Micropuncture study of composition of proximal and distal tubular fluid in rat kidney

KARL J. ULLRICH, BODIL SCHMIDT-NIELSEN, ROBERTA O’DELL, GUNDULA PEHLING, CARL W. GOTTSCHALK, WILLIAM E. LASSITER, AND MARGARET MYLLE

Department of Zoology, Duke University, Durham, and Department of Medicine, University of North Carolina, Chapel Hill, North Carolina

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4 Career Investigator, American Heart Association.

5 Established Investigator, American Heart Association. Formerly Research Fellow, Life Insurance Medical Research Fund.

performing multiple microdeterminations on each sample of tubular fluid. The fraction of the filtered substance remaining in the tubule as well as its concentration could thus be determined. The effect of rate of urine flow on the movements of water and solutes was investigated by studying rats with either low or high rates of urine flow, the latter state resulting from loading with mannitol or urea.

METHODS

Rats of the Wistar strain, 200–500 g in weight, were anesthetized with intraperitoneal sodium pentobarbital, 35 mg/kg body weight, and the left kidney exposed through an abdominal incision. Carbon 14-labeled urea, about 40 μCi/100 g body weight, was injected through the jugular vein. A priming dose of inulin in saline was followed by a maintenance infusion calculated to maintain a plasma inulin concentration of approximately 75 mg/100 ml. The infusion rate varied in different experiments from 0.0085 to 0.085 ml/min according to the rate of urine flow desired and the size of the rat. Diuresis was induced in some animals by the infusion of 20% mannitol or 10% urea solution at 0.085 ml/min. Beginning approximately 1 hr after the maintenance infusion had been started, three to five samples of tubular fluid were collected by micropuncture from surface tubules in each experiment. To prevent backflow the tubules were blocked distal to the site of puncture by the injection of a droplet of mineral oil, and the rate of collection adjusted to maintain the droplet at a constant position. Approximately 0.15–0.20 μl of tubular fluid was collected. In the animals with low urine flow the collection time varied from 30 to 60 min; in the experiments with osmotic diuresis, only 10–15 min was required. Blood samples were collected from the vena cava before and after each microsample, and urine from the experimental kidney was collected simultaneously via a ureteral catheter.

The microsamples were deposited under oil on a de-
pression slide, and 0.001–0.001 μl withdrawn for freezing point determination. Small glass capillary pipettes 0.05–0.10 μl in size (7) were used for measuring and transferring the remainder of the sample. Transfer of the sample from the droplet in the depression slide was accomplished as follows. A water-acetone cleaned capillary pipette was filled with chloroform, and the tip immersed in the oil. The chloroform was blown out into the oil in the vicinity of the sample, and when the first air bubbles appeared, the tip was immersed in the sample. After release of pressure applied by mouth, the pipette filled quickly by capillary attraction. The entire procedure was carried out under the microscope to insure that the capillary filled properly. For each set of analyses a complete set of standards was measured with the same capillary pipette used for the unknown samples. Duplicate determinations were always made for insulin, and sometimes for urea and sodium. All values are presented as determined, and no factors have been introduced for plasma water concentration or Donnan effect.

Insulin (0.05-μl sample). This was determined according to the anthrone method described in detail by Hilger, Klümpner, and Ullrich (7). The only modification was that 6 μl instead of 12 μl of anthrone reagent were used, and the microcuvette described by Ullrich and Hampel (8) was rinsed with part of the 6-μl reagent divided by air in several fluid columns. Insulin in plasma and urine (after proper dilution) was determined on 2-μl samples. For precipitation of protein, 8 μl of water were placed on a piece of parafilm, 2 μl of sample (plasma, urine or standard) were pipetted into the droplet of water, and 2 μl of 10% zinc sulphate solution and 2 μl of 0.5 N sodium hydroxide were placed on the parafilm next to the water droplet. The three droplets were then mixed with a glass rod and drawn into a glass capillary 1.2 mm in diameter. The ends of the capillary were fused in a microflame and the capillary centrifuged. In spite of the evaporation that takes place during the pipetting and mixing on the parafilm, the method was extremely accurate. The explanation for this is that with some practice the time for each precipitation is so constant that all samples are exposed to essentially the same amount of evaporation. Eight microliters of supernatant were subsequently pipetted into 200 μl of the anthrone reagent which had been placed in the middle of a 3-mm diameter glass tube. The ends of this glass tube were then sealed in a microflame, and the fluid was mixed by shaking. All samples and standards were then heated in a water bath for 10 min at 50 C. The optical density was measured in the same manner as the microsamples.

Urea. Approximately 0.08-μl samples of tubular fluid and plasma were collected on chemically clean stainless steel planchets, dried overnight, and their radioactivity determined in a windowless flow proportional counter as described by Lassiter, Gottschalk, and Mylle (3). Urea was determined chemically in plasma and urine by the Conway method (9).

Osmolality. The osmolality of tubular fluid, urine, and plasma was determined by the ultramicrocryoscopic method of Ramsay and Brown (10).

Sodium. After determining the radioactivity in the samples of tubular fluid, the dry material on the planchets was dissolved in 20 μl of distilled water. This solution was pipetted into 1 ml of freshly distilled acetone, and sodium was determined with a Beckman flame photometer by the micromethod described by Hilger, et al. (7). The error of this method is ±2.1%. Sodium concentration was determined directly in plasma and urine samples diluted 1 to 12,000 in water.

RESULTS

Antidiuretic rats. (Fig. 1 and Table 1.) Seven samples of proximal fluid were collected from three rats. All samples were isosmotic with plasma. The fluid/plasma (F/P) sodium ratio averaged 0.99 and varied from 0.93 to 1.01. The F/P inulin ratios varied from 1.5 to 3.1, indicating reabsorption of water from each tubule proximal to the site of collection. The urea concentration was measured in four samples and was higher than in plasma, but in only one sample was the F/P urea ratio as high as that for inulin. In the others, it was one-half or less.

Eight samples of distal fluid were collected from four rats. The samples were either hypo-osmotic or
isosmotic with plasma. The sodium concentration ranged from 71 to 111 mEq/liter, corresponding to F/P ratios of 0.50-0.79 (avg. 0.62). The inulin ratios varied from 5.7 to 1.7, all of which are at least twice as high as that of the highest proximal sample. There was no apparent increase in the inulin ratio along the distal convolutions in these few samples. In contrast to the proximal samples, the urea F/P ratios were equal to or somewhat higher than the inulin ratios and ranged from 6.3 to 8.1, indicating that at least as much urea was present as had been filtered.

Osmotic diuresis. (Figs. 2 and 3 and Table 1.) Fourteen samples of proximal fluid were collected from seven rats made diuretic by the infusion of a hypertonic solution of mannitol or urea. All samples were isosmotic with plasma. In each case, the sodium concentration of the tubular fluid was lower than that of plasma. This was especially true during mannitol diuresis when F/P sodium ratios varied from 0.96 to as low as 0.78, and averaged 0.90. During urea diuresis, they ranged from 0.99 to 0.91, and averaged 0.95. The F/P inulin ratios ranged from 1.25 to 3.02. Six samples from rats undergoing mannitol diuresis all had F/P urea ratios lower than their inulin ratios. The urea-C\textsuperscript{14} concen-

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TABLE 1. Results of analyses of tubular fluid, urine, and blood

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Location, % Tubule</th>
<th>GFR, ml/100 g min</th>
<th>Inulin, Osmolality, mOsm/kg H\textsubscript{2}O</th>
<th>Sodium, mEq/l</th>
<th>Potassium, mEq/l</th>
<th>Urea, mEq/l</th>
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<tr>
<td>1</td>
<td>P 16</td>
<td>0.80</td>
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<tr>
<td>2</td>
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<td>1.52</td>
<td>138</td>
<td>303</td>
<td>303</td>
</tr>
<tr>
<td>3</td>
<td>P 45</td>
<td>0.51</td>
<td>1.33</td>
<td>349</td>
<td>297</td>
<td>297</td>
</tr>
<tr>
<td>4</td>
<td>P 29</td>
<td>0.43</td>
<td>2.30</td>
<td>320</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
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<td>1.66</td>
<td>852</td>
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<tr>
<td>7</td>
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<td>6.17</td>
<td>173</td>
<td>305</td>
<td>265</td>
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<tr>
<td>8</td>
<td>D 48</td>
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<tr>
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<tr>
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</tr>
<tr>
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<td>P 29</td>
<td>0.43</td>
<td>2.30</td>
<td>320</td>
<td>300</td>
<td>300</td>
</tr>
</tbody>
</table>

P indicates proximal tubular sample. D indicates distal tubular sample.
The concentration of tubular fluid was not determined during urea diuresis because of failure to achieve equal specific activity in urine and blood under this condition. Ten samples of distal fluid were collected. Samples from the early distal convolution were hypo-osmotic but were approximately isosmotic in the late portion. The sodium concentration was quite low in all samples and ranged from 22 to 53 mm/liter, corresponding to F/P ratios of 0.16-0.38 (avg. 0.24). The lowest ratios were in samples from the late distal convolution. The F/P inulin ratio of six samples from the first half of the distal convolution averaged 4, and was 5.9 for four samples from the last half. The F/P urea ratio was the same as the F/P inulin ratio in one sample and approximately half as much in the other two distal samples from animals in mannitol diuresis.

**DISCUSSION**

Sodium and water reabsorption. In these experiments the sodium concentration of proximal fluid was essentially the same as that of plasma in antidiuretic rats, but significantly lower than plasma when osmotic diuresis was induced with mannitol or urea. These observations are consistent with the fact that proximal fluid is isosmotic with plasma (1, 3, 5, 6). They are also consistent with the two proximal sodium determinations of Walker et al. (1), and with the sodium 22 F/P ratios reported by Windhager and Giebish (2). The latter authors reported ratios not significantly different from unity during infusion of 0.9% sodium chloride, but as low as 0.87 during infusion of 20% mannitol solution. Our results differ from those of Litchfield and Bott (4), who report proximal sodium ratios greater than unity in nonenduretic animals and close to unity during mannitol diuresis. It is difficult to reconcile the last set of observations with the apparent high water permeability of the proximal epithelium. Sodium is, of course, the predominant cation in both plasma and tubular water, and presumably appears in the filtrate in concentrations predicted by the Donnan equilibrium (i.e., about 0.95 X concentration in plasma water or about 1.02 X concentration in whole plasma). Since subsequent tubular reabsorption of sodium, as for any other solute, should lead to equivalent loss of water, the tubular sodium concentration should remain essentially the same as that of plasma unless a nonreabsorbable solute, such as mannitol, is present in significant concentration. In the latter circumstance the concentration of sodium in tubular fluid must fall since all solute present obligates water, and the fraction of total solute now represented by sodium is reduced. According to this interpretation, data as reported by Litchfield and Bott...
(4) during mannitol diuresis are to be expected only if there is no proximal reabsorption of sodium and water.

The reabsorption of sodium against a chemical gradient as well as an electrical gradient (11) demonstrates the capability of the proximal epithelium to transport sodium actively. Indeed, Windhager and Giebisch have recently concluded from measurements of the short circuit current "that the major portion, possibly all, of the proximal sodium reabsorption in the rat kidney is active in nature" (12).

In the distal convolution the F/P sodium ratio averaged 0.6 in nondiuretic rats, but was as low as 0.16 during osmotic diuresis, even though the osmolality of the fluid was the same under both conditions. The difference in sodium concentration reflects the presence of additional water obligated by the osmotically active mannitol and urea, and does not necessarily indicate any difference in the activity of the sodium pump itself. The low sodium concentration in fluid entering the distal convolution presumably reflects active transport of sodium out of the ascending limb of the loop of Henle, and the slight fall in sodium concentration and rise in inulin ratio seen along the distal convolution during osmotic diuresis indicates active transport of sodium by this epithelial membrane. Further reabsorption of sodium, presumably active, occurred in the collecting ducts.

These relatively few F/P inulin ratios are in general agreement with other available estimates of regional tubular water reabsorption (1-3). The average proximal water reabsorption in our antidiuretic rats was small, however, since two late proximal samples had low inulin ratios. All investigators report considerable variation in per cent water reabsorption in individual tubules, and our average value would presumably have been greater if a larger population of nephrons had been studied.

Urea. It has been shown previously by Lassiter et al. (3), that in the nondiuretic rat approximately one-half of the filtered urea is lost from the proximal tubule, but that a similar amount or more is gained in the descending limb of the loop of Henle, since at least as much urea as filtered is present in the early distal convolution. A large amount of urea is also lost out of the collecting ducts, and apparently it is this urea that is added to the loop fluid and recirculated. The present observations confirm these observations and are of special value, since the inulin and urea determinations were done on the same samples of fluid. The few data obtained during mannitol diuresis suggest that less urea was lost from the proximal convolution and collecting ducts in this condition, and less was added to loop fluid. The urea clearance was much higher. These data are consistent with the hypothesis that with less water reabsorption along the nephron and collecting ducts, the concentration gradients favoring diffusion of urea out of the nephron are lower, and thus loss should be less transtubularly and more excreted in the final urine. Also, with diminished loss from the collecting ducts one should not expect to gain as much urea in the loop. Unfortunately, these data give little insight into the problem as to whether all movement of urea in the mammalian kidney is passive or whether active transport of urea also occurs.

REFERENCES