Osmotic Concentration and Dilution in the Mammalian Nephron

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The micropuncture evidence relating to the location and the mechanism for concentration and dilution of the urine is reviewed. As required by the countercurrent hypothesis for urine concentration, these data demonstrate that in the presence of antidiuretic hormone, the tubular urine is first concentrated in the descending limb of the loop of Henle and then diluted in the ascending limb of the loop before its final concentration in the collecting ducts. The loop of Henle is believed to function as a countercurrent multiplier system, and the vasa recta as countercurrent diffusion exchangers. Additional data are required during water diuresis before the course of events in this condition is established.

The micropuncture technic which was originally applied to the study of kidney function by Wearn and Richards has been particularly fruitful in elucidating the course of events as they occur in concentration and dilution of the urine. Over 20 years ago Walker and his collaborators demonstrated that proximal tubular fluid in frogs and Necturus was isosmotic with plasma, and that the hypo-osmotic urine characteristic of these animals first appeared in the distal tubule. In 1941 Walker, Bott, Oliver and MacDowell demonstrated that a large percentage of the glomerular filtrate was reabsorbed proximally by an isosmotic process in rats, guinea pigs, and 1 opossum. They were able to collect only 3 samples of fluid from distal convolutions. One of these was isosmotic, but the other 2 were hypo-osmotic. This latter observation was rather surprising, as the final urine was hyperosmotic. They were reluctant to attach too much significance to their few analyses of distal fluid, but pointed out that the findings suggested that the site of active chloride reabsorption was proximal to that of pure water.

More recently, Wirz reported that proximal tubular fluid was also isosmotic in rats undergoing a water diuresis, and that fluid in the early distal convolution was hypo-osmotic under all conditions. During water diuresis, the fluid remained hypo-osmotic throughout the distal convolution and collecting ducts, but became isosmotic by the end of the distal convolution when the final urine was hyperosmotic. Miss Mylle and I have been able to confirm most of the above results, and to extend them. This is fortunate, for the 3 groups have worked independently and have been widely separated, either by the years, or by many miles, and this confirmation lends credence to the results. It is only proper to point out that although the results obtained by micropuncture may be very helpful, the methods are so difficult that they present many potential pitfalls.

We have also found (fig. 1) the proximal tubular fluid to be isosmotic, regardless of whether the final urine was hyper- or hypo-osmotic. In hyperpenic rats elaborating markedly concentrated urine, the fluid in the early distal convolution is consistently hypo-osmotic, and in the second half of the distal convolution, isosmotic, but never hyperosmotic (fig. 2). Clearly, the hyperosmotic phase of urine concentration is established in the collecting ducts.

The hypotonicity of the fluid entering the early distal convolution can be explained on
the basis of hyperosmotic reabsorption of solute in the loop of Henle, or secretion of water into the loop. If it is due to reabsorption of solute in excess of water, the only solute present in sufficient quantity to explain this degree of hypotonicity is sodium chloride. Accordingly, in an attempt to differentiate between these 2 possible mechanisms, osmotic diuresis was induced with the nonreabsorbable solute, mannitol, and with sodium chloride (fig. 3).

When a 25 per cent solution of mannitol was infused intravenously, the rate of urine flow increased up to 80 times that of the hydropenic state, and the urine/plasma osmolality ratios were correspondingly low. Again, early distal fluid was hypo-osmotic, but the osmolality ratio was not less than 0.6, and late distal samples were isosmotic or nearly so. Similar results were obtained during glucose diuresis.

When sodium chloride, given as a 5 or 7 per cent solution, was the loading solute, comparable urine flows were achieved, but the degree of hypotonicity of the fluid in the early distal convolution increased. Here, a large percentage of the samples had fluid/
plasma osmolality ratios less than 0.6; the minimum fluid/plasma ratio was 0.3. As before, fluid from the late distal convolution was isosmotic. The increase in the degree of hypotonicity with sodium chloride is impressive and constitutes, we believe, strong evidence that the hypotonicity of the fluid in the early distal convolution is due to hyperosmotic reabsorption of sodium chloride in the loop of Henle.

All of the data so far can be explained by the conventional theories for the reabsorption of sodium and water, i.e., the proximal "obligatory" reabsorption and the distal "facultative" reabsorption of sodium and water, with the hyperosmotic phase probably resulting from active transport of water by the cells of the collecting ducts. This interpretation, however, neglects the countercurrent hypothesis of Kuhn and collaborators, which was first proposed in 1942 but which had few proponents until recently. This new and revolutionary theory, treated in detail in 1951 by Hargitay and Kuhn, proposes that the hairpin-like loop of Henle functions as a countercurrent multiplier system in which a small concentration gradient, at any given level in the loop, is multiplied as the tip of the loop is approached. This system was believed to result in an increasing osmotic pressure in the medulla of the kidney. The theory further proposes that the concentration of the urine results from the passive withdrawal of water from the collecting ducts as they traverse this area. Their original experimental evidence, gained in conjunction with Wirz, was a cryoscopic study of rat kidney slices. They found that, at any given level, the osmotic pressure of the fluid was identical in the lumen of all the tubules. There was also an increasing osmotic gradient from the cortex, which was isosmotic with arterial plasma, to the tip of the papilla. Although the recent analyses of distal tubular fluid do not invalidate the concept of an increasing osmotic gradient from cortex to papilla in at least some of the medullary structures, they do demonstrate that all tubular fluid at a given level in the kidney does not have exactly the same osmolality. They also suggest to us that postmortem diffusion probably accounted for this aspect of the results of Wirz, Hargitay and Kuhn. Since some doubt remained that the osmolality in all of the medullary structures exceeds that of the cortex, additional direct sampling and measurement of osmolality seemed to be required. Wirz accomplished this for the medullary blood vessels.
by demonstrating that blood from the vasa recta at the tip of the hamster papilla had the same osmolality as that of the final urine. More recently, we accomplished the same for fluid from the loop of Henle. Under conditions of proper illumination, it is possible to identify loops of Henle in the living hamster papilla and to sample their contents. Fluid from the bends of the loops, or very close thereto, had essentially the same osmolality as that from a collecting duct at the same level (fig. 4). These results demonstrate that the urine is first concentrated and then diluted before its final concentration, as required by the countercurrent hypothesis. We were also able to confirm Wirz's observation that the osmolality of blood from the vasa recta is the same as that of fluid from a collecting duct at the same level.

These observations indicate to us that a countercurrent mechanism is operative in the nephrons of the kidney, as well as in the vasa recta. The details of the mechanism remain to be established, and one can propose numerous variations of the countercurrent hypothesis that will explain the facts that are now available. We agree that the active transport on which the system depends is performed by the nephron, and that the loop of Henle functions as a countercurrent multiplier system. The exchanges in the vasa recta, as in other blood vessels, are probably passive and they are be-

Figure 4
Relation between the osmolality of collecting duct fluid, fluid from the loops of Henle, and vasa recta blood in various desert rodents. (Republished by permission of the American Journal of Physiology.)
OSMOTIC REGULATION IN THE MAMMALIAN NEPHRON

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. per Kg. of water 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 antidiuretic hormone is believed to be water permeable and functionally sodium impermeable (net transport is 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 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. 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 isosmotic level of the fluid presented to the collecting ducts. As suggested by Schmidt-Nielsen, urea is probably also involved in some 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. 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, Klümpir and Ullrich indicates that active reabsorption of sodium by the epithelium of the collecting duct also occurs. This reabsorption 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 cells of the collecting duct would lead to the isosmotic reabsorption of water at the level at which the reabsorption of the solute 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; this view is consistent with the observations of Wirz 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 and now confirmed by us, and apparently function as countercurrent diffusion exchangers as shown in figure 5. (See Scholander for a discussion of this general biologic 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 his co-workers, 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 the blood in the vasa recta with medullary interstitial fluid in all likelihood is not due 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. 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

*Figure 5*

*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 epithelium of the collecting duct is based on the work of Hüger, Klümpеr and Ulrich, has recently considered the theoretic aspects of the activation of the counter-current multiplier by active sodium transport. (Republished by permission of the American Journal of Physiology.)*

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from the thin descending limbs of the loops of Henle and the collecting ducts. This water, with solutes isosmotic 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.\textsuperscript{15} The 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.\textsuperscript{17}

As noted above, many hypotheses are consistent with the presently established facts. For example, the hypothesis of 
Berlin et al.,\textsuperscript{14} in which both the descending and ascending limbs of the loop of Henle were postulated to be relatively water-impermeable and actively to transport sodium from lumen to interstitium, is compatible with present information if the thin descending limb is assumed to be water-permeable in the presence of antidiuretic hormone. In such a system, the volume of fluid reaching the tip of the loop would be somewhat smaller than in the hypothesis detailed above, because transport of sodium out of the thin descending limb would lead to further extraction of water.

Ullrich\textsuperscript{10} has proposed that the active transport of sodium in the medulla is limited to the epithelium of the thick ascending limb and collecting ducts, and that exchanges in the thin limbs are passive. He visualizes that the osmolality of the loop and of the interstitial fluids is the same throughout the inner medulla, and that osmotic equilibration with collecting duct fluid lags behind so that the fluids in the loop and in the collecting duct have the same osmolality only at the tip of the papilla. We believe this hypothesis is unlikely, since we have recently been able to demonstrate in a few loops in the papilla of the desert rodent, \textit{Psammomys obesus},\textsuperscript{*} an increase in osmolality in the direction of their tips.

\textbf{Antidiuretic Hormone}

The micropuncture findings are consistent with the hypothesis that the epithelium of the collecting ducts, and perhaps the distal convolution, is rendered freely permeable to water by the presence of antidiuretic hormone,\textsuperscript{4, 14} but we agree with Wirz\textsuperscript{4} that antidiuretic hormone may have an additional effect on the concentrating mechanism. If a change in the permeability to water of the epithelium of the collecting duct and of the distal convolution were its only effect, the interstitium of the medulla could be even more hypoosmotic during water diuresis than during hydropenia, for less water is probably extracted from the collecting ducts during water diuresis than during hydropenia. Among other possible mechanisms whereby antidiuretic hormone could facilitate the operation of the counter-current system are 1 or more of the following actions: (1) increased transport of sodium by the thin, ascending limb; (2) increased permeability to water of the thin, descending limb; or (3) decreased medullary blood flow.

\textbf{References}


\*Kindly supplied by Dr. Bodil Schmidt-Nielsen.
The First Micropuncture Studies of the Kidney

In the spring of 1921 I had the privilege of seeing a demonstration of Professor Robert Chambers of New York in which, by means of micropipettes, he injected various substances into the cell bodies of amoebae. It excited me to believe that the frog's kidney, already made accessible to direct vision, might become accessible also to instrumental manipulation. Dr. J. T. Wearn, learning of this thought, crystallized it by suggesting that it might be possible to draw fluid from the intracapsular space within the malpighian body and thus to compare its chemical composition with that of the blood plasma from which it is derived. If such a project could be successfully and comprehensively accomplished, we should gain knowledge of the most direct character by which to estimate the nature of the processes concerned in the separation from the blood within the glomerulus of the fluid which is eventually to become urine: and a far more satisfactory basis for study of function of the tubules would be laid than existed previously because, until we are definitely certain concerning the amount and composition of the fluid which constitutes the head water of the stream flowing down the tubule, we can scarcely hope to decide with certainty concerning the changes to which this fluid is subjected during its passage through the tubule.—A. N. Richards. Methods and Results of Direct Investigations of the Function of the Kidney. The Beaumont Foundation Lectures, series 8. Baltimore, Williams and Wilkins, 1929, p. 15.
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