>Medulla</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DI</td>
<td>716 ± 110*</td>
<td>610 ± 16</td>
</tr>
<tr>
<td>DI-ADH</td>
<td>1087 ± 65</td>
<td>655 ± 21</td>
</tr>
<tr>
<td>Papilla</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DI</td>
<td>1036 ± 134*</td>
<td>911 ± 60 *</td>
</tr>
<tr>
<td>DI-ADH</td>
<td>3125 ± 94</td>
<td>1360 ± 91</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DI</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>DI-ADH</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* Significantly different from ADH-treated DI rats, p < 0.05.

utilized, changes in renal hemodynamics may have occurred, resulting in a reduction in the amount of sodium presented to the long loops of Henle for reabsorption. Such an occurrence would diminish the ability of ADH to increase interstitial sodium content, by a mechanism opposite to that which we have proposed may occur during potassium loading.

As to the mechanism by which ADH exerts its sodium-sequestering action, all explanations are still speculative. It had been proposed as early as 1962 by Levitin et al.\textsuperscript{a} that ADH might enhance active sodium reabsorption by the loops of Henle or collecting tubules, analogous to the manner in which it increases active sodium transfer across the frog skin\textsuperscript{10} and toad bladder.\textsuperscript{27} Micropuncture studies,\textsuperscript{18-22} however, had until recently consistently failed to show such an action of ADH. In 1980 an in vitro effect of ADH on chloride transport of isolated perfused medullary thick ascending limbs of the mouse was demonstrated by Hall and Varney.\textsuperscript{23} In the same year Sasaki and Imai\textsuperscript{24} reported that ADH stimulated NaCl transport across isolated perfused medullary thick ascending limbs of mouse, rat, and rabbit. The biochemical basis for such an effect had already been shown to exist by Imbert, Morel, and colleagues in a series of publications.\textsuperscript{23-31} They have shown the presence of ADH-sensitive adenyl cyclase activity in both thin and thick ascending limbs of rat\textsuperscript{28,29} and rabbit\textsuperscript{26,27} and in the thick ascending limbs of mouse.\textsuperscript{81} No such activity could be demonstrated in the human,\textsuperscript{30} a fact that may partially explain the inferior concentrating ability of man compared to the rodent and rabbit.

These findings once again allude to the possible importance of a direct effect of ADH on active sodium reabsorption. Even barring such an effect, however, ADH might still affect the medullary sodium gradient according to the model of passive sodium reabsorption in the inner medulla proposed by Kokko and Rector in 1972.\textsuperscript{82} By increasing the urea concentration in the interstitium surrounding the loops of Henle of deep nephrons, ADH will also enhance the abstraction of water from descending limbs, resulting in an increased gradient for the passive diffusion of sodium out of thin ascending limbs. A failure to find increased interstitial sodium content after ADH administration would, in fact, challenge the importance of this mode of sodium reabsorption in the inner medulla.
It is also possible that ADH may alter the deposition of sodium in the medullary interstitium indirectly, through alterations in medullary blood flow and/or filtration rate of juxtamedullary nephrons. It has been shown that medullary blood flow is lower in antidiuresis than in water diuresis, and thus the countercurrent mechanism of solute deposition would operate more efficiently in the presence of ADH. Measurements of single nephron glomerular filtration rate in rats show that the filtration rate of deep nephrons is higher in antidiuresis than in the absence of ADH. Thus more sodium might be sequestered in the medullary interstitium simply because a greater fraction of filtered sodium is delivered to that region in the presence of ADH.

Finally, it has been reported recently that, compared to normal Long-Evans rats, DI rats have a deficiency of glycosaminoglycans in the papillary interstitium. It has been suggested that these polyanionic molecules may act as ion exchangers, sequestering cations in the interstitium, and may also sterically exclude water from interstitial spaces. If the concentration of papillary glycosaminoglycans can be restored to normal by ADH treatment, an ADH-dependent role for these molecules in the maintenance of the medullary sodium gradient may be established.

In addition to the question of the role of ADH in the maintenance of the interstitial solute gradient, it is also unclear to what extent ADH, or the absence of it, affects urinary sodium excretion ($U_{\text{Na}}V$) and sodium balance. It is generally accepted that antidiuretic hormone has a natriuretic action in dog and rat, including the Brattleboro rat, but this is normally seen at supraphysiological doses and may be related to systemic effects of ADH rather than a direct action of ADH on renal sodium reabsorption. Nevertheless, it is relevant to consider the possibility of physiologically important effects of ADH on renal sodium handling and sodium balance, particularly in view of the evidence concerning the effects of ADH on the intrarenal deposition of sodium.

Another of Valtin's and Schroeder's early findings was that urinary sodium excretion was about 50% higher in DI rats than in Long-Evans controls. Friedman and Friedman reported shortly thereafter that food intake was also higher in the DI rat and this could have accounted for the elevated sodium excretion seen by Valtin. Subsequent studies by Balment and Haack, however, showed that the DI rat excretes a greater fraction of its sodium intake than normal rats and may have a food intake either equal to or less than that of normal rats. In 1974, Möhring showed dose-dependent sodium retention in DI rats in response to single injections of 250 or 500 mU of ADH, but no effect with lower doses. The evidence for sodium retention was a decrease in the fraction of ingested sodium that was excreted. Prolonged administration of low doses of ADH also had no effect on sodium balance. A dose of 500 mU/day for seven days caused sodium retention on the first day only, followed by cycles of positive and negative sodium balance. In contrast, Balment et al. reported a decrease in the excreted fraction of ingested sodium in male DI rats from a pretreatment value of 82.5% to 63.3% after one week of treatment with one unit of ADH per day. As shown in Table 5, we have also observed a significant decrease in urinary sodium excretion in DI rats on varying potassium intakes, after three weeks of treatment with 100 mU ADH/100 g body weight/day. Although on a normal diet this reduction in $U_{\text{Na}}V$ was associated with a reduction in sodium intake, on a high potassium diet there was no significant change in sodium intake and on a potassium-free diet sodium intake was actually increased after ADH treatment. In all cases, the fraction
of ingested sodium that was excreted in the urine was decreased as a result of ADH administration. The effect of ADH to decrease sodium excretion was greatest on a high potassium diet.

It should be noted that any large differences between sodium intake and urinary sodium excretion must represent either a transient state of sodium retention or increased loss of sodium in the feces.

It seems clear, therefore, that ADH can, in some circumstances, cause sodium retention, an action that is influenced by the state of potassium balance. The same mechanisms previously discussed, by which ADH may enhance medullary sodium trapping, could also explain its effects on sodium excretion.

Mohring measured serum sodium concentration in DI rats during seven to nine days of treatment with 100 mU or 500 mU of ADH per day. Serum sodium concentration was significantly reduced by both doses of ADH, despite differing effects on sodium balance, as discussed earlier. Although plasma volumes were not measured in these experiments, the fall in plasma sodium concentration was most likely the result of a rise in plasma volume following ADH treatment. In another study, Möhring measured actual changes in plasma volume in DI rats after a single injection of 125 mU of Pitressin. Plasma volume increased significantly (4.14 to 4.74 ml/100 g body weight), accompanied by a significant decrease in hematocrit but, surprisingly, no change in plasma osmolality. Similar findings were associated with chronic ADH treatment.

In our experiments, using doses comparable to Möhring’s for a longer period of time, we could demonstrate no significant effect of ADH on plasma sodium concentration (Table 4). In view of the concurrent finding of reduced urinary sodium excretion, our failure to see a fall in plasma sodium concentration may actually indicate a net increase in the amount of extracellular sodium. It should be emphasized, however, that without precise measurements of plasma or extra-

### Table 5

**Urinary Sodium Excretion (U_Na, V) and Intake of Untreated (DI) and ADH-Treated (DI-ADH) DI Rats with Different Potassium Intakes**

<table>
<thead>
<tr>
<th></th>
<th>U_Na, V or Na intake (µEq/100 g bw/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DI</td>
</tr>
<tr>
<td>Normal diet</td>
<td></td>
</tr>
<tr>
<td>Na intake</td>
<td>1096 ± 59</td>
</tr>
<tr>
<td></td>
<td>(1510 ± 78)</td>
</tr>
<tr>
<td></td>
<td>(72.5%)</td>
</tr>
<tr>
<td>High K diet</td>
<td></td>
</tr>
<tr>
<td>Na intake</td>
<td>1340 ± 85</td>
</tr>
<tr>
<td></td>
<td>(1390 ± 40)</td>
</tr>
<tr>
<td></td>
<td>(96.4%)</td>
</tr>
<tr>
<td>K-free diet</td>
<td></td>
</tr>
<tr>
<td>Na intake</td>
<td>798 ± 12</td>
</tr>
<tr>
<td></td>
<td>(843 ± 37)</td>
</tr>
<tr>
<td></td>
<td>(94.7%)</td>
</tr>
</tbody>
</table>

* Significantly different from untreated DI rats, p < 0.05. Numbers in parentheses are (U_Na, V/Na intake) × 100.
cellular fluid volume, changes in serum sodium concentration are at best a poor index of whether or not sodium retention has occurred in response to ADH treatment. The values of plasma sodium concentration, which are shown in TABLES 3 and 4, merit further discussion. Plasma sodium concentration varied from 136 ± 3 mEq/L to 148 ± 1 mEq/L in untreated DI rats, depending on potassium intake. We have generally found a reciprocal relationship between plasma sodium and potassium concentration; hence plasma sodium concentration was highest on a K⁺-free diet. A reciprocal relationship was also seen in these studies between sodium and potassium content of skeletal muscle. In addition, our values of plasma sodium concentration are lower than those reported by others.¹, ², ¹⁵, ⁴⁷, ⁵², ⁶³, ⁶⁵ We have indeed found at times that plasma sodium concentration of the DI rat was not elevated compared to Long-Evans rats. In agreement with our data, Balment et al. reported values of plasma sodium concentration of 141.9 ± 1.7 mEq/L in male and 137.6 ± 1.2 mEq/L in female DI rats compared to 144.1 ± 2.0 and 137.7 ± 1.2 mEq/L in male and female heterozygous rats.⁵¹ There are many possible explanations for these differences including biological variation among the different colonies of DI rats studied, as well as differences in environmental and dietary conditions and methods of blood-sampling. In addition the state of hydration of the rat at the time of blood sampling may be important (e.g. dehydrated versus overhydrated) and this may be related to the time of day at which the sample is obtained. Thus even a simple measurement such as serum sodium concentration may be complicated by the peculiar physiology of the DI rat. Standardized experimental conditions and appropriate controls are essential.

In summary, studies in the Brattleboro rat indicate that physiological levels of ADH may play a direct role in the maintenance of sodium and water balance through actions on renal sodium handling and renal tissue sodium deposition, effects that are modified by the state of potassium balance. In addition, sodium balance may be affected indirectly by changes in blood pressure, and extracellular fluid volume secondary to the well-known effects of ADH on water balance.

**Potassium Balance**

In his early descriptions of the Brattleboro rat, Valtin¹ noted that, in addition to an elevated urinary sodium excretion, DI rats excreted significantly more potassium in the urine than did Long-Evans rats. The same constraints on interpretation that applied to the finding of increased sodium excretion, however, were also applicable in the case of potassium. In the face of reports of a greater than normal food intake in DI rats,⁵⁰ the higher than normal excretion of potassium might simply reflect a greater ingestion of potassium. This topic was not given much attention until 1972 when Möhring⁵⁵ first reported that DI rats were hypokalemic. Further studies⁵¹ confirmed this finding revealing, as well, a reduced potassium content of skeletal muscle of DI rats. Balment et al.⁵¹ later found that female DI rats had a greater urinary potassium loss than female heterozygous rats. As shown in TABLE 6, when expressed as a fraction of dietary intake, female DI rats excreted more potassium in the urine than did heterozygous females. No such difference was found, however, between DI and heterozygous males. The authors suggested that this difference in potassium handling might be due to sex differences in mineralocorticoid levels.
In an effort to determine whether the hypokalemia of DI rats might be secondary to excessive loss of potassium in the urine we compared food intake and electrolyte excretion of DI and Long-Evans rats housed in metabolism cages for periods of three and nine days. The urinary potassium excretion of DI rats was found to be higher than that of Long-Evans rats on the first four days of study, but not significantly different on the remaining days. The difference in urinary potassium excretion could not be accounted for by differences in food intake between the two groups. As shown in Table 7, measurements of plasma potassium concentration and muscle potassium content revealed that the DI rats were potassium deficient at the start of the balance study and after three days, but by nine days of study were potassium repleted, as evidenced by normal plasma potassium concentration and muscle potassium content.

We also studied the alterations in renal potassium content and concentration of DI and Long-Evans rats, which occurred between three and nine days of balance study. After three days in metabolism cages both the potassium content and concentration of the renal medulla and papilla of DI rats were elevated compared to Long-Evans rats. By nine days, however, there were no differences in either of these measurements. The major change between three and nine days had been a decrease in the medullary and papillary potassium concentration of DI rats, primarily due to an increase in the water content of these regions.

Valtin did not note differences in tissue potassium between DI and Long-Evans rats after a brief period in metabolism cages. This, however, may have been due to his method of analysis. Wet and dry weights were determined on

### Table 6

**Urinary Potassium Excretion, as a Fraction of Potassium Intake, of DI and Heterozygous Brattleboro Rats**

<table>
<thead>
<tr>
<th></th>
<th>DI</th>
<th>Hetero</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>86.9 ± 1.1 *</td>
<td>78.0 ± 1.8</td>
</tr>
<tr>
<td>Male</td>
<td>74.3 ± 1.4</td>
<td>72.4 ± 2.4</td>
</tr>
</tbody>
</table>

* Significantly different from heterozygous rats of the same sex. Data from Balment et al.

### Table 7

**Plasma Potassium Concentration and Muscle Potassium Content of DI and Long-Evans (LE) Rats Prior to and After 3 and 9 Days, of Balance Study**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>Start</th>
<th>3 days</th>
<th>9 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DI</td>
<td>3.6 ± 0.1 †</td>
<td>3.7 ± 0.2 ††</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>LE</td>
<td>—</td>
<td>4.3 ± 0.2</td>
<td>4.3 ± 0.1</td>
</tr>
<tr>
<td>Muscle K⁺</td>
<td>DI</td>
<td>362 ± 6 * †</td>
<td>442 ± 8 * ††</td>
<td>468 ± 5</td>
</tr>
<tr>
<td>(mMoles/Kg FFDW)</td>
<td>LE</td>
<td>495 ± 3</td>
<td>465 ± 8</td>
<td>456 ± 5</td>
</tr>
</tbody>
</table>

* Significantly different from Long-Evans rats studied at the same time, \( p < 0.05 \).
† Significantly different from DI rats at 9 days, \( p < 0.05 \).
a different group of kidneys than those used for chemical analysis, a procedure that may have obscured small differences in potassium concentration. In our studies, wet and dry weights were obtained on the same tissue used for measurement of electrolytes.

It should be recalled that after nine days in metabolism cages urinary potassium excretion of DI rats was not different from that of normal rats. Thus a prolonged period in metabolism cages had resulted in spontaneous potassium repletion as well as normalization of the rate of potassium excretion in DI rats.

Interestingly, the decrease in potassium excretion after several days in metabolism cages occurred regardless of the potassium intake. As shown in Figure 1, despite a wide range of urinary potassium excretion rates, a significant decrease in the $U_K/V$ of DI rats was always observed after three to five days in metabolism cages. It should be noted also, that DI rats on a potassium-free diet reduced their urinary potassium to levels even lower than those observed in normal rats on the same diet. This suggested to us that the transiently high urinary potassium excretion observed early in balance studies was not due to a primary defect in renal potassium handling. We postulated, instead, that these alterations in tissue and urinary potassium might be secondary to changes in water balance when DI rats are moved from their regular habitation cages to individual metabolism cages. If this were so, then mild dehydration would be expected to enhance urinary potassium excretion and mimic the observed tissue changes.

When DI rats were acutely dehydrated by deprivation of water for five to eight hours, urinary potassium excretion was increased compared to DI rats drinking ad lib. Not only was basal $U_K/V$ higher in dehydrated DI rats but also the excretion of potassium in response to a KCl load (200 $\mu$Eq/100 g body weight, by gavage). In addition, as shown in Table 8, five hours of water deprivation significantly decreased the water content of renal papilla and increased the potassium concentration of both medulla and papilla. No changes were observed in gastrocnemius muscle. These results are in agreement with those of Valtin who found an increase in potassium concentration of medulla and papilla of DI rats after 12 hours of dehydration. A more chronic dehydration in the form of water rationing was also found to enhance potassium output. In summary, the potassium deficiency of DI rats originally reported by Mohring was shown to be spontaneously reversible and associated with a transient elevation in urinary potassium excretion. The elevation in potassium excretion may occur when DI rats are forced to compete with other rats for drinking water and thus are more apt to experience periods of dehydration. This may result in increased potassium concentration of renal medulla and papilla through a reduction in water content. The higher potassium concentration, in turn, may augment the gradient for potassium secretion in the loops of Henle and collecting ducts and result in inappropriate renal potassium loss and hypokalemia. The simple procedure of allowing DI rats non-competitive access to drinking water reverses this chain of events and results in potassium repletion.

In some of our studies of tissue composition it was noted that alterations in water balance affected not only the concentration of potassium in renal tissue but also the potassium content. Jamison et al. have shown that, in the rat, there is medullary recycling of potassium from the collecting tubule to the descending limb of the loop of Henle. The degree of recycling was dependent on the concentration of potassium in the collecting duct fluid. Thus
during mild dehydration, medullary potassium recycling might be enhanced, thereby increasing the medullary and papillary potassium pool.

Our results also explain a previous discrepancy in the literature between the data of Möhring and an earlier study by Friedman and Friedman in which muscle and plasma potassium levels of DI rats were found to be normal. Friedman's rats, however, had been housed in metabolism cages for more than three weeks prior to study and thus, according to our findings, would already have been potassium repleted.

**TABLE 8**

**Renal Water Content and Potassium Concentration of Control (Cont.) and Dehydrated (Dehyd.) DI Rats**

<table>
<thead>
<tr>
<th></th>
<th>H$_2$O Content (ml/100 g wet wt.)</th>
<th>[K] (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cont. 80.0 ± 1.5</td>
<td>98.7 ± 2.0</td>
</tr>
<tr>
<td>Medulla</td>
<td>Dehyd. 78.0 ± 2.5</td>
<td>152.4 ± 30.0*</td>
</tr>
<tr>
<td></td>
<td>Cont. 84.0 ± 2.0</td>
<td>73.1 ± 2.1</td>
</tr>
<tr>
<td>Papilla</td>
<td>Dehyd. 78.4 ± 2.5 *</td>
<td>87.6 ± 2.1 *</td>
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* Significantly different from values in control DI rats, p < 0.05. Data from Fernandez-Repollet et al.
The next logical question is whether ADH administration exerts an effect on potassium balance and whether such an effect is physiological or pharmacological in nature. As was the case with sodium, ADH has also been shown to be kaliuretic. 42, 44, 48, 40 Since a recent report has shown that DDAVP, a non-pressor analogue of arginine vasopressin did not cause either natriuresis or kaliuresis, 48 these may be effects secondary to vasopressor-related actions of ADH.

Mohring 53 studied the acute effects of ADH on potassium balance in Brattleboro rats. Single injections of Pitressin, between 50 and 500 mU, caused a dose-dependent potassium retention, evidenced in a decrease in the fraction of ingested potassium that appeared in the urine. This potassium retention was transient, potassium excretion returning to normal by the third to fourth day after injection. There was no effect of ADH on potassium balance in heterozygous rats. A single injection of 100 mU of vasopressin significantly increased the serum potassium concentration of DI rats (3.32 ± 0.06 to 3.44 ± 0.09 mEq/L).

Mohring also observed that with more prolonged treatment of DI rats with ADH, 47 a dose-dependent retention of potassium occurred on the first day of treatment. Potassium excretion rates then returned to normal despite continued ADH treatment. Kaliuresis occurred after cessation of treatment. Balment 64 could demonstrate no difference in potassium balance of untreated or oil-treated DI rats and DI rats after one week of ADH treatment. This is not really contradictory to Mohring’s results, however, since even if potassium had been retained early in treatment, the escape observed by Mohring would have occurred by the time of study.

Mohring 53 initially attributed the effects of ADH on potassium balance to alterations in distal tubular flow rate secondary to effects of ADH on water reabsorption. In support of this he showed a significant inverse correlation between the degree of potassium retention and urine flow rate, during single injections of varying doses of ADH. Such reasoning may be specious, however, and the physiologically significant correlation may, in fact, have been between potassium retention and dose of ADH. This interpretation is supported by Mohring’s later findings 47 in which the magnitude of potassium retention was greater but the duration shorter, at a higher dose of ADH. This suggests to us that the degree of potassium retention is dose-dependent but limited to an absolute net potassium gain, which may be determined by the degree of potassium deficiency. It should be recalled that potassium retention did not occur in potassium-replete heterozygous rats. 53 In addition, we have shown 66 that DI rats retain more of an oral potassium load than do normal rats.

Furthermore, we have studied the effects of three weeks of ADH treatment on $U_KV$ of DI rats maintained on normal, high potassium, or potassium-free diets. In DI rats on a potassium-free diet ADH caused a slight but not significant decrease in urinary potassium excretion. On a normal diet, ADH significantly decreased $U_KV$ but potassium intake was also decreased and there was no significant change in the fractional excretion of potassium. In contrast, in DI rats on a high potassium diet ADH significantly increased both $U_KV$ and the fraction of ingested potassium that appeared in the urine. Thus ADH had different effects on potassium balance and urinary potassium excretion depending on the state of potassium balance at the time of administration.

The escape from potassium retention observed by Mohring 47 could then be due simply to potassium repletion and restoration of normal serum and tissue
potassium levels, although Möhring suggested that this escape may be mediated by the increase in plasma mineralocorticoid concentration, which also occurs in response to ADH treatment.

We have studied the effects of three weeks of ADH treatment on potassium concentration and content of renal tissue in varying states of potassium balance. As shown in Table 9, ADH treatment of DI rats on a normal diet caused a significant increase in the potassium content of renal medulla and papilla. This increase, however, was not reflected by changes in potassium concentration, since water content of these zones was also significantly increased by ADH treatment. This increase in water content, though contradictory to Valtin's observations, and paradoxical in view of the finding of reduced water reabsorption into the inner medulla during antidiuresis, was nonetheless consistently observed by us. Whatever the mechanism, this may be a long-term effect of ADH, and thus not seen in acute studies of the transition to antidiuresis or after short-term treatment with ADH.

On high potassium and potassium-free diets (Table 9), ADH increased papillary renal potassium content, and in the high potassium group an increase was observed in the medulla as well. As might be anticipated, the increase in potassium content was also much greater on a high potassium intake. In the papilla the change in potassium content was large enough to significantly increase the potassium concentration despite an increase in water content of this zone. No changes were seen in the cortex on any dietary potassium intake as a result of ADH treatment.

In summary, both ADH and the lack of it, affect potassium balance and the deposition of potassium in the renal medulla and papilla. The mechanism for these effects is not known, but it is interesting to speculate that ADH might stimulate the medullary potassium recycling described by Jamison. A role of systemic effects of ADH and/or alterations in renal hemodynamics and filtration rate cannot, however, be ruled out.

OTHER INFLUENCES ON SODIUM AND POTASSIUM BALANCE IN THE DI RAT

Having reviewed the current state of our knowledge of sodium and potassium balance in the Brattleboro rat and of the effects of ADH on the balance of these cations, it would seem of interest to mention those unusual characteristics of the DI rat that have a proven or potential influence on their electrolyte balance. Reduced blood pressure and altered renal hemodynamics may be important, particularly insofar as glomerular filtration rate has been shown to be reduced in the DI rat. Not only gross changes but more subtle differences, such as the lack of nephron heterogeneity recently reported, may affect renal electrolyte handling.

It has been known for some years that the renin-angiotensin system is also markedly altered in the DI rat, either as a direct consequence of the lack of ADH, which is known to inhibit renin secretion in a variety of species or indirectly, as a result of the diabetes insipidus. It is well established that plasma renin activity, plasma renin concentration, and plasma angiotensin II concentration are all increased in the DI rat. Although it has been reported that the increase in renin is confined to the male, this has not been confirmed by others.

ADH treatment of DI rats, either a single injection, or more chronic
<table>
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<th>[K] (mEq/L)</th>
<th>K content (mMoles/Kg FFDW)</th>
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<tr>
<td></td>
<td>K-free</td>
<td>Normal</td>
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<tr>
<td><strong>Medulla</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DI</td>
<td>63.7</td>
<td>± 2.4</td>
</tr>
<tr>
<td>DI-ADH</td>
<td>61.3</td>
<td>± 5.6</td>
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<tr>
<td><strong>Papilla</strong></td>
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<td></td>
</tr>
<tr>
<td>DI</td>
<td>78.3</td>
<td>± 1.7</td>
</tr>
<tr>
<td>DI-ADH</td>
<td>67.6*</td>
<td>± 3.3</td>
</tr>
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Values are means ± SEM.
* Significantly different from untreated DI rats, p < 0.05.
reduced plasma renin activity, plasma renin concentration, and plasma angiotensin II concentration. A sex difference in the renin response to ADH has also been noted, but not consistently.

Not only is there an increased release of renin and generation of angiotensin II in the DI rat, but an increased storage of renin in the kidneys, as well. Renal renin content was found to be greater in kidneys of DI rats. It is interesting that even when female DI rats had normal plasma renin activity, renal renin content was increased.

Finally, this hyperactivity of the renin angiotensin system is not limited to basal conditions. DI rats also have an exaggerated renin response to common stimuli of renin release, such as dehydration and renal artery clamping.

Despite a hyperactive renin-angiotensin system, Brattleboro rats have been shown to suffer from adrenal insufficiency, specifically, reduced plasma aldosterone and corticosterone concentrations and adrenal weights. They also had a diminished corticosterone response to mild stress that was not corrected by two to three weeks of ADH treatment. ADH treatment did restore plasma mineralocorticoid levels and adrenal weights to normal despite a concomitant reduction in angiotensin II concentration.

In addition to the possibility that ADH or the lack of it may directly affect the adrenal gland, reduced adrenal function could also be secondary to the mild potassium deficiency and/or the elevated serum sodium concentration found in the DI rat. Pituitary ACTH content was also lower in DI rats and this may play an important role in the adrenal insufficiency.

Thus, although the renin-angiotensin system is highly stimulated in the Brattleboro rat, adrenal function is subnormal. It is not known to what extent these alterations affect electrolyte balance, although Balment did not note any significant changes in sodium or potassium balance of DI rats after adrenalectomy. It is also not known what role the high plasma angiotensin II levels may play in preventing an even greater adrenal insufficiency.

Finally, the Brattleboro rat shows a significant reduction in the urinary excretion of prostaglandins E and F. The excretion rate of these prostaglandins in the DI rat was only about 20% of the rate observed in Long-Evan rats, and was increased in both DI and normal rats after ADH or DDAVP treatment. Renal prostaglandin synthesis may be diminished in the DI rat due to the absence of a stimulatory effect of ADH on prostaglandin synthesis. Although one might have expected to see an increased prostaglandin production in the DI rat due to hypokalemia, potassium deficiency did not increase prostaglandin excretion in normal rats.

Bankir and coworkers also showed a decreased rate of synthesis of PGE by the papilla, but an increased synthesis by glomeruli, of the DI rat in vitro. The finding of a decreased rate of papillary synthesis correlates well with morphological studies, which have shown both gross and fine structural changes in the papillae of DI rats. Notably, interstitial cells were markedly affected even at the subcellular level and were shown to contain fewer lipid droplets, presumably a reflection of decreased prostaglandin synthesis. The known effects of prostaglandins on renal sodium excretion and renin release make this an important area for further study in the DI rat.

The many hemodynamic, morphological and hormonal changes listed here are no doubt manifestations of the complex interaction of multiple homeostatic mechanisms. It is to be hoped that a study of these interactions in the DI rat will provide useful information concerning the function of these same mechanisms in the normal rat, and ultimately, man.
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