Defect in the Renal Tubular Reabsorption of Water Associated With Potassium Depletion in Rats

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ABSTRACT

HOLLANDER, WALTER, JR., ROBERT W. WINTERS, T. FRANKLIN WIL- LIAMS, JOHN BRADLEY, JEAN OLIVER AND LOUIS G. WELT. Defect in the renal tubular reabsorption of water associated with potassium depletion in rats. Am. J. Physiol. 189(3): 557-563. 1957.—The effect of graded degrees of K depletion on the ability to produce a concentrated urine was studied in Sprague-Dawley rats. With increasing degrees of K depletion, as measured by the concentration of K in fat-free skeletal muscle, there was a progressive decrease in the maximum urinary concentration. This defect of the renal concentrating mechanism appeared to be better correlated with the degree than with the duration of potassium depletion and could be demonstrated either by the use of exogenous vasopressin or by water deprivation. The potassium-deficient rats in at least one experiment developed a significant polydipsia. The data do not allow any conclusions with respect to the relationship of the polydipsia to the renal concentrating defect except that the latter at least was not severe at the onset of the increased water intake.

In 1937, Schrader, Prickett and Salmon (1) reported lesions in the renal tubular epithelium of rats fed a diet deficient in potassium. This observation has been amply confirmed, although there has been no agreement as to the nature or location of the histologic abnormalities (2-4). In recent years much evidence has been obtained which suggests that depletion of potassium may result in derangements of renal function. A defect in the renal concentrating mechanism has been specifically suggested by the studies of Smith and Lasater (5), of Brokaw (6), of Spargo (4) and particularly by the recent studies in man of Relman and Schwartz (7).

The present experiments represent an attempt to study the effects of graded degrees of potassium depletion on the renal concentrating mechanism of the rat. A separate report (J.O.) will deal with the associated renal pathology.

METHODS AND MATERIALS

Experiment A. The animals were male, Sprague-Dawley rats with initial weights of 300-400 gm, housed in individual cages. All rats had free access to distilled water and to a basal diet which was deficient in potassium, sodium, phosphate and chloride, and which contained 4% urea. The composition of the diet is shown in table 1. All rats were given 5 ml of an electrolyte solution once each day by gavage, control groups receiving normal amounts of potassium chloride and of a neutral mixture of monosodium and disodium phosphates, potassium-deficient groups receiving sodium bicarbonate and the same mixture of sodium phosphates. The quantities of each ion received in this manner are shown in table 2. The total solute load administered by gavage was the same for control and for experimental rats.

After 1 and 2 weeks on the experimental regimen (groups A-I and A-2), the ability of each rat to form a maximally concentrated urine was tested with exogenous vasopressin as described below. Approximately 24 hours after completing the renal concentration test, during which time the rats were again allowed free
access to the basal diet and to water, the rats were anesthetized with hexobarbital sodium (Evipal) administered intraperitoneally, blood was obtained anaerobically from the abdominal aorta, the kidneys were removed for pathologic studies, and 15–25 gm of skeletal muscle were removed from the paraspinal and hind leg areas (as free of blood and connective tissue as possible) for determination of potassium concentration.

**Experiment B.** This was identical with experiment A except that the basal diet did not contain urea and the ability to form a maximally concentrated urine was tested after three and four weeks of potassium depletion (groups B-3 and B-4). The urines collected during the renal concentration test at 4 weeks were analyzed for their concentration of sodium, potassium, and urea plus ammonia nitrogen. In order to exclude abnormal absorption or inactivation of vasopressin as a factor in the results, the renal concentrating mechanism of one group of rats was tested during the last 12 hours of a 20-hour period of dehydration and without exogenous vasopressin. These rats were not killed and are not reported in detail; however, they can be considered as part of experiment B-3 since they were part of that group until the rats of group B-4 were separated for the vasopressin test at the end of 4 weeks. The water deprivation test was performed several days later at 4½ weeks.

**Experiment C.** This study was designed to test the effect of loading with bicarbonate in the absence of potassium depletion. The plan was identical with experiment B except that the electrolyte solution administered to the experimental animals contained both sodium bicarbonate and potassium chloride as well as the sodium phosphate mixture. The amount of sodium bicarbonate was the same as that given to the potassium-deficient rats in experiments A and B; the amount of potassium chloride was the same as that given to the controls in experiments A and B. Control rats were treated as in experiment B. The ability to produce a maximally concentrated urine was tested after 5 weeks on the regimen.

**Experiment D.** The animals were female Sprague-Dawley rats with an initial weight of 100–125 gm, housed as in experiment B with free access to distilled water and with the same basal diet as in experiment B. The control and potassium-deficient animals were divided into two groups: 1) in this group the potassium-deficient rats had free access to the basal diet but the controls were individually paired with experimental rats and each control rat was allowed only the quantity of diet which its experimental pair had consumed during the previous 24 hours. The food containers could not be tipped but there was some food scattering. Electrolytes were administered by gavage as shown in table 2. The ability to form a concentrated urine was tested after 2½ weeks but these rats were not killed. 2) Identical to group D-1 except that these rats were not tested for concentrating ability but instead were killed at the time of the renal concentration test on group D-1. Studies on blood and tissues were performed as in experiment A. In this group, the control rats were not pair-fed.

The ability of each rat to form a maximally concentrated urine was tested as follows: at some time during the afternoon, 50 μu of vasopressin-in-oil (Parke, Davis & Co. Pitressin-in-oil lot S-480 B) were administered subcutaneously and drinking water was withheld. In order for the vasopressin to become fully effective, an interval of approximately 5 hours was allowed, after which the rats were stimulated to empty their bladders by covering their heads with an ether cone. This method was usually successful in stimulating the passage of some urine; it is impossible to know that the bladders were emptied. The rats were then placed in individual screen-bottomed metabolism cages without food or water. All urine passed during the subse-
URINE CONCENTRATION IN POTASSIUM DEPLETION

An aliquot of the basal diet was analyzed for its actual content of sodium and potassium using an internal-standard flame photometer, and the results are included in Table 1.

Serum was analyzed for potassium with an internal standard flame photometer and for total CO2 content by the method of Van Slyke and Neill (9). The concentration of potassium was determined by flame photometry on a dilute (0.75 N) nitric acid digest of dry fat-free muscle. The urine specimens of experiment B-4 were analyzed for sodium and potassium using a flame photometer and for total urea plus ammonia nitrogen by the method of Conway (16).

Water intake in experiment D-I was measured by weighing water bottles daily.

Statistical analysis of the results was performed using Student's t test, a probability (P) of t of less than 0.05 being considered necessary to demonstrate a significant difference.

RESULTS

The results of all studies are presented in Table 3.

Weight. The potassium-deficient rats of experiments A, B and C appeared to eat less than the controls and gained weight at a slower rate. Although food consumption was made...

The results of the various analyses are given in Table 3.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>No. of Rats/Group</th>
<th>Dur., wk.</th>
<th>Body Weight, gm</th>
<th>Serum CO2, mm./l.</th>
<th>Muscle Kc, mm/100 gm f. f.s.</th>
<th>Urine During Concentration Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>11</td>
<td>1</td>
<td>324 ± 25</td>
<td>P &gt; 0.05</td>
<td>26.6 ± 1.2</td>
<td>47.6 ± 1.2</td>
</tr>
<tr>
<td>A</td>
<td>5</td>
<td>2</td>
<td>306 ± 25</td>
<td>P &gt; 0.05</td>
<td>24.0 ± 1.2</td>
<td>48.2 ± 1.2</td>
</tr>
<tr>
<td>B</td>
<td>5</td>
<td>3</td>
<td>334 ± 25</td>
<td>P &gt; 0.05</td>
<td>25.8 ± 1.2</td>
<td>45.8 ± 1.2</td>
</tr>
<tr>
<td>B</td>
<td>5</td>
<td>4</td>
<td>342 ± 25</td>
<td>P &gt; 0.05</td>
<td>25.8 ± 1.2</td>
<td>45.8 ± 1.2</td>
</tr>
<tr>
<td>C</td>
<td>5</td>
<td>5</td>
<td>319 ± 25</td>
<td>P &gt; 0.05</td>
<td>20.5 ± 1.2</td>
<td>49.1 ± 1.2</td>
</tr>
<tr>
<td>D-1</td>
<td>6</td>
<td>1/2</td>
<td>113 ± 7.6</td>
<td>P &gt; 0.05</td>
<td>23.3 ± 1.2</td>
<td>46.9 ± 1.2</td>
</tr>
<tr>
<td>D-2</td>
<td>5</td>
<td>1/2</td>
<td>126 ± 7.6</td>
<td>P &gt; 0.05</td>
<td>23.3 ± 1.2</td>
<td>46.9 ± 1.2</td>
</tr>
</tbody>
</table>

* Group means, standard deviations, and P values. Figures in parentheses (exp. A-1) represent mean values for the cumulative control data of exp. A, B and C. In these three experiments, statistical analysis and the P values reported utilize this cumulative control data because for the particular parameters involved, analysis of variance revealed no significant difference among the several control groups, and because statistical sensitivity is enhanced by using the pooled control data.
equal in experiment D-1, and although there was no recognizable diarrhea, the potassium-deficient rats in this study also gained less weight than did their control pairs. It would appear, therefore, that the decreased growth of potassium-depleted rats is at least partly the result of a diminished utilization of food and not merely due to a smaller food intake.

Degree of Potassium Depletion. All groups subjected to a potassium-deficient intake became significantly depleted of potassium, as measured by the concentration of potassium in skeletal muscle. The rats of experiment A became depleted more rapidly than did those in experiment B, probably due to some diarrhea in the former group which was thought to be caused by the urea content of their diet. The rats of experiment D were females rather than males, were smaller than those in experiments A and B, were given more sodium bicarbonate than was used in the other studies, and became more potassium depleted in 18 days than did the rats of group B-4 in 28 days.

Ability to Form a Concentrated Urine. The effect of potassium depletion upon the ability to elaborate a concentrated urine is shown in figure 1. The concentrating ability of the control rats was quite reproducible in all experiments. With increasing potassium depletion, there was a progressive decrease in the maximum urinary concentration achieved, and this concentrating defect appears to be almost linearly related to the degree of potassium depletion (as measured by the concentration of potassium in muscle). The concentrating defect does not seem to be well correlated with the duration of potassium depletion or with the sex or initial weight of the rats.

Rate of Excretion of Solutes. The measurements of urine volume were probably only accurate to ±0.5 ml and hence the reported rates of solute excretion are imprecise. However, within the context of this limitation, the average rates of excretion of solutes during the urinary concentration tests were never significantly different in the two groups.

Bicarbonate Loading Without Potassium Depletion. The rats in experiment C had a normal concentration of potassium in muscle and produced a normally concentrated urine after 5 weeks on a diet which was adequate in potassium but which contained an amount of sodium bicarbonate comparable to that given the potassium-depleted rats in experiments A and B.

Alkalosis. The potassium depletion was generally accompanied by alkalosis, as judged by the serum CO₂ content. A relationship similar to that depicted in figure 1 can be shown to exist between the serum CO₂ content and the maximum achievable urinary concentration, except that potassium-depleted rats of group A-1 did not have a serum CO₂ content significantly above control levels.

Composition of the Urine. Figure 2 presents the mean concentration of the principal urinary solutes for experiment B-q. It will be noted that the proportional decrease in the concentration of ‘urea’ was approximately the same as the proportional decrease of twice the sodium plus potassium. It is therefore likely that the renal concentrating defect...
related to the total solute concentration that the kidney could achieve rather than to the concentration of any particular solute.

**Serum Solute Concentration.** The osmolality of the blood was not determined in most instances. In a few such determinations (B-4), the sera of two control rats had a slightly higher average concentration than that of three potassium-depleted rats (295 and 285 mOs, respectively).

**Water Intake.** As shown in figure 3, daily measurements of water intake (D-1) confirm the observations of previous investigators (4-6) that animals on a potassium-deficient diet may develop polydipsia. The relationship of this abnormality to the defective renal concentrating process cannot be ascertained from the current data, but it will be noted that the polydipsia developed almost immediately after beginning the potassium-deficient intake at a time when the renal concentrating defect surely was not severe and perhaps was not yet present.

**Water Deprivation Test.** The rats subjected to a urinary concentration test using water deprivation rather than exogenous vasopressin had an average maximum urinary concentration of 1924 mOs which was significantly lower than that of their controls (2424 mOs).

**Pathologic Lesions.** There are unequivocal lesions visible by conventional staining techniques in the renal tubules of most of the potassium-deficient rats. A future report (J.O.) will deal with detailed studies of renal pathology, including microdissection of nephrons. On the basis of the findings to date, the most definite and consistent lesion appears to be in the collecting tubules.

**DISCUSSION**

The current studies indicate that in rats, an increasing degree of potassium depletion is accompanied by a progressive decrease in the maximum achievable urinary concentration. The possibility of such a relationship was suggested by Ferrebee et al. (11) in 1941;
however, the polyuria and polydipsia which they observed in dogs receiving desoxycorticosterone acetate was not prevented by protecting against potassium depletion (11).

Smith and Lasater (5) observed polydipsia and polyuria in dogs on a potassium-deficient diet (without exogenous adrenal steroids) but renal concentrating ability was not specifically reported. Brokaw (6) observed a similar increase in the water turnover of rats on a potassium-deficient diet but could not demonstrate a defective renal concentrating mechanism. Since the method of testing did not prevent evaporation, interpretation of the results is difficult. Furthermore, the test involved 36 hours of water deprivation which (particularly in the potassium-depleted rats) may have resulted in decreased rates of glomerular filtration (12), which in turn might have caused the production of a more concentrated urine than would otherwise have occurred. Finally, the measurement of density (specific gravity) rather than a colligative property could have masked a difference in concentration.

Several reports of human patients in which the data suggest a renal concentrating defect associated with potassium depletion have been reviewed by Relman and Schwartz (7) along with a report of their own studies on potassium-depleted patients in whom multiple derangements of renal function were demonstrated, including a diminished maximum urinary concentration.

The rate of glomerular filtration in the current studies may have been lower in the potassium-depleted than in the control rats (12). However, if a reduction in filtration rate had any influence on the maximum urinary concentration, it probably would be to increase it (13), and hence, this factor is not likely to have exaggerated the difference in concentrating ability which was observed.

The total electrolyte load administered by gavage in experiments A, B and D was the same for experimental and control rats, and the approximate rates of total solute excretion were never significantly different in the two groups. However, since the number of functioning nephrons may have been decreased, it is possible that the ‘normal’ rate of solute excretion represented a larger than normal solute load per nephron in the potassium-depleted rats with a consequent reduction in the maximum obtainable urinary concentration. This explanation of the data cannot be excluded. However, in a concomitant study (unpublished observations, Winters, Hollander, Williams and Welt) on two human patients in which the experimental design is based on that of Baldwin et al. (14), it has been demonstrated that the ratio $T_{m}^{H_2O}/C_{In}$ (14) was significantly less than normal in association with potassium depletion and that subsequent partial repletion of potassium was associated with an increase in the ratio $T_{m}^{H_2O}/C_{In}$ toward normal without any increase in $C_{In}$. It appears likely, therefore, that in these two human beings the diminished renal concentrating ability was at least partly due to a tubular defect.

The results were not due to the high intake of sodium bicarbonate per se in view of the results of experiment C. The role of alkalosis cannot be as readily dismissed since potassium depletion was generally associated with alkalosis in the current experiments. However, a renal concentrating defect can also be produced in rats made potassium-deficient but not alkalotic (electrolyte-free diet without sodium loading) (personal communication, Holliday). It should also be noted that in the potassium-depleted subjects studied by Relman and Schwartz (7), a renal concentrating defect
was observed in the absence of significant alkalosis.

The possibility of some nutritional deficiency in addition to that of potassium cannot be wholly excluded in experiments A and B since the potassium-deficient rats ate less food than the controls. That it did not play a necessary role in the results, however, can be inferred from experiment D in which the control and experimental rats were pair-fed and in which a marked impairment of urinary concentrating ability developed in the potassium-deficient rats while none appeared in the controls. A possible role of chloride deficiency (15, 16) is excluded by experiment D, in which the potassium-deficient rats received chloride. The results obtained with the rats subjected to water deprivation make it unlikely that factors such as poor absorption or unusually rapid inactivation of exogenous vasopressin account for the results when vasopressin was used.

The polydipsia associated with potassium depletion does not have a known explanation. It appears likely from experiment D-I that the polydipsia is not merely the result of a severe defect of the renal concentrating mechanism and that it may even precede any diminution in maximum achievable urinary concentration. If this were so, it would seem possible that the increased water intake itself might have conditioned the diminished renal response to vasopressin. It is planned to investigate this possibility further since it cannot be excluded with the available data.

The process by which water is abstracted from the renal tubular urine is unknown; hence it is impossible to know why it should be defective in association with potassium deficiency. Since certain enzymatic reactions are known to be potassium dependent (17, 18), it is intriguing to speculate on the possibility that potassium depletion may interfere with the integrity of chemical reactions either directly related to the concentrating mechanism or involved in the provision of energy for this process. It is presumed but unproved that the renal tubular cells participate in the depletion of potassium.

The authors thank Mr. Edmund Gehan of the Department of Biostatistics, University of North Carolina, for assistance with statistical considerations.

REFERENCES

6 The basal diet was initially thought to be adequate in all constituents except the specific electrolytes previously outlined. It has been realized subsequently that the quantities of copper and manganese are inadequate by some standards. Additional studies utilizing diets designed to be deficient in copper, manganese, or both have not demonstrated any defect of the renal concentrating mechanism.