Micropuncture study of composition of loop of Henle fluid in desert rodents

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According to the countercurrent hypothesis for concentration of the urine, the tubular urine is first osmotically concentrated in the descending limb of the loop of Henle, then diluted in the ascending limb of the loop before its final concentration in the collecting ducts (1, 2). Previous studies of the composition of loop of Henle fluid relating to its osmolality, fluid/plasma (F/P) inulin and urea concentration ratios, and preliminary data on sodium concentration support this hypothesis (3, 4). The present report concerns these same parameters, but multiple analyses were performed on the same samples of fluid, thereby facilitating their comparison, rather than on separate samples as in the previous experiments. In addition, more definitive data on the sodium concentration of loop fluid are presented.

METHODS

Adult Mesocricetus auratus (golden hamsters) and Psammomys obesus were anesthetized with sodium pentobarbital, 35 mg/kg body weight, and given a priming intravenous injection containing 25 µC of urea-C14 and inulin, 50 mg/kg body weight. This was followed by a constant intravenous infusion containing inulin, 2 mg/min kg body weight. After allowing about 1 hr for equilibration, approximately 0.15 µl of fluid was collected by micropuncture from the bend, or very close thereto, of the loops of Henle at the tip of the exposed papilla as previously described (3). In each case the lumen of the loop was 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 in the loop. Collections lasted approximately one-half hour. Generally, before and after each collection, urine was collected in a micropipette from the mouth of a nearby collecting duct, and blood from the inferior vena cava. The samples were divided under oil and osmolality determined microcryoscopically (5), inulin spectrophotometrically (6), and urea-C14 in a windowless flow proportional counter (4). Following determination of their radioactivity, the samples which had been dried on stainless steel planchets were dissolved in water and their sodium concentration determined by flame photometry (6). The plasma urea concentration was also determined chemically (7) in order to calculate molal concentrations from the urea-C14 F/P and U/F ratios.

The animals had been fed for 4 months either a high-protein diet, consisting of soybean meal with a 4% urea solution for drinking, or a low-protein diet, consisting of barley and lettuce with tap water for drinking.
RESULTS

The results of the multiple analyses on 12 samples of loop of Henle fluid and 20 samples of collecting duct urine are presented in Table 1. The data are presented as obtained, and no factors have been introduced for plasma water concentration or Donnan effect.

Loop of Henle fluid. The osmolality of loop fluid varied from 588 to 1256 mOsm/kg H2O and averaged 836 mOsm/kg H2O.

<table>
<thead>
<tr>
<th>Sample Description</th>
<th>Inulin U/P</th>
<th>F/P Sodium</th>
<th>Urea U/P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD</td>
<td>1.20</td>
<td>1.03</td>
<td>0.37</td>
</tr>
<tr>
<td>Loop</td>
<td>1.90</td>
<td>1.07</td>
<td>0.35</td>
</tr>
<tr>
<td>CD</td>
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Collecting duct urine. The osmolality of collecting duct urine ranged from 577 to 1355 mOsm/kg H2O and averaged 882 mOsm, nearly the same as that of loop fluid in spite of the differences in times of collection. In contrast to loop fluid, however, sodium was present in low concentration with an average U/P ratio of 0.3, and urea was the major solute present, with an average U/P ratio of 3.9. In contrast to loop fluid was the average inulin U/P ratio of 109, approximately ten times that of loop fluid.

The average U/P urea ratio of 30 in the collecting duct urine is undoubtedly spuriously low because of absorption of radiation from the urea-C4 by the large amount of inulin present in these samples. The absorption of beta radiation is proportional to the weight of solute present and not simply to the number of molecules present, and thus is no major problem in analyses of loop fluid, where the inulin concentration is only one-tenth that of the final urine. In the previous series of experiments in which only the osmolality and urea-C4 or inulin-C1400H F/P ratios were determined (4), the collecting duct U/P urea ratio averaged 54, or nearly twice that of the present series, but the other concentration ratios for loop fluid and final urine were remarkably similar to those in this study.

Osmolality and sodium concentration gradients in papillary loops of Henle. In the inner zone of the medulla the epithelium of both descending and ascending limbs of the loop of Henle is very thin, and the role played by the thin ascending limb in the countercurrent mechanism has remained obscure, and hence controversial. This part of the loop has been considered either to participate actively in the countercurrent multiplier by the active transport of sodium out of the ascending limb (3, 8, 9) or to function passively as a countercurrent diffusion exchanger (6). In the latter case it has been suggested (10) that the osmolality and sodium concentration may be constant throughout this part of the loop and that the increase in osmolality of urine in the collecting ducts in the inner zone of the medulla represents a delay in reaching diffusion equilibrium which is approximated only at the tip of the papilla.

In an effort to elucidate these problems micro-punctures were performed in Psammomys on loops of Henle both at the tip of the papilla and as close to the base of the papilla as possible. The apical one-third, approximately, of the papilla is accessible to micro-

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TABLE 1. Composition of loop of Henle fluid and collecting duct urine

<table>
<thead>
<tr>
<th>Sample Description</th>
<th>Inulin, F/P</th>
<th>Osmolality (mOsm/kg H2O)</th>
<th>Sodium (mmol/l)</th>
<th>Urea (mmol/l)</th>
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CD = collecting duct.
puncture in this fashion. The results of the osmolality determinations are presented in Fig. 2. As is usual with this type of preparation, the osmolality of the final urine fell with time, but in each case loop fluid from near the tip of the papilla had approximately the same osmolality as the final urine, while that from limbs (3 descending, 1 ascending) nearer the base of the papilla was less concentrated than the final urine. The sodium concentration was determined in two of the loop samples, and the values are included in Fig. 1. In a sample from the tip of the papilla, the sodium concentration was 286 mM/liter or 30% of the osmolality and 225 mM/liter or 32% of the osmolality in a sample from a descending limb nearer the base of the papilla.

In another Psammomys, fluid from a descending limb high on the papilla had an osmolality of 740 mOsm, and fluid obtained by transmural micropuncture of an adjacent collecting duct at that level had an osmolality of 762 mOsm. The osmolality of urine from a terminal duct of Bellini was 1,078 mOsm before and 1,040 mOsm after the above collections. Thus, there was a gradient of approximately 300 mOsm along the exposed papilla, but the osmolality of descending loop and collecting duct fluids was essentially the same at the same level. Later, fluid from another descending limb high on the papilla had an osmolality of 677 mOsm and that from an adjacent collecting duct was 692 mOsm, again essentially the same.

Injection of oil into the lumen of the loops in the two Psammomys also clearly demonstrated that the lumen of the ascending limb is wider than that of the descending limb, as previously reported for the hamster (3). A similar difference in respect to external diameter has also been observed in maceration preparations of dissected nephrons from rat kidneys (unpublished observations). These observations may be relevant to the proposed difference in function of these two limbs of the loop.

DISCUSSION

These results lend further support to the counter-current hypothesis for urine concentration and demonstrate that sodium and attendant anions are the major solutes in the hyperosmotic loop of Henle fluid and that they, together with urea, constitute most of its osmotically active solute. In contrast, although collecting duct urine was equally hyperosmotic, sodium was present in low concentration, and urea was the major solute present. The high concentration of sodium in loop fluid probably results, at least in large part, from the diffusion of water out of the thin descending limb of the loop of Henle into the hyperosmotic medullary interstitium. The basic process leading to medullary hypertonicity appears to be the active transport of sodium out of the water-impermeable ascending limb of the loop of Henle, with countercurrent diffusion in the vasa recta minimizing subsequent water loss out of the water-permeable thin descending limb of the loop as proposed by Berliner (11), in addition to the water loss resulting from medullary hypertonicity. This follows since, when compared with fluid from the end of the proximal convolution, the F/P sodium ratio appears to increase less than that for inulin as the fluid flows down the loop. Detailed comparison of loop fluid with available data on proximal and distal fluids is of limited value, however, in elucidating the mechanism of operation of the countercurrent system, since the fluids have been obtained from two different types of nephrons, i.e., those with long versus short loops, from different species, and, in addition, the data from the proximal convolution must be extrapolated to the end of the proximal tubule which in itself is quite hazardous.
LOOP OF HENLE FLUID

loss of solute from the medulla. Furthermore, the finding of an osmotic gradient in that portion of the loop with only thin epithelium indicates that the thin, as well as the thick, segment of the ascending limb actively participates in the process. Analysis of fluid from the first part of the distal convolution provides clear evidence that the thick ascending limb actively transports sodium.

If, as seems likely, the F/P inulin concentration ratio is a measure of the net transtubular movement of water, the average ratio of 1.1 in this and the previous study (4) indicates that 9% of the filtered water reached the tips of the long loops of Henle under these conditions. The ratio of 1.1 is higher than that of fluid in the beginning of the distal convolution in nondiuretic rats, which averages 0.7 (4, 12). This is as expected, since the long loops dip deeper into the increasingly hyperosmotic medulla, and more water should be extracted from them than from the short loops of the superficial nephrons studied in the rat. There may be species differences as well.

Urea. At any point along the nephron, the urea ratio divided by that for inulin is a measure of the amount of urea present relative to that filtered. Since the average loop F/P urea ratio was 1.2 and that for inulin 1.1, it immediately follows that approximately as much urea as filtered was present in the fluid at the bend of the long loops of Henle under these conditions, a finding similar to that of the previous study (4). The amount of urea present in the loop under other conditions remains unknown, and it may exceed the amount filtered when the countercurrent system is operating most effectively and the urine concentration is maximal. Indeed, the urea ratio exceeded that of inulin in some of the samples in this study, but we are reluctant to attach too much significance to individual results in view of the potentially large experimental error. The findings on the loop fluid are consistent with what is known to occur in the superficial nephrons of the nondiuretic rat (4, 12), where half of the filtered urea is lost out of the proximal convolution, but regained in the loop since as much urea as filtered is present in the fluid in the early distal convolution. Presumably, loss of urea from the collecting ducts results in a relatively high concentration of urea in the interstitium of the medulla and, in turn, diffusion into the water-permeable descending limb of the loop (3, 4). Unfortunately, our results provide little insight into the mechanism of transport of urea by the mammalian kidney, and active transport of urea by some portion (or all) of the nephron has neither been demonstrated nor excluded.

Except for the difference in blood urea concentrations (4.2 and 9.4 mm/liter in the hamsters on a low-protein, and 9.6, 15, 23, and 51 mm/liter in those on a high-protein diet), there was little apparent effect of the two dietary regimens. This should not be interpreted to signify that such diets are without effect, since there are but few data, and they were obtained under the stressful conditions of anesthesia and operation. It is of interest but of unknown significance that the loop sample from the hamster with a blood urea of 51 mm/liter had the highest urea percentage of osmotically active solute and the lowest F/P urea ratio. The U/P urea ratios for the final urine were also the lowest. This was not a function of diminished water reabsorption, since the U/P inulin ratios were not unusual.

REFERENCES