Micropuncture study of the mammalian urinary concentrating mechanism: evidence for the countercurrent hypothesis

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In their classical mammalian micropuncture study, Walker et al. (1) demonstrated that fluid from Bowman's capsule and the first two-thirds of the proximal tubule of rats, guinea pigs and one opossum, was isosmotic with plasma while the kidney was elaborating hyperosmotic urine. They also collected three samples from distal convolutions, two of which were hypo-osmotic, the third, isosmotic. The physiological condition of these animals is probably best characterized as a state of mild diuresis, resulting from the intravenous infusion of 10% sucrose or 0.9% sodium chloride solution. Earlier, Walker et al. (2) had demonstrated that fluid from proximal convoluted tubules of Necturi and frogs was essentially isosmotic with plasma, while fluid from distal convolutions and the urine were hypo-osmotic. Much more recently, Wirz (3) has reported on the osmolality of eight samples of distal tubular fluid in hydropenic rats elaborating hyperosmotic urine. Fluid from the early distal convolution was hypo-osmotic, and from the late distal, isosmotic. Six samples from rats undergoing a water diuresis were hypo-osmotic throughout the distal convolution, and five proximal samples were isosmotic.

In 1951 Hargitay and Kuhn (4) introduced a new theory for the mechanism of urine concentration based on the premise that the hairpin-like loop of Henle acts as a countercurrent multiplier system. The original experimental observations in support of this concept were those of Wirz, Hargitay and Kuhn (5) who concluded from freezing-point studies of slices from concentrating rat kidneys that the osmolality was identical for all adjacent tubular structures at any level in the kidney, and that there was a steadily increasing osmotic gradient from the cortex to the tip of the papilla. The cortex itself was found to be isosmotic with plasma. Subsequently, Wirz (6) showed that the medullary blood took part in this mechanism by demonstrating that blood from the vasa recta at the tip of the hamster papilla was as hyperosmotic as the urine. Although not invalidating the concept of an increasing osmotic gradient from cortex to papilla, the recent analyses of distal tubular fluid by Wirz (3) and those reported in this paper demonstrate that all tubular fluid does not have exactly the same osmolality at a given level in the kidney, and suggests to us that post-mortem diffusion probably accounted for this aspect of the results of Wirz, Hargitay and Kuhn (5).

Due to the fundamental importance of these data and their limited number, we have reinvestigated this subject, and have confirmed and extended the previous micropuncture experiments. Furthermore, we have obtained evidence for the first time, by direct sampling and analysis that fluid from the bend of the loop of Henle is as hyperosmotic as that from a collecting duct at the same level in the concentrating kidney.
White Rats

Male rats of the Wistar strain, 150–380 gm in weight, were thirsted (except for the two with diabetes insipidus noted below) and fasted for 18–24 hours and anesthetized with intraperitoneal sodium pentobarbital, 40 mg/kg, or intravenous sodium pentothal, 30 mg/kg of body weight. The left kidney was exposed, and micropuncture performed as previously described (γ), except that mineral oil bathed the kidney instead of Ringer’s solution. Samples of fluid $1 \times 10^{-8}$ to $1 \times 10^{-7}$ ml in volume were collected from surface segments of tubules in mineral oil-filled, Pyrex micropipettes, 5μ in outside diameter at the tip. Following collection, the pipettes were sealed with mineral oil from the quartz-rod illuminator and used directly for the osmolality determination, thus eliminating the necessity for fluid transfer and its attendant hazards. Although the small size of the samples obviated the danger of collecting fluid distal to the site of puncture, as a further check, approximately one-half of the tubules were blocked distally by the injection of oil prior to the collection. There was no difference in the osmolality of samples from blocked or unblocked tubules. The majority of the collections were from proximal convolutions, identified in viva by criteria we have previously described (B), but a smaller number of proximal convolutions were collected from surface segments of tubules in hypertonic glucose diuresis. Different symbols refer to different rats.

Simultaneous samples of urine were collected under oil from the ureter of the punctured kidney, and heparinized blood was collected under oil from the inferior vena cava below the renal veins. Urine flow was measured from the opposite ureter with a drop recorder, the values doubled and expressed as microliters per kilogram per minute. In previous experiments there has been no evidence that micropuncture affected the rate of urine flow.

The freezing point depression of tubular fluid, plasma and urine was determined microcryoscopically by the method of Ramsay and Brown (g) using samples $1 \times 10^{-6}$ to $1 \times 10^{-7}$ ml in volume. Although the standard deviation for this method is $\pm 0.003^\circ$C, determinations were rounded off to the nearest $0.01^\circ$C. The instrument was calibrated with known molar and molar solutions of sodium chloride and urea, using data from the International Critical Tables (10). All results are expressed in terms of osmolality except for rates of solute excretion and osmolar clearance (urine flow × osmolality ÷ plasma osmolality). These were calculated from osmolar equivalents of freezing point depression as urine volume was measured and not the weight of urinary water.

Loading solutes were administered intravenously at 0.085 ml/min. except in the two instances noted otherwise in table 2. The infusions were allowed to proceed approximately one hour before any samples of tubular fluid were collected.
Other Rodents

Golden Hamsters (Mesocricetus auratus), 50-100 gm in weight and one 80-gm kangaroo rat (Dipodomys spectabilis) were utilized in order to collect fluid from structures in the papilla of the kidney. Their preparation was similar to that of the rats except that all were not thirsted and the upper portion of the left ureter was opened in order to expose the extrarenal portion of the papilla.

Another desert rodent, Psammomys obesus, was also studied as described below.

RESULTS

White Rats

Proximal tubule. HYDROPEMIC RATS. Fluid from the first two-thirds, the pars convoluta, of 22 proximal tubules of 14 hydropenic rats with and without superimposed osmotic diuresis, was isosmotic (fig. 1 and tables 1-5). The last one-third of the proximal tubule, the pars recta, is not accessible to micropuncture since it forms the first part of the loop of Henle. Two samples of fluid from Bowman's capsule were also isosmotic.

DIABETES INSIPIDUS. Two rats were studied that had diabetes insipidus as the result of hypothalamic coagulation lesions. They were allowed free access to water before the experiment and one was infused with 0.7% sodium chloride, and the other with 4% mannitol solution. Five samples of proximal tubular fluid were obtained, all of which were isosmotic, while the urine was hypo-osmotic (fig. 1).

Distal convolution. HYDROPENIA. Twenty-three samples of fluid were collected from the distal convolutions of seven hydropenic rats (table 1). As shown in figure 2, samples from early distal convolutions were quite hypo-osmotic, even though the simultaneous urine was markedly hyperosmotic. The tubular fluid had again reached, or closely approximated, the isosmotic value by the end of the distal convolution, and in no instance did it exceed the isosmotic value. The hyperosmotic concentration of the urine, which averaged 2229 mOsm/kg H2O in this group, obviously occurred beyond the distal convolution, in the collecting ducts.

HYPERTONIC MANNITOL DIURESIS. When 25% mannitol solution was infused intravenously into six hydropenic rats, the urine flow was increased up to 80 times that of the hydropenic state, and the urine/plasma (U/P) osmolality ratios were low. Again (fig. 3 and table 2), early distal fluid was hypo-osmotic, and samples from the late distal tubule were isosmotic, or nearly so. The limit of the early distal hypertonicity was equal to a fluid/plasma (F/P) osmolality ratio of 0.6.

HYPERTONIC GLUCOSE DIURESIS. During the infusion of 25% glucose solution into six rats, the results were much the same (fig. 4 and table 3) and the limit of the early distal hypertonicity was again an F/P osmolality ratio of approximately 0.6. Fluid from the late distal was isosmotic or approximately so, as was one sample from a surface segment of a collecting duct.

HYPERTONIC SODIUM CHLORIDE DIURESIS. When sodium...
chloride was the loading solute, given as 5 or 7% solution to eight rats, the degree of the early distal hypotonicity was much greater (fig. 5 and table 4). Thirteen of nineteen samples from the first half of the distal convolution had F/P osmolality ratios below 0.6, and reached a minimum of 0.3. Nevertheless, the tubular fluid was again isosmotic by the mid-point of the distal convolution. It is also interesting to note that the U/P osmolality ratios were higher than when mannitol or glucose were the loading solutes, even though the range of solute excretion was the same.

HYPERTONIC UREA DIURESIS. In five rats, 5, 7 or 10% urea in 0.9% saline was infused intravenously (fig. 6 and table 5). Fluid from the first half of the distal convolution was hypo-osmotic with one exception and isosmotic in the second half, with three exceptions.

Osmotically Active Solute Excretion and Urine Flow. The relationship between the rate of excretion of osmotically active solute and rate of urine flow is shown in figure 7. When sodium chloride and urea were the loading solutes, the urine was more concentrated at a given rate of solute excretion, than with glucose and mannitol. The regression equations relating solute excretion to urine flow were the same for mannitol and glucose, and when treated as a group, y = 1.75x + 5.46. For sodium chloride, y = 1.57x + 53.10, and for urea, y = 1.67x + 50.8.

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URINARY CONCENTRATING MECHANISM

a slightly truncated conc. Many vasa recta were visible on its surface and in its interior, due to their rich content of red cells (6). In a healthy preparation, blood flow was rapid in the long vessels which ran parallel to the long axis of the papilla and many anastomoses were obvious. Large collecting ducts were readily apparent. Close inspection, under proper illumination, also revealed an occasional small tubular structure filled with clear fluid. When punctured and outlined by the injection of a large amount of dye, they were seen to form typical hair-pin loops without anastomosis. Although the papillary portion of the loops consisted entirely of thin segments, the lumen of the ascending limb appeared somewhat wider than that of the descending limb. Flow in the loop was rapid. Occasionally it was possible subsequently, to macerate the kidney and isolate the juxtamedullary nephron into whose loop of Henle a large amount of dye, they were seen to form typical hair-pin loops without anastomosis. (We have seen no evidence of red cells or anastomosis. (We have seen no evidence of such a structure as the latter.) In order to prove that a structure punctured in the papilla was doubt that the structure punctured in the papilla was a loop of Henle and not an unusual vas rectum without red cells or anastomosis. (We have seen no evidence of such a structure as the latter.) In order to prove that a sample of fluid was tubular fluid and not plasma, it was sufficient to test it qualitatively for protein. This was done routinely by heating the sample in its pipette in boiling water or adding an equal part of 20% trichloracetic acid (11), and examining it for a precipitate and became quite opaque.

Loop of Henle. Fourteen samples of fluid were collected from, or very close to, the bend of the thin limb of loops of Henle in eight hamsters and one kangaroo rat. The osmolality of fluid from the loop was the same, or almost the same as fluid from an adjacent collecting duct at the same level (fig. 10 and table 6). In those cases where the osmolality differed, the difference is of questionable significance, as a considerable time period sometimes elapsed between the two collections, and a steady state was difficult to achieve.

In another hamster, collections were made from cortical convolutions demonstrating clearly that the osmolality of their proximal and distal fluid was similar.
to that in rats (fig. 11 and table 1), while the urine was highly concentrated. Subsequent microdissection confirmed Sperber’s statement that all loops had exceedingly long, thin segments, and extended into the papilla. It was also possible to collect one sample from the bend of a loop of Henle at the tip of the papilla, the osmolality of which was the same as that of fluid from an adjacent collecting duct (table 6).

**DISCUSSION**

These experiments confirm the previous mammalian micropuncture findings (1, 3) that proximal tubular reabsorption is an isosmotic process; that in the presence of antidiuretic hormone (ADH), early distal fluid is hypo-osmotic but is again isosmotic as it leaves the distal convolution and enters the collecting tubules, in which the hyperosmotic phase of urine concentration occurs, as had been predicted by Smith (13). This was true, not only during hydropenia, but also during osmotic diuresis of considerable magnitude. It is important to emphasize that these relations exist in the hydropenic animal elaborating highly concentrated urine in both nephrons with short loops extending to the boundary of the inner and outer zones of the medulla as well as in those with long loops extending into the papilla.

Furthermore, these experiments provide strong evidence as to the cause of the hypotonicity of the fluid in the early distal convolution. As pointed out by Smith (13), if the urine is made hypo-osmotic by hyperosmotic solute reabsorption, the only solute present in sufficient quantities, the reabsorption of which could lead to a marked degree of hypotonicity, is sodium chloride. The alternative explanation is the secretion of water into the tubular lumen. In order to differentiate between these two possible mechanisms, diuresis was induced with sodium chloride, which is probably hyperosmotically reabsorbed by the amphibian distal tubule (2), and with glucose and mannitol, which are probably not reabsorbed beyond the proximal tubule. The increased extent of the early distal hypotonicity with sodium chloride load- ing is impressive (fig. 12), and constitutes, we believe, strong evidence that the hypotonicity is indeed the result of hyperosmotic reabsorption of sodium chloride in the loop of Henle. If secretion of water into the loop were the sole operative mechanism, its extent would probably be independent of the nature of the solutes present.

One might question why the early distal hypotonicity was not so marked during hydropenia when sodium chloride was also the major solute present. This is probably because only a small volume of fluid entered the distal convolution during hydropenia (8), and sufficient water had diffused out to raise appreciably the osmolality of the remaining tubular fluid before it could be sampled. Had it been possible to sample the tubular fluid at the point where it was most hypo-osmotic, the F/P osmolality ratio would probably have been the same under both conditions, as there was no evidence that a transfer maxi-

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### Table 5. Tubular Fluid, Plasma and Urine Osmolarities, and Urine Flow During Hypertonic Urea Diuresis in Hydropenic Rats

| Tubular Fluid | Convolution, % proximal or distal | mOsm/kg H₂O | F/P Osm | mOsm/kg H₂O | U/P Osm | Flow, ml/kg/min. |
|--------------|----------------------------------|------------|--------|------------|--------|----------------|---|
| 315-gm Rat 10% Urea in 0.9% Saline | D 77 | 357 | 1.00 | 357 | 758 | 2.1 | 191 |
| | D 67 | 182 | 0.50 | 323 | 790 | 2.2 | 318 |
| | P 73 | 171 | 0.47 | 293 | 833 | 2.3 | 267 |
| | P 53 | 293 | 1.00 | 293 | 823 | 2.3 | 318 |
| | D 74 | 210 | 0.58 | 293 | 822 | 2.3 | 343 |
| | D 26 | 198 | 0.54 | 258 | 893 | 2.3 | 361 |
| 365-gm Rat 10% Urea in 0.9% Saline | D 79 | 374 | 1.00 | 374 | 772 | 2.1 | 377 |
| | Glomerulus | 305 | 1.00 | 305 | 762 | 2.0 | 377 |
| | D 28 | 306 | 1.00 | 396 | 779 | 2.0 | 377 |
| | D 57 | 232 | 0.59 | 396 | 795 | 2.0 | 377 |
| | D 42 | 216 | 0.54 | 401 | 900 | 2.2 | 302 |
| 410-gm Rat 10% Urea in 0.9% Saline | P 47 | 294 | 1.01 | 294 | 1104 | 4.9 | 178 |
| | D 27 | 322 | 0.58 | 324 | 1050 | 3.0 | 163 |
| | D 31 | 317 | 0.53 | 352 | 1056 | 3.0 | 163 |
| | D 80 | 247 | 0.53 | 439 | 822 | 1.9 | 387 |
| | P 66 | 374 | 1.00 | 374 | 1079 | 3.1 | 426 |
| | D 29 | 352 | 0.67 | 367 | 1100 | 3.4 | 366 |
| 420-gm Rat 10% Urea in 0.9% Saline | D 71 | 374 | 1.00 | 374 | 708 | 1.8 | 74 |
| | D 61 | 198 | 0.53 | 379 | 730 | 1.9 | 77 |
| | D 21 | 248 | 0.54 | 390 | 719 | 1.8 | 92 |
| | D 33 | 308 | 0.86 | 383 | 740 | 1.9 | 62 |
The details of how this complex mechanism operates are unknown, but it appears to depend primarily on active ion transport by one or more of the involved structures throughout the entire depth of the medulla, in which the osmotic gradient is established. The great theoretical advantage of the countercurrent mechanism is that at no level in the kidney need there be large osmotic differences maintained by tubular structures only one cell layer thick since the gradients are established in the longitudinal axes of their straight portions. According to both theoretical considerations (4) and experimental observations (11, 13), the longer these straight medullary segments, the higher the concentration achieved. Another general feature of this mechanism is that all water movement occurs secondary to solute transport, and it is unnecessary to postulate active water transport. Although future observations will unquestionably demand revisions, our present conception of the operation of the countercurrent multiplier system follows (fig. 13), and is much as proposed by Hargitay and Kuhn (4), and modified by Wirz (3).

Sodium, by an unknown active mechanism, and chloride, as a result of the electrochemical gradient established, are believed to be transported out of the relatively water impermeable ascending limb of the loop of Henle into the interstitium of the medulla until a gradient of perhaps 200 mOsm/kg H2O has been established between the fluid of the ascending limb and interstitium. This single effect is multiplied as the fluid in the thin descending limb comes into osmotic equilibrium with the interstitial fluid by the diffusion of water out of and probably the diffusion of some sodium chloride into the descending limb thus raising the osmolality of the fluid presented to the ascending limb. In this fashion an increasing osmotic gradient is established in the direction of the tip of the papilla and yet at no level is there a large osmotic difference between luminal and interstitial fluid. In contrast, the epithelium of the collecting ducts in the presence of ADH is believed to be water permeable and functionally sodium impermeable (net transport probably small although there may be diffusion into and active transport out of the collecting ducts). This results in diffusion of water out of the collecting ducts into the hyperosmotic medullary interstitium until the fluid remaining in the collecting ducts becomes correspondingly concentrated. It is readily apparent that in order for the urine to be significantly concentrated, the flow through the loops of Henle must considerably exceed the flow through the collecting ducts (4). This is accomplished under the
influence of ADH by diffusion of water out of the distal convolution into the interstitium of the cortex, reducing the volume and increasing the osmolality to the isoosmotic level of the fluid presented to the collecting ducts. As suggested by Schmidt-Nielsen (16), urea is probably also involved in some as yet unknown fashion. It appears most likely to us that urea diffuses into the descending limb, contributing to the osmotic gradient established in the loop, and diffuses out of and/or is actively transported out of the ascending limb. Further, and unlike sodium, depending on the circumstances, there may be net diffusion of urea from the interstitium into the collecting ducts (exaltation), or vice versa, as water is extracted from them.

Recent work by Hilger, Klümper and Ullrich (17) indicates that active reabsorption of sodium by the collecting duct epithelium also occurs. This may play an important role in maintaining a high concentration of sodium in the interstitium of the medulla by preventing loss of sodium in the urine. In the presence of ADH, reabsorption of sodium, or other solute, by the collecting duct cells would lead to reabsorption of water, isoosmotic for the level at which the solute reabsorption occurred, and a reduction in the volume of fluid presented to any more distal portion of the collecting ducts. In the absence of ADH, reabsorption of solute would lead to further dilution of the collecting duct fluid consistent with the observations of Wirz (3) and ourselves (unpublished observations) on the osmolality of fluid from the end of the distal convolution and of urine.

The vasa recta also participate in this mechanism, as first shown by Wirz (6) and now confirmed by us, and apparently function as countercurrent diffusion exchangers. (See Scholander (18) for a discussion of this general biological principle.) They make the entire mechanism far more effective, resulting in a higher osmotic gradient, by tending to trap sodium, urea and other diffusible solutes in the medulla. This aspect of the mechanism has recently been clearly discussed by Berliner and co-workers (14), who have emphasized the importance of a low effective medullary blood flow in establishing a high osmotic gradient. We would point out, however, that the osmotic equilibration of vasa recta blood with medullary interstitial fluid in all likelihood is due not only to the diffusion of solute into their descending and out of their ascending limbs but also results in large part from the diffusion of water in the opposite direction (fig. 19). This short-circuiting of water across the tops of the vascular loops may be at least in part responsible for the seemingly rich content of red cells in the vasa recta at the tip of the papilla. The efficiency of the countercurrent exchange in the vasa recta is critical for they probably remove not only the blood entering the medulla, but also the water that diffuses from the thin descending limbs of the loops of Henle and the collecting ducts. This water, with solutes isoosmotic for the particular level of the medulla, presumably moves into the vasa recta because of the gradient of its chemical potential established by the colloid osmotic pressure of the plasma proteins, since the hydrostatic pressures in the capillaries and interstitium are the same (7). More nearly the osmotic pressure of the blood leaving the medulla approaches that of the blood entering it, the less solute will be lost from the medulla, and hence the higher the osmotic gradient established.

A great deal of confirmatory evidence for the countercurrent theory, particularly in the form of analyses of medullary slices, is present and rapidly accumulating in the literature. Some years ago, Glimstedt (19) and Ljungberg (20) reported an increasing concentration of chloride in the medulla as the papilla was approached, and more recently Ullrich and Jarausch (21) and others (14, 16) have made similar observations in respect to sodium, chloride, urea and creatinine. Ullrich and Jarausch (21) also made the important observation that the final concentration of the urine is a linear function of the sodium concentration in the tip of the papilla, even though the concentration of sodium in the urine was far less than in the papilla. It is interesting to review the work of Hirokawa (22), who studied the osmotic relations of cortex and medulla in various animals, using the

![Figure 9](http://ajplegacy.physiology.org/doi/10.1152/ajprenal.1964.84.3.784/CARL-W-GOTTSCHALK...)

**FIG. 9.** Photographs of a maceration preparation of a hamster kidney showing A, a loop of Henle that was punctured at its bend and injected with a large amount of dye which also filled B, the pars recta of the proximal and distal tubules of this juxta-medullary nephron. Much of the dye escaped from one limb of the loop post mortem.

![Figure 10](http://ajplegacy.physiology.org/doi/10.1152/ajprenal.1964.84.3.784/CARL-W-GOTTSCHALK...)

**FIG. 10.** Relation between the osmolality of collecting duct fluid and blood from loops of Henle and vasa recta blood in 9 hamsters, I kangaroo rat, and I Psammomys obesus.
osmotic slice technique which we now realize gives at best only comparative values. Yet, in 1908, he wrote that "the urine present in the medulla has a much higher osmotic pressure than that of the convoluted tubules of the cortex; therefore, the osmotic pressure of the urine increases considerably during its passage through the loops of Henle and collecting tubules." He also found that the osmotic pressure of the cortex was very constant, that the osmotic pressure of the cortex was very constant, therefore, the osmotic pressure of the urine that the osmotic pressure of the cortex was very constant, therefore, the osmotic pressure of the urine that the osmotic pressure of the cortex was very constant, therefore, the osmotic pressure of the urine that the osmotic pressure of the cortex was very constant, therefore, the osmotic pressure of the urine that the osmotic pressure of the cortex was very constant, therefore, the osmotic pressure of the urine that the osmotic pressure of the cortex was very constant, therefore, the osmotic pressure of the urine that the osmotic pressure of the cortex was very constant, therefore, the osmotic pressure of the urine that the osmotic pressure of the cortex was very constant, therefore, the osmotic pressure of the urine that the osmotic pressure of the cortex was very constant, therefore, the osmotic pressure of the urine that the osmotic pressure of the cortex was very constant, therefore, the osmotic pressure of the urine.

**TABLE 6. Osmolality of Fluid From Loops of Henle and Collecting Ducts and of Vasa Recta Blood**

<table>
<thead>
<tr>
<th>Loop of Henle Fluid</th>
<th>Collecting Duct Fluid</th>
<th>Vasa Recta Blood</th>
<th>Inferior Vena Cava Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>mOsm/kg H2O</td>
<td>mOsm/kg H2O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50-gm Hamster-10% Glucose i.v.</td>
<td>407</td>
<td>450</td>
<td>406</td>
</tr>
<tr>
<td>60-gm Hamster</td>
<td>80a</td>
<td>843</td>
<td>347</td>
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<tr>
<td>60-gm Hamster</td>
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<td>599</td>
<td>599</td>
</tr>
<tr>
<td>80-gm Hamster</td>
<td>953</td>
<td>768</td>
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<td>80-gm Hamster</td>
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<td>1087</td>
<td>325</td>
</tr>
<tr>
<td>80-gm Kangaroo Rat</td>
<td>530</td>
<td>509</td>
<td>483</td>
</tr>
<tr>
<td>105-gm Psammomys Obesus</td>
<td>538</td>
<td>616</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 12.** Comparison of the osmolality ratios of fluid from the distal convolution and of urine during hypertonic sodium chloride diuresis with those during hypertonic mannitol and glucose diuresis.

**Fig. 13.** Diagram depicting the countercurrent mechanism as it is believed to operate in a nephron with a long loop and in the vasa recta. The numbers represent hypothetical osmolality values. No quantitative significance is to be attached to the number of arrows and only net movements are indicated. As is the case with the vascular loops, all loops of Henle do not reach the tip of the papilla and hence the fluid in them does not become as concentrated as that of the final urine, but only as concentrated as the medullary interstitial fluid at the same level. The active sodium transport by the collecting duct epithelium is based on the work of Hilger, Klimper and Ullrich (17).

One may propose numerous variations of the countercurrent mechanism which will explain the facts already established, e.g., active sodium transport out of the ascending and into the descending limb of the loop of Henle; recirculation of sodium and little loss of water from the loop; the possibility that the thin limbs of the loop of Henle function as a countercurrent diffusion exchanger, etc. Until definitive evidence becomes available, we prefer the hypothesis illustrated above.

and "in contrast, the osmotic pressure of the medulla is extraordinarily variable; it is almost without exception higher than that of the cortex, and is higher, the more concentrated the excreted urine." (Translation ours.)

**Osmotic Diuresis**

During osmotic diuresis, an increased volume of isosmotic fluid is presented to the loop of Henle as a result of the failure of proximal reabsorption of solute and hence water. Although reduced in volume as it flows through the distal convolution by diffusion of water into the interstitium of the cortex, an increased volume of isosmotic fluid also remains to flow through the collecting ducts. As is clear from the mathematical treatment of Hargitay and Kuhn (4), both of these factors result in a decrease in the osmotic gradient established, and hence a reduction in urine concentration. Although no quantitative measurements were made, we (8) have described the increase in proximal and distal flow during osmotic diuresis, in preparations similar to the present ones. An increase in medullary blood flow may occur as well, and would have a similar effect by removing more solute.
The increased osmotic concentration at which a given load of sodium chloride was excreted, compared to mannitol and glucose, is readily explained by the countercurrent theory. Assuming little net reabsorption of solute in the collecting ducts, the volume of isosmotic fluid entering them would be equal in both cases per unit of osmotically active solute excretion. The more dilute early distal fluid during sodium chloride diuresis indicates greater reabsorption of sodium chloride by the loops of Henle and hence a higher urinary osmotic pressure to be expected. The excess water entering the distal convolution diffuses into the interstitium of the cortex and does not impair the efficiency of the concentrating mechanism. Tending to offset this somewhat is the effect of the increased volume flow through the loops of Henle with sodium chloride loading per unit of solute excretion.

The urine was also more concentrated per unit of osmotically active solute excretion with urea loading than with mannitol or glucose. Kellogg and Koike (23) have also reported this for the rat. Schlegel and Stone (24) reported similar relations between urine volume and solute excretion in rats loaded with urea and sodium chloride. We also agree with Wirz (3) that ADH probably acts in some other manner in addition to the above, in establishing the countercurrent multiplier system, for if this were its only effect, the interstitium of the medulla would be even more hyperosmotic during water diuresis than during hydrogenuria, since less water is removed from the collecting tubules. Although there are no available data on this point, among other possible effects by which ADH could facilitate the operation of the countercurrent system are a) increased sodium transport in the thin ascending limb, as found by Leaf, Anderson and Page (26) in the toad bladder. Sodium transport in the thick portion of the ascending limb would seem to be independent of ADH activity, as Wirz (3) and we (unpublished observations) have found the early distal fluid hypo-osmotic when the urine was hypo-osmotic b) increased water permeability of the descending limb, or c) decreased medullary blood flow.

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