Micropuncture study of net transtubular movement of water and urea in nondiuretic mammalian kidney\textsuperscript{1, 2}

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LASSITER, WILLIAM E., CARL W. GOTTSCHALK, AND MARGARET MYLLE. Micropuncture study of net transtubular movement of water and urea in nondiuretic mammalian kidney. Am. J. Physiol. 200(6) : I 139-I 146. 1961.—Anesthetized, nondiuretic rats and hamsters were infused with C\textsuperscript{14}-labeled inulin-carboxylic acid or urea, and fluid was subsequently collected by micropuncture from surface tubules in the rats and from loops of Henle and collecting ducts in the hamsters. Osmolality and radioactivity of tubular fluid, ureteral urine, and plasma were determined. There was net loss of both water and solute from all segments of the nephron. In the loop of Henle, water loss occurred primarily from the descending limb and solute loss from the ascending limb. Urea was reabsorbed from the proximal and distal convolutions and collecting ducts but was added to the tubular fluid in the descending limb of the loop of Henle. These results lend support to the countercurrent theory of urine concentration and indicate that the osmotic gradient in the medulla is established by active transport of solute out of the ascending limb of the loop of Henle. The results are compatible with passive movement of water and urea, but the possibility of active transport of urea is not excluded.

The first micropuncture study of water reabsorption in the proximal tubule of the mammalian kidney was reported in 1941 by Walker and his associates (1). These investigators employed exogenous creatinine as an indicator of water reabsorption, or glucose in phlorizinized animals, and estimated that 60\% of filtered water is reabsorbed by the midpoint of the proximal tubule in the rat. Some of their animals, however, were made moderately diuretic by infusion of 10\% sucrose or 0.9\% sodium chloride, which might have tended to limit water reabsorption, and Fingl (2) has presented evidence for the tubular secretion of creatinine in the rat kidney. Also, their rats were unilaterally nephrectomized, which might have modified glomerular filtration and tubular function in the remaining kidney. We have therefore reinvestigated the question of proximal water reabsorption in strictly nondiuretic animals, using inulin as a more reliable indicator of water reabsorption, and have extended the observations to the other parts of the nephron. Our findings lend further support to the countercurrent hypothesis for urine concentration (3) and throw additional light on the mode of action of the countercurrent system.

Since urea is of recognized importance in the formation of a concentrated urine (4-7) and its movement is probably conditioned importantly by water reabsorption, a study of the transtubular movement of urea was included in this investigation. Micropuncture studies have previously been reported (8) of the urea concentration in renal tubules of amphibia, but not in those of mammals.

\textbf{METHODS}

\textit{White Rats}

Normally hydrated, nondiuretic male rats of the Wistar strain, 200-350 g in weight, were anesthetized by intraperitoneal injection of sodium pentobarbital, 35 mg/kg body wt, and the left kidney was exposed through an abdominal incision. The kidney was bathed in mineral oil, and fluid samples were collected by micropuncture from surface tubules, as previously described (9, 10). To prevent retrograde flow, tubules were blocked by injection of a droplet of mineral oil, and the rate of

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\textsuperscript{2} This investigation was supported by American Heart Association grant 59 G 151 and by Public Health Service grant H-2334 (C3).


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\textsuperscript{5} Established Investigator, American Heart Association.
collection was adjusted to maintain the droplet at a constant position just distal to the puncture site. From 0.05 to 0.10 μl of tubular fluid could be collected in this manner in 20 to 60 min. Each sample was then discharged under mineral oil into a siliconed glass dish and a small aliquot (10^-8 to 10^-4 μl) used for determination of osmolality by the ultramicrocryoscopic method of Ramsay and Brown (11). The remainder of the sample was aspirated into calibrated, constant-bore Pyrex capillary tubing, between layers of chloroform to prevent evaporation, and volume was estimated by measurement of the length of the sample in the capillary with an eyepiece micrometer. The fluid was then analyzed for either inulin or urea. Tracer compounds labeled with carbon 14 were used to facilitate analysis of the very small samples.

At the conclusion of each experiment the kidney was removed and macerated, and micropuncture sites were localized by microdissection (9). As in previous studies, the proximal tubule is considered to extend from the glomerulus to the beginning of the thin descending limb of the loop of Henle; the distal convolution, from the macula densa to its junction with one or more other distal convolutions to form a collecting duct.

In the inulin studies rats were given a priming injection of 15 μc inulin-C14 carboxylic acid through an indwelling jugular cannula and were then infused at the constant rate of 25 μc in 0.5 ml isotonic saline/hr. In most instances reasonably constant levels of plasma radioactivity were obtained. Experiments in which the inulin levels in consecutive plasma samples differed by more than 10% were discarded. In the urea studies animals were given a single intravenous injection of 100 μc urea-C14, and at least 1 hr was allowed for isotopic equilibration before micropuncture.

After estimation of volume each sample was plated on an aluminum planchet and dried overnight at room temperature, and radioactivity was determined in a Packard windowless flow proportional counter. Self-absorption corrections were unnecessary because of the small size of the samples. Similar determinations were made on ureteral urine and on plasma obtained from the inferior vena cava before and after each micropuncture. Sufficient radioactivity was present in most samples to count at a rate of at least twice background. In the urea studies samples were acidified by addition of a drop of 0.015 N HCl to each planchet, in order to eliminate any C14-labeled bicarbonate which might have been produced in vivo by bacterial degradation of the urea.

**Hamsters**

Golden hamsters, 50-125 g in weight, were prepared in a fashion similar to the rats, except that the upper part of the left ureter was opened to expose the tip of the papilla. Inulin-C14 OOH was infused intravenously at the rate of 12.5 μc in 0.25 cc saline/hr, after a priming dose of 7.5 μc. It proved more difficult in these animals than in the rats to maintain a constant plasma level of inulin, and only those experiments in which the level varied less than 15% in consecutive plasma samples are included. In the urea studies, 50 μc of urea-C14 was given in a single intravenous injection. Fluid was collected by trans-tubular micropuncture from loops of Henle near their

**TABLE 2. Tubular fluid to plasma inulin and osmolality ratios**

<table>
<thead>
<tr>
<th>Rat No. of Tubule</th>
<th>Proximal % F/P Osm.</th>
<th>F/P Inulin</th>
<th>Distal % Conv. F/P Osm.</th>
<th>F/P Inulin</th>
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<tbody>
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<tr>
<td>14</td>
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**TABLE 1. Comparison of excretion of stable inulin and inulin-C14OOH in rats**

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Source of Sample</th>
<th>Counts/min</th>
<th>Inulin, mg%</th>
<th>Specific Activity, counts/mg % Inulin</th>
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<td>179</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>Urine 1</td>
<td>52,075</td>
<td>674</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>Urine 2</td>
<td>160,000</td>
<td>2,030</td>
<td>78</td>
</tr>
<tr>
<td>2</td>
<td>Infusion</td>
<td>34,516</td>
<td>80</td>
<td>976</td>
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<td>Urine 1</td>
<td>13,402</td>
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<td>282</td>
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<tr>
<td></td>
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<td>Blood</td>
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<tr>
<td></td>
<td>Urine 2</td>
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<td>18,400</td>
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<td>Urine 3</td>
<td>124,264</td>
<td>17,500</td>
<td>7-1</td>
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bends and from adjacent collecting ducts or from the orifices of terminal collecting ducts, and analyses were performed as noted above.

RESULTS

White Rats

Inulin. Specific Activity. To test the validity of the radioisotope measurements as a true index of inulin excretion, two rats were infused with isotonic saline containing from 0.5 to 2.0 mg stable inulin and 2.0 μC inulin-C14 OOH/ml. The infusion solution and bladder urine were then analyzed for both radioactive and stable inulin. A third animal received an infusion containing 100 mg stable inulin and 50 μC inulin-C14 OOH/ml, and samples were collected from proximal tubules for analysis. Stable inulin was determined by the ultramicro method of Hilger, Klümper, and Ullrich (19). Results of the three experiments are summarized in Table 1. In each experiment the specific activity of inulin in tubular fluid or urine agrees with that of plasma or the infusion fluid to within 16%, except where self-absorption interferes, as is the case with the high urinary inulin concentration in the third experiment. In the experiments to follow the quantity of inulin infused was always small, and significant self-absorption did not occur.

Proximal Tubule. Samples of proximal tubular fluid were collected for determination of osmolality and inulin content from 16 tubules in 9 rats. The inulin fluid to plasma concentration ratios, corrected to plasma water, are summarized in Table 2. All samples were isosmotic with plasma. Although there was wide variability among individual tubules, the average inulin concentration increased progressively along the proximal tubule, as shown in Fig. 1, in which the fluid to plasma inulin concentration ratio is plotted, after correction to plasma water, as a function of distance along the tubule. The representation of the inulin ratio as a linear function of distance is intended merely to indicate the trend. Because of the variability among different tubules, the true shape of the curve cannot be determined with certainty. At the two-thirds point, that is, at the end of the convoluted portion of the tubule, the inulin concentration ratio reached approximately 3. In Fig. 2 the same data are plotted in reciprocal fashion to indicate the fraction of filtered water remaining at each level in the tubule. No
and urea-F4 in rats

distal convolution had an osmolality averaging 0.7 times
recorded in Table 2. Fluid from the first third of
original glomerular filtrate.

those based on ch .emical determinations.
ratios computed from radioisotope measu
stable urea determinations were performed on urine and


TABLE 3. Comparison of excretion of stable urea
and urea-C14 in rats

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Source</th>
<th>Urea U/P Ratio</th>
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<td>4</td>
<td>Urine 1</td>
<td>204</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>115</td>
</tr>
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</table>

collections were made from the final third of the proximal
tubule, the pars recta, since this portion of the tubule
plunges deep into the kidney substance and is not accessible
to micropuncture.

DISTAL CONVOLUTION. Fifteen samples of fluid from the
distal convolution were obtained in nine rats. Results of
the determinations of osmolality and the inulin ratio are
recorded in Table 2. Fluid from the first third of the
distal convolution had an osmolality averaging 0.7 times
that of plasma, and the inulin ratio in these samples
averaged 6.9. The inulin concentration increased pro-
gressively along the distal convolution, as is shown in
Fig. 3. Fluid from the last third of the convolution was
isosmotic with plasma and had an average inulin concen-
tration ratio of 14.9.

URETERAL URINE. The average of 60 determinations of
urine osmolality in these rats was 6.4 times that of plasma
(σ = 0.3). The inulin ratio averaged 690 (σ = 45),
indicating a mean excretion of 0.14% of the water in the
original glomerular filtrate.

UREA. SPECIFIC ACTIVITY. Simultaneous radioactive and
stable urea determinations were performed on urine and
plasma in four rats. Urine to plasma ratios are recorded in
Table 3. No consistent difference was noted between
ratios computed from radioisotope measurements and
those based on chemical determinations.

PROXIMAL TUBULE. Collections for urea determination
were made from 2 glomeruli and 21 proximal tubules in
12 rats. Results are shown in Table 4. All proximal
samples were isosmotic with plasma. The urea concentra-
tion in the two samples of glomerular fluid was the same
as that in plasma water. As shown in Fig. 4, the urea
ratio increased to about 1.5 in the first quarter of the
tubule but then increased little more along the remainder
of the accessible portion of the tubule, indicating loss of
urea from the tubular lumen.

DISTAL CONVOLUTION. The osmolalities and urea ratios
of 32 collections of distal fluid from 17 rats are also shown


in Table 4. Wide variation in the urea content of different
tubules was noted, particularly among late distal samples.
The average urea ratio of fluid from the first third of the
convolution was 7.7. Although there was wide scatter
among late distal samples, the trend was for the urea
concentration to increase along the convolution, and the
average ratio of the final third was 10.5.

URETERAL URINE. The urea ratio of the ureteral urine,
determined in 44 samples from 18 rats, averaged 90
(σ = 6).

Hamsters

The results of collections made from loops of Henle and
adjacent collecting ducts in the exposed tip of the ham-
ster papilla are summarized in Table 5. The osmolality
of fluid from the loop of Henle was essentially identical
to that in adjacent collecting ducts, as previously re-
ported (10), but the two fluids differed markedly in the
fraction of filtered water remaining, as indicated by the
inulin concentration ratios, and in chemical composition.
In the final urine the most important osmotically active
constituent was urea, but urea contributed much less to
the osmotic activity of the loop of Henle fluid, where Na+
is present in high concentration (13). Two collections
were made from loops in a hamster infused with NaCl,
and the results confirm the presence of a high sodium
concentration in fluid at the bend of the loop (Table 5).

DISCUSSION

Validity of Methods

WATER. The inulin clearance has long been accepted as
a reliable measure of glomerular filtration. Hendrix,
Westfall, and Richards (14) demonstrated by micro-
puncture that inulin is completely filtrable by the glo-
meruli in frogs and Necturus, and Richards, Bott, and West-
fall (15) showed that no tubular secretion of inulin occurs
in the intact kidneys of frogs, dogs, and rabbits. Much
further evidence supporting the reliability of inulin as an
index of glomerular filtration has been reviewed in detail
by Smith (16). Cotlove (17 and personal communication)
has shown that in the steady state the renal clearance of
inulin-C14OOH is identical to that of regular inulin, and
our experience has been similar (1 Table 1). Our measure-
ments of the concentration of inulin-C14OOH in tubular
fluid are thought, therefore, to provide a reliable index
of the net transtubular movement of water.

UREA. In the case of labeled urea, the tracer is added
to a large body pool of urea, and the problem of equilibra-
tion must be considered. If urea-C14 is administered in a
single intravenous injection and sufficient time is allowed
for equilibration, the specific activity of the urea pool
should decrease exponentially as labeled urea is excreted
and new, unlabeled urea is synthesized by the body.
Walser and Bodenlos (18) administered urea-C14 to
humans and found that, although both urine and plasma
urea specific activity decreased exponentially, the specific
activity of urinary urea remained consistently higher.
TRANSTUBULAR MOVEMENT OF WATER AND UREA

than that of simultaneously obtained plasma, the difference corresponding to a "urinary delay time" of about 1 hr. They considered this to indicate that the excreted urea was in equilibrium, not with plasma, but with possibly a pool of urea in the renal parenchyma, and that "a fall in specific activity, therefore, would not be reflected in the excreted urea until the specific activity of this pool had reached the same value." Although the data are not extensive, in our studies we found no consistent discrepancy between the urine:plasma urea ratios measured chemically and with the C14-labeled tracer (Table 3). The relatively low urea clearance and slow fall in plasma radioactivity in our animals would tend to minimize the effect of the "urinary delay time" on the validity of the urea-C14 determinations in tubular fluid.

**Proximal Tubule**

*Water.* Water loss occurred progressively along the convoluted portion of the proximal tubule, as indicated by the rise in inulin concentration (Fig. 1), and at the end of the convolution only one-third of the filtered water remained within the tubular lumen. This is in reasonable agreement with the estimate of proximal water reabsorption made by Walker et al. (1) with creatinine (Fig. 5). These authors, by extrapolating the water reabsorption curve to the end of the tubule, estimated that approximately 80% of the glomerular filtrate is reabsorbed in the proximal tubule. The final third of the tubule, the pars recta, however, plunges deep into the kidney and differs anatomically and possibly functionally from the convoluted portion on the kidney surface. Possibly countercurrent diffusion occurs here as in the other parts of the loop of Henle. We feel it is quite hazardous, therefore, to extrapolate beyond the experimentally determined points, and the inulin concentration at the very end of the proximal tubule remains a matter for speculation.

Since proximal tubular fluid is always isosmotic with plasma (1, 10), solute is reabsorbed from the tubule in proportion to water; thus about two-thirds of filtered solute is reabsorbed in the convoluted portion of the proximal tubule. The strictly isotonic character of proximal fluid suggests that the proximal tubule is freely permeable to water and that water reabsorption at this site is passive and is a consequence, in large measure, of the reabsorption of solute.

*Urea.* A large fraction of the filtered load of urea is reabsorbed in the proximal tubule. Urea and inulin concentration ratios are plotted together in Fig. 6, and it will be noted that at the end of the convoluted portion of the tubule the urea ratio was only half the inulin ratio, indicating loss of 50% of the filtered urea. It seems likely that urea diffuses out of the proximal tubule along its concentration gradient, but the precise concentration of urea in the interstitial fluid of the kidney cortex is not actually known, so the possibility of active urea transport in the proximal tubule is not excluded.

**Loop of Henle**

*Water.* The loop of Henle of the surface tubule in the rat is not accessible to micropuncture, but comparison of late proximal with early distal fluid permits deductions regarding its over-all function. The countercurrent multiplier mechanism for urine concentration in the loop of Henle could in theory be activated either by active

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**Table 4. Tubular fluid to plasma urea and osmolality ratios**

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Proximal, % of Tubule</th>
<th>F/P Osm</th>
<th>F/P Urea</th>
<th>Distal, % of Conv.</th>
<th>F/P Osm</th>
<th>F/P Urea</th>
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<td>5.1</td>
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<td>0.94</td>
<td>5.1</td>
</tr>
<tr>
<td>35</td>
<td>21</td>
<td>0.87</td>
<td>2.5</td>
<td>21</td>
<td>0.87</td>
<td>2.5</td>
</tr>
<tr>
<td>36</td>
<td>61</td>
<td>1.00</td>
<td>1.6</td>
<td>61</td>
<td>1.00</td>
<td>1.6</td>
</tr>
<tr>
<td>37</td>
<td>30</td>
<td>0.73</td>
<td>9.0</td>
<td>30</td>
<td>0.73</td>
<td>9.0</td>
</tr>
<tr>
<td>38</td>
<td>51</td>
<td>1.02</td>
<td>1.7</td>
<td>51</td>
<td>0.67</td>
<td>7.6</td>
</tr>
<tr>
<td>39</td>
<td>30</td>
<td>0.67</td>
<td>7.6</td>
<td>30</td>
<td>0.67</td>
<td>7.6</td>
</tr>
<tr>
<td>40</td>
<td>40</td>
<td>0.67</td>
<td>7.2</td>
<td>40</td>
<td>0.67</td>
<td>7.2</td>
</tr>
</tbody>
</table>
transport of water from the medullary interstitium into the ascending limb of the loop of Henle or by active sodium transport out of an ascending limb which is relatively impermeable to water. In either case, early distal fluid would be hypertonic to plasma. If active transport of water into the loop occurs as the primary event, more water should be present at the beginning of the distal convolution than at the end of the proximal tubule. Comparison of late proximal and early distal inulin ratios, however, indicates that this is not so (Fig. 7, Table 6). Indeed, the inulin ratio of hypertonic fluid from the first third of the distal convolution is more than twice that of late proximal fluid. Since water is lost, not gained, in the loop, the hypertonicity of early distal fluid must then be a consequence of the removal of solute in excess of water from the loop of Henle.

Analysis of fluid collected at the bend of the loop in the hamster papilla indicates that loss of water occurs primarily from the descending limb and loss of solute from the ascending limb of the loop, as predicted by the countercurrent theory. As is shown in Table 5, fluid at the bend of the loop is hypertonic to plasma, its osmolality being comparable to that of urine in adjacent collecting ducts, and solute removal therefore occurs beyond the bend, in the ascending limb of the loop. The relatively high inulin ratio at the bend of the loop indicates water loss beyond the proximal convolution, from the descending limb of the loop. Urea, although present in increased concentration in the loop, cannot account for the high concentration of solute in loop fluid which, according to countercurrent theory, should form the most part be Na\(^+\) and associated anions. This is demonstrated by our finding of an elevated loop sodium concentration on two determinations with Na\(^+\).

Long loops of juxtamedullary nephrons in the hamster cannot of course be directly compared with surface tubules in the rat, but it is probably safe to assume that the descending limbs of long loops function in a manner qualitatively, although not quantitatively, similar to the shorter loops of cortical nephrons. Thus, at the bend of the short loops, the sodium concentration and inulin, urea, and osmolality ratios should all be somewhat lower. Comparison of events in the ascending limbs of long and short loops is more difficult, for only the long loop has both a thin and a thick ascending segment, and the functions of the thin ascending segment remain speculative.

Urea. Throughout most of the mammalian nephron, concentration gradients are thought to favor the diffusion of urea out of the tubule into the surrounding interstitium, paralleling the diffusion of water. The loop of Henle dips down into a region of presumably increasing interstitial urea concentration (19), however, and our results indicate that, although water diffuses out of the descending limb of the loop of Henle, there is net movement of urea in the opposite direction, into the lumen of the tubule. Urea and inulin concentration ratios at the end of the proximal convolution and in early distal fluid are compared in Table 6. The urea ratio divided by the inulin ratio at any point in the nephron is a measure of the amount of urea reaching that point, relative to the amount filtered. Thus, at the end of the proximal convolution the urea ratio is one-half the inulin ratio, indicating that a quantity of urea equivalent to half that filtered reaches the end of the convolution. Although half the filtered urea is lost from the proximal convolution, an amount at least equal to the filtered load reaches the distal convolution, indicating net movement of urea into the loop of Henle. That the addition of urea to the tubular fluid occurs primarily in the ascending limb of the loop is indicated by the relatively high urea concentration at the bend of the loop in the hamster papilla (Table 5).

The demonstration that urea is added to the tubular fluid in the loop of Henle should not be considered evidence for active transport of urea. Although the urea concentration in the interstitial fluid of the medulla is unknown, the overall urea concentration in tissue slices has been shown to increase progressively from the outer medulla to the tip of the papilla (7, 19). The collecting duct is permeable to urea when antidiuretic hormone is present, and diffusion of urea from the collecting duct, where it is present in high concentration, almost surely contributes to the maintenance of a high urea concentration in the medullary interstitium. Active transport of urea by the renal tubule has been shown to occur in elasmobranch fishes (20, 21) and in frogs (8, 22). In mammals, on the other hand, active transport has not been conclusively demonstrated, although the studies of Schmidt-Nielsen (23, 24) suggest that it may occur, at least in desert rodents. The results of the present study do not exclude the possibility of active urea transport. However, the quantity of urea lost from the urine beyond the late distal convolution appears to be sufficient to account at least in large measure for the gain in urea between the late proximal and early distal convolutions (Table 6). Whether the addition of urea to the loop can thus be explained entirely by passive diffusion or whether active transport of urea is involved remains a matter for speculation, however. Only superficial proximal and distal tubules are accessible to micropuncture, and the extent to which urea is concentrated and reabsorbed may be different in the deeper nephrons of the kidney.

In brief, our results lend support to the following conception of the function of the loop of Henle as a countercurrent system. Water is removed from the descending limb of the loop by passive diffusion out of the loop into the hypertonic medullary interstitium, and urea diffuses into the loop at this site. Active transport of Na\(^+\) out of the ascending limb of the loop, which must be relatively

### Table 5. Composition of loop of Henle fluid and collecting duct urine in the hamster papilla

<table>
<thead>
<tr>
<th></th>
<th>Loop Fluid</th>
<th>Collecting Duct Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>F/P osmolality</td>
<td>2.8 (29 samples)</td>
<td>3.0 (35 samples)</td>
</tr>
<tr>
<td>F/P inulin</td>
<td>11 (11 samples)</td>
<td>12 (14 samples)</td>
</tr>
<tr>
<td>F/P urea</td>
<td>17 (9 samples)</td>
<td>54 (9 samples)</td>
</tr>
<tr>
<td>F/P Na(^+)</td>
<td>2.2 (2 samples)</td>
<td>0.25 (2 samples)</td>
</tr>
</tbody>
</table>
watertight, activates the countercurrent multiplier system and thus leads to the establishment and maintenance of hypertonicity in the medulla. The hypertonicity of early distal fluid is thus a consequence of the removal of sodium (and chloride) without water from the ascending limb of the loop. From our data one cannot exclude the possibility that active transport of Na⁺ also occurs out of the descending limb of the loop, but certainly water loss predominates at this site.

**Distal Convolution**

**Water.** In the non-diuretic state, water loss continues in the distal convolution, as indicated by the progressive rise in inulin concentration along the convolution (Fig. 3). In normal, non-diuretic rats, the hypotonic fluid entering the distal convolution invariably becomes isosmotic with plasma before it reaches the collecting duct (10, 25); thus water is lost from the distal convolution in excess of solute, in contrast to the situation in the loop of Henle, where solute is extracted from the tubule in excess of water. In this manner solute, for the most part Na⁺ plus anion, is retained in the medulla, where it contributes to the high interstitial osmolality, and the “free” water remaining in the tubule is delivered to the distal convolution, from which it is subsequently lost into the interstitium of the cortex, where it does not dilute the high osmolality being maintained in the medulla by the countercurrent multiplier system.

Although water loss predominates in the distal convolution, solute removal continues as well. As seen in Table 6, the inulin ratio increased by more than twofold along the distal convolution, but the increase in osmolality was less, indicating removal of solute from the tubular fluid.

**Urea.** The urea ratio in hypotonic samples collected from the first part of the convolution was similar to the inulin ratio (Table 6), but the variability in the urea ratio in late distal samples was much greater than that of the inulin ratio, suggesting varying degrees of permeability to urea among different convolutions. At least one other parameter of kidney function, the pH of tubular fluid, has been noted to exhibit more natural variability in the distal tubule than in other parts of the nephron (26). Despite the great variability among individual nephrons, the urea concentration tended to increase along the distal convolution, and, on the average, urea equivalent to 70% of the filtered load reached the end of superficial convolutions to be presented to the collecting ducts.

**Collecting Ducts**

**Water.** In the non-diuretic animal, fluid entering the collecting ducts from superficial distal convolutions is isosmotic with plasma; therefore, the final elaboration of a concentrated urine occurs in the collecting ducts (10, 25). According to countercurrent theory, this is achieved by passive reabsorption of water as the urine within the collecting ducts achieves osmotic equilibrium with the surrounding hypertonic interstitium in the medulla. Our results indicate that, of the roughly 5% of glomerular filtrate which reaches the end of the surface distal convolution, nearly all appears to be reabsorbed in the collecting ducts, only about 0.15% of the original glomerular water appearing as urine, on the average, in the rats in our study. Caution must be exercised, however, in comparing fluid from individual surface tubules with the final urine, for the urine reflects the over-all activity of all the nephrons in the kidney, and fluid entering the collecting ducts from deeper nephrons almost certainly has a higher inulin ratio than that from surface tubules.

Although the inulin concentration ratio in urteral urine was some 40% greater than that in late distal fluid, the average urine osmolality in these rats was 1,900, only 6.4 times greater than the late distal osmolality. This indicates that, in addition to water, there was also considerable reabsorption of solute, amounting to roughly 85% of the total solute delivered to the collecting ducts. Solute reabsorption from the collecting ducts has also been noted by Hilger, Klümpér, and Ullich (12). These investigators obtained fluid by microcatheterization from various levels in collecting ducts of hamsters and found evidence of active sodium reabsorption.

**Urea.** Comparison of the urea and inulin ratios in late distal fluid and urteral urine (Table 6) indicates that the urea clearance under the experimental conditions of this study much of the urea entering the collecting ducts was reabsorbed and did not appear in the final urine. Urea reabsorption at this site has also been demonstrated in the hamster kidney by Klümpér et al. (27). As already noted, urea was lost from the collecting ducts contributes to the maintenance of a high interstitial urea concentration in the medulla, and much of it apparently diffuses ultimately into the descending limb of the loop of Henle and is recirculated.

The urea clearance in these experiments averaged only 13% of the glomerular filtration rate, as indicated by comparison of the urea and inulin ratios in the final urine (Table 6). This is lower than the ratio observed by others in unanesthetized rats (23, 28) and is believed to be a consequence of unusually efficient water conservation in these anesthetized animals. If the collecting ducts are permeable to urea, as is believed to be the case when antidiuretic hormone is present, factors promoting the reabsorption of water would tend to increase the intraluminal concentration of urea in the collecting ducts and thus its rate of back-diffusion. As we have noted, nearly all the water delivered from the distal convolution to the collecting ducts was reabsorbed in our rats. By contrast, the inulin ratio in hamster urine was much lower, only

### Table 6. Average fluid to plasma osmolality, inulin, and urea ratios in the rat kidney

<table>
<thead>
<tr>
<th>Source</th>
<th>Osmolality F/P</th>
<th>Inulin F/P</th>
<th>Urea F/P</th>
<th>Inulin Ratio</th>
<th>Urea Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early proximal</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Late proximal</td>
<td>1.0</td>
<td>3.0</td>
<td>1.5</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Early distal</td>
<td>0.7</td>
<td>6.9</td>
<td>7.7</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>Late distal</td>
<td>1.0</td>
<td>14.9</td>
<td>10.5</td>
<td>0.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Uretical urine</td>
<td>6.4</td>
<td>690</td>
<td>90</td>
<td>0.13</td>
<td></td>
</tr>
</tbody>
</table>
REFERENCES

have observed by microdissection that proximal tubules of the rat kidney to be isosmotic with plasma, and we Wirz, Hargitay, and Kuhn (30) found the entire cortex
ences should be minimal in the proximal tubule, for that observed in more superficial nephrons. These differ-
dent, and it is probable that water and solute reabsorp-
enter the inner medulla and possess a thin ascending seg-}

in the rat kidney have long loops, that is, loops which
osmotic gradients established by solute movement. Filtered water is reabsorbed in the convoluted portion of
passive, conditioned by membrane permeability and the
tonic by the end of the convolution. In all parts of the
nephron transtubular movement of water is thought to be
variably isosmotic with plasma (I, I0); hence, in this seg-
ment solute reabsorption parallels water reabsorption. In
the loop of Henle, solute is lost in excess of water, and
the reverse occurs in the distal convolution, leaving about 5% of the original volume of water to enter the
collecting ducts. Fluid in the proximal convolution is
variably isosmotic with plasma (1, 10); hence, in this segment solute reabsorption parallels water reabsorption.
In the loop of Henle, solute is lost in excess of water, and
the reverse occurs in the distal convolution, since distal fluid is initially hypotonic to plasma but becomes iso-
tonic by the end of the convolution. In all parts of the
nephron transtubular movement of water is thought to be
passive, conditioned by membrane permeability and the
osmotic gradients established by solute movement.
According to Sperber (29), about 98% of the nephrons
in the rat kidney have long loops, that is, loops which
enter the inner medulla and possess a thin ascending seg-
ment, and it is probable that water and solute reabsorp-
tion in these deep nephrons differs quantitatively from
that observed in more superficial nephrons. These differ-
ences should be minimal in the proximal tubule, for Wirz, Hargitay, and Kuhn (30) found the entire cortex of
the rat kidney to be isosmotic with plasma, and we have observed by microdissection that proximal tubules

TABLE 7. Estimated segmental contributions to net water reabsorption in superficial nephrons

<table>
<thead>
<tr>
<th>Source</th>
<th>Inulin F/P</th>
<th>Water Remaining in Tubule, % of Glomerular Filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerulus</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Late proximal convol.</td>
<td>3</td>
<td>33</td>
</tr>
<tr>
<td>Distal convol., beginn.</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>Distal convol., end</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>Ureteral urine</td>
<td>690</td>
<td>0.14</td>
</tr>
</tbody>
</table>

120 (Table 5), and, although the urea ratio was no higher than that seen in the rats, this indicates excretion of a larger fraction of filtered urea in the hamsters.

Segmental Contributions to Water and Solute Reabsorption

Both water and solute are lost from all segments of the mammalian nephron, but it is apparent that the bulk of reabsorption occurs in the proximal tubule. Net water loss from the various segments of surface nephrons is indicated in Table 7. Values for the inulin ratios at the beginning and end of the distal convolution are estimated by extrapolation. On the average, about two-thirds of the filtered water is reabsorbed in the convoluted portion of the proximal tubule, another 10-15% in the loop of Henle, and 15% from the distal convolution, leaving about 5% of the original volume of water to enter the collecting ducts. Fluid in the proximal convolution is invariably isosmotic with plasma (1, 10); hence, in this segment solute reabsorption parallels water reabsorption. In the loop of Henle, solute is lost in excess of water, and the reverse occurs in the distal convolution, since distal fluid is initially hypotonic to plasma but becomes isotonic by the end of the convolution. In all parts of the nephron transtubular movement of water is thought to be passive, conditioned by membrane permeability and the osmotic gradients established by solute movement. According to Sperber (29), about 98% of the nephrons in the rat kidney have long loops, that is, loops which enter the inner medulla and possess a thin ascending segment, and it is probable that water and solute reabsorption in these deep nephrons differs quantitatively from that observed in more superficial nephrons. These differences should be minimal in the proximal tubule, for Wirz, Hargitay, and Kuhn (30) found the entire cortex of the rat kidney to be isosmotic with plasma, and we have observed by microdissection that proximal tubules

of superficial and juxtaglomerular nephrons are similar in length and appearance (unpublished observations). The long loops of Henle of the deep nephrons, however, dip into a region of higher interstitial osmolality in the inner medulla, and water reabsorption in these loops almost certainly exceeds that observed in surface nephrons; thus the inulin ratio at the beginning of distal convolutions of deep nephrons is probably higher than that observed in surface convolutions. On the other hand, the osmolalities of early distal fluids in the two groups of nephrons may be similar, for early distal fluid is uniformly hypotonic to plasma in the desert rodent Psammomys obesus, an animal in which all the nephrons have long loops. We (13) have observed an increment in osmolality toward the tip of the papilla along thin segments of loops in the Psammomys, in a region where there are no thick ascending segments. It is therefore probable that the thin segment participates in the active transport of sodium out of the ascending limb of the loop and that water and solute losses are both increased in long loops.

Urea is lost in surface nephrons from the proximal and distal convolutions and added to tubular fluid in the descending limb of the loop of Henle (Table 6). Transtubular movement of urea in deep nephrons probably differs, at least in degree, from that observed in surface tubules. The urea concentration in slices of renal medulla, and presumably in the interstitial fluid of the medulla, increases progressively toward the tip of the papilla (19). More urea appears to diffuse into the descending limb of long loops, and, although the high urea concentration in early distal fluid in surface nephrons suggests that the thick portion of the ascending limb is relatively impermeable to urea, this may not be true of the thin segment; thus some urea may be trapped in the inner medulla by countercurrent diffusion from the thin ascending limb of the loop into the descending limb. Although we cannot be certain of the over-all magnitude of urea reabsorption in deep nephrons, it is improbable, unless there is active urea transport, that it exceeds that observed in surface nephrons, and the quantitative importance of the collecting ducts in urea reabsorption in the nondiuretic kidney, indicated in Table 6, is emphasized.

In the specific activity studies we are indebted to Drs. Karl J. Ullrich, Bodil Schmidt-Nielsen, and Roberta O'Dell, who performed the chemical analyses for inulin and urea. Inulin-C14 carboxylic acid was supplied by New England Nuclear Co., Boston, Mass.

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