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History of the urinary concentrating mechanism

CARL W. GOTTSCHALK

Department of Medicine (226H), School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Although the processes that generate the osmotic gradients in the inner medulla remain controversial, the countercurrent mechanism for the osmotic concentration and dilution of urine is now generally accepted. It was not always so.

The mechanism for urinary dilution posed no conceptual difficulties for renal physiologists. Active transport of sodium chloride by a nephron segment whose epithelium had restricted water permeability in the absence of antidiuretic hormone (ADH), presumably located in the "distal" portion of the uriniferous tubule, was logical and based on proven and analogous processes. It was also obvious that when its water permeability was increased by ADH, water transport would be closely coupled to solute transport and reabsorbate and tubular fluid would be isosmotic. It appeared necessary to postulate the active transport of water as the final step in the production of urine which was hyperosmotic to the body fluids. Despite the fact that there was no proven example of active water transport in the animal kingdom, active water transport by the cells of the collecting ducts was proposed and was generally accepted. The simple biological solution of establishing by solute transport a hypertonic environment in an anatomically restricted portion of the kidney such that all water transport could be postulated as passive was not obvious and was scorned when proposed.

Smith's hypothesis

For many years the conventional hypothesis for urinary concentration was that proposed by Homer W. Smith, the then dean of renal physiology, and his colleagues. They integrated the concepts that flowed from their extensive investigations of kidney function by clearance techniques with the available micropuncture results and other relevant data. In his monumental monograph, *The Kidney: Structure and Function in Health and Disease* [1] published in 1951, Smith wrote:

> "In the current view, the reabsorption of water by the renal tubules involves at least two more or less independent processes: (1) passive water reabsorption in the proximal tubule and thin segment (proximal system), and, under appropriate circumstances, in the distal tubule; and (2) active water reabsorption that is presumably confined to the distal system, i.e., in the distal tubule and possibly in the collecting ducts also. So far as is known only active water reabsorption is under rapidly variable hormonal control."

As the concept was developed about 85% of the filtered water was presumed to be reabsorbed proximally ("obligatory reabsorption") in association with the reabsorption of sodium and other solutes. Reabsorption of approximately 15% of the filtered water was subject to physiological control through antidiuretic hormone and was postulated as occurring in the distal segment. These ideas are presented in the well known schema showing reabsorption of sodium and water in the proximal and distal segments (Fig. 1). Not shown in the schema is the final hyperosmotic phase of urine concentration resulting from the active reabsorption of water in the most distal segments.

Smith and colleagues also developed the concepts of "free water clearance," C_{H_2O}, in the diluting operation

$$C_{H_2O} = V - C_{Osm}$$

and of "solute–free water reabsorption," $T^c_{H_2O}$ in the concentrating operation

$$T^c_{H_2O} = C_{Osm} - V.$$

In his very influential *Principles of Renal Physiology*, [2] published in 1956, Smith wrote:

> "The concentrating operation appears to consist of a continuing constant reabsorptive operation which removes an approximately constant quantity of solute–free water ($Tm^c_{H_2O}$) from the antecedent isosmotic tubular urine so long as the volume of the latter exceeds the $Tm^c_{H_2O}$.
>
> It is not determined whether this concentrating operation is located in the most distal portion of the distal segment, or in the collecting ducts, though fragmentary evidence favors the latter. Neither is it known whether the concentrating operation is activated by ADH: the induction of antidiuresis by this hormone, by abolishing water diuresis, may simply expose the concentrating operation to view."

The functions, C_{H_2O} and $T^c_{H_2O}$, were easily and precisely measurable. Not surprisingly, for a decade most of the investigators of urinary concentration and dilution involved their measurements in humans and experimental animals and integration of their findings into the Smithian concept.

The countercurrent hypothesis

Dr. Werner Kuhn, Professor of Physical Chemistry at the University of Basel, Switzerland, originated the countercurrent multiplier concept for urine concentration. In a paper [3] that appeared in the German literature during World War II and was generally overlooked by renal physiologists, certainly English speaking ones, Kuhn and Ryffel presented a model with possi-

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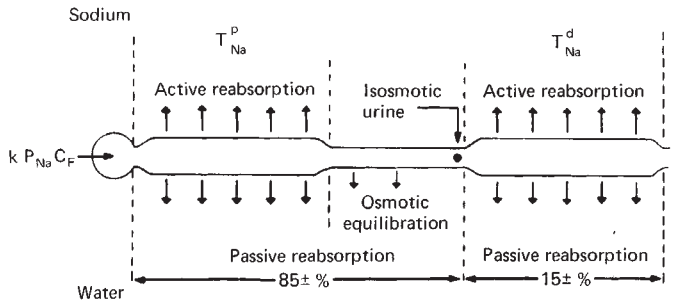

Fig. 1. *Smith's schema for salt and water reabsorption in the rectilinear nephron [2].*

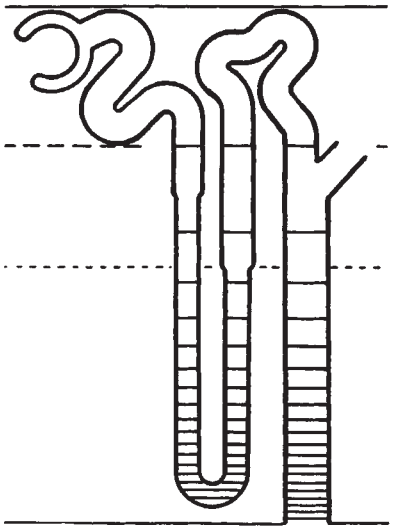

Fig. 2. *Variation of osmolality in a single nephron and collecting duct as described by Wirz, Hargitay and Kuhn [5].*

ble relevance to the kidney. In a two–solute, multicompartment system with differential membrane permeability, they demonstrated osmotic concentration of fluid in one compartment in the absence of hydrostatic driving force or membrane activity. They pointed out that the small effect could be multiplied in a countercurrent system and suggested its possible relevance to the osmotic concentration of urine by the kidney.

In 1951 Hargitay and Kuhn published the seminal paper entitled "Das Multiplikationsprinzip als Grundlage der Harnkonzentrierung in der Niere" [4]. This paper presented in detail a totally new concept for the mechanism of urine concentration. The theoretical treatment was accompanied by data obtained on a working model. Simultaneously, Wirz, Hargitay and Kuhn presented cryoscopic data on kidney slices (Fig. 2) demonstrating that the osmolality of the fluid in all tubes in the renal medulla increased in the direction of the tip of the papilla [5]. Hargitay and Kuhn's experimental model operated on the basis of a hydrostatic pressure differential causing filtration of water through a semipermeable membrane in the direction corresponding to transport from descending to ascending limb of the loop of Henle. Although these workers explicitly ruled out the possibility that hydrostatic pressure was the driving force in the kidney, and presumed that "an electro-osmotic pressure" was operative, this feature of the model resulted in much misunderstanding. Hargitay and Kuhn pointed out that the countercurrent multiplier could operate equally well on the basis of salt transport in the opposite direction, that is, from ascending to descending limbs of the loop. In a paper published in 1959, Kuhn and Ramel [6] proved mathematically the validity of a countercurrent model based on active salt transport and speculated that this was the likely mechanism in the kidney. In their original 1951 publication, they proposed that the countercurrent mechanism created a milieu of increasing hypertonicity toward the tip of the papilla and that the final osmotic concentration of the urine would be due to the passive flow of water out of the collecting ducts down an osmotic gradient into the medullary interstitium, an integral part of the countercurrent system to this day.

In 1953 Wirz [7] demonstrated by micropuncture that blood collected from vasa recta near the tip of the papilla had practically the same osmotic pressure as the urine simultaneously produced. The osmolality of vasa recta plasma was assumed to be similar to that of the interstitial fluid in the spaces surrounding the blood vessels. These results provided strong evidence against the hypothesis that the final concentration of urine in the collecting ducts was due to the active removal of water. If that were the case, blood in the vasa recta would of necessity be hypotonic to systemic blood. The hypotonicity might be slight if the blood supply to the papilla was large as compared to urine flow, but such a process could never cause the papillary blood to become hypertonic.

In 1956 Wirz [8] presented the first direct measurements of the osmolality of proximal and distal tubular fluid from rats elaborating hyper- or hypo-osmotic urine. Five samples of proximal fluid were isosmotic at a time when the urine was markedly hypo-osmotic. Earlier Walker, Bott, Oliver, and MacDowell [9] had presented data demonstrating that proximal tubular fluid was isosmotic in the rat when the urine was hyperosmotic. Wirz also collected a small number of samples from the distal convolution. Samples from the first half of the distal convolution were hyposmotic irrespective of whether the urine was hyper- or hypo-osmotic. When the urine was hypo-osmotic, the fluid remained hypo-osmotic throughout the distal convolution. When the urine was hyperosmotic, the tubular fluid was isosmotic at the end of the distal convolution. Clearly, these data demonstrated that the final hyperosmotic phase of urine concentration occurred beyond the distal convolutions and, by definition, in the collecting ducts. Wirz interpreted these results along with his earlier results as providing strong evidence for countercurrent function of the loop of Henle, with the single effect being due to sodium chloride reabsorption by the ascending loop of Henle. Others did not find this interpretation convincing.

Henle's loop and urine concentration

Kuhn and collaborators were by no means the first to relate the loop of Henle to the process of urine concentration. Peter, [10] in 1909 had pointed out a correlation between the length of the loop and maximum urine concentration in various species. This correlation was further developed by Sperber in 1944 [11]. Sperber thought that both thin and thick segments were related to water reabsorption, since both segments were longer in

animals excreting a concentrated urine. He did not think that the thin segment could be the main site in which hypertonicity of the urine developed.

In 1927, Marian M. Crane, in a study entitled "Observations on the Function of the Frog's Kidney," [12] reported that the osmotic pressure of the urine from frogs was never higher than that of their blood. In the discussion of her results, Crane speculated:

> "Since the chief anatomical difference between a frog's kidney and that of a mammal is the absence of the loop of Henle in the former, the failure of the frog's tubule to reabsorb water against osmotic pressure suggests that it may be in the loop of Henle that such reabsorption takes place. If such is the case, the power to excrete a hypertonic urine should appear first in those animals in which a loop is first developed. Huber has described a very definite loop in the bird's kidney, so according to this theory the bird should be able to excrete a urine hypertonic to the blood. d'Errico reports freezing point determinations indicating that the osmotic pressure of the chicken's urine is a little higher than that of the blood."

Crane worked in the laboratory of E.K. Marshall at Johns Hopkins, and in 1933 Burgess, Harvey and Marshall conducted a comparative study [13] of the effect of the pituitary extract in animals with and without loops of Henle. Their studies were done on fish, frogs, alligators, chickens, dogs, and humans. They reported that the typical antidiuretic action (that is, not dependent on a decrease in glomerular filtration rate) following administration of pituitary extract was limited to animals with loops of Henle. They concluded:

> "Since it is apparent that the presence of the thin segment of the loop of Henle is the only new development of the avian and mammalian kidney in comparison to that of the lower vertebrates, it would appear reasonable to localize the site of the antidiuretic pituitary action on this segment of the renal tubule."

The suggestion that urinary concentration occurred in the loop of Henle was dropped when in 1941 Walker, Bott, Oliver, and MacDowell [9] reported on the osmolality of three samples of fluid collected from the distal convolution of rats. One sample was isosmotic, and two were slightly hypo-osmotic, suggesting to the investigators that the two latter might have traversed the loop "with abnormal rapidity". But "insofar as they permit a suggestion it must be that the site of water reabsorption is in the late distal tubule or even in the post–distal connecting tubule rather than in the loop of Henle." In all early studies just discussed relating the loop of Henle to the process of urine concentration, the authors focused their attention on the development of a thin segment of the loop in animals with the ability to elaborate a hyperosmotic urine. In no case was attention drawn to the U-shaped configuration of the loop of Henle and its possible functional significance. To my knowledge the first suggestion of the physiological importance of the hairpin configuration was provided by Kuhn, Wirz and collaborators.

Medullary solute concentration

In support of the medullary localization of the concentrating process in the kidney, Wirz, Hargitay and Kuhn in their paper [5] on direct cryoscopy of kidney slices referred to earlier studies on medullary composition. Using osmometric methods which we now know give only comparative and not absolute values, Filehne and Biberfeld in 1902 and Hirokawa in 1908 reported that the osmotic pressure was higher in slices of medullary tissue than in slices of cortical tissue [14, 15]. Hirokawa conducted more extensive studies and his discussion of those results was remarkably prescient. (My translation).

> "The osmotic pressure of kidney cortex is very constant and is in all species examined (pig, cattle, rabbit, cat) within the limits of the osmotic pressure of a 1 to 2% sodium chloride solution. It is independent of the concentration of the excreted urine and does not reach higher values when the osmotic pressure of the excreted urine increases to a very high level.
>
> In contrast, the osmotic pressure of kidney medulla is extraordinarily variable; it is almost without exception higher than that of the kidney cortex, and is the higher the more concentrated is the excreted urine.
>
> When the excretion of a strongly diluted urine is produced by infusion of water or weak salt solution, the osmotic pressure of kidney medulla can be lowered to the level of the osmotic pressure of the kidney cortex.
>
> Our observations show unequivocally that the urine present in the medulla has a much higher osmotic pressure than the urine that is in the convoluted tubules of the kidney cortex; therefore, the osmotic pressure of the urine increases considerably during its passage through Henle's loops and collecting tubules."

In the 1940's Glimstedt and Ljungberg reported that the chloride concentration of medullary tissue of rabbit kidneys increased to very high levels as the tip of the papilla was approached [16, 17]. In the 1950's Ullrich et al observed that the osmotic pressure of tissue slices from kidneys of thirsted dogs rose steadily from the outer medullar toward the tip of the papilla as did the concentration of sodium, chloride, urea, and creatinine [18, 19]. Ullrich and colleagues also demonstrated by microcatheterization of collecting ducts an increase in osmolality of tubular urine flowing down the collecting ducts [20].

The Berliner hypothesis

In 1958 Berliner, Levinsky, Davidson, and Eden presented an alternative hypothesis for the function of the loop of Henle [21]. It rejected a countercurrent multiplier function of the loop, but held to passive countercurrent exchange in the vasa recta. "Instead of behaving as a countercurrent multiplier, the loop is viewed as a source of sodium salts and the fact that the loops dip deep into the medulla provides the means of delivering sodium salts deep into the medullary tissue." No functional differentiation was postulated for ascending and descending limbs. The entire loop was assumed to transport salt from lumen to peritubular fluid; its epithelium was assumed to have a very low permeability to water and be unaffected by ADH. The vasa recta were considered as an efficient countercurrent exchanger minimizing loss of solute from the medulla via the blood vessels.

This important paper also assigned a specific or unique position for urea in the concentrating operation of the kidney. The proposal was that "urea in the urine can add to the osmotic pressure of the urine without being balanced by an osmotically equivalent amount of sodium chloride in the medullary intersti-

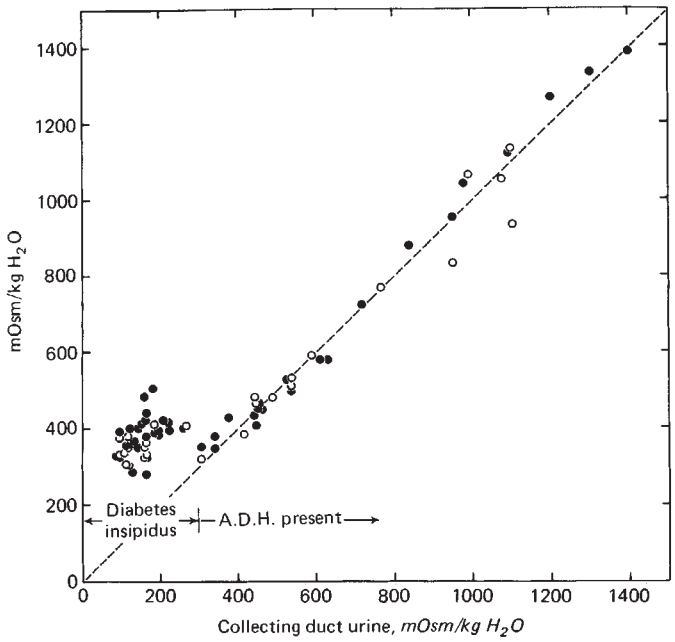

Fig. 3. *Relation between the osmolality of collecting duct urine and fluid from loops of Henle (●) and vasa recta blood (○) in various normal desert rodents and in hamsters with experimental diabetes insipidus [25].*

tial fluid." In the presence of ADH the collecting ducts were assumed to be freely permeable to urea as well as to water. Countercurrent diffusion in the vasa recta of urea lost from the collecting ducts was postulated to result in a high concentration of urea in the medullary interstitial fluids, and with sodium chloride to account for their high osmolality. Although the unique position assigned to urea remains an integral part of current ideas of the function of the countercurrent mechanism, the hypothesis that both ascending and descending limbs of the loop of Henle are freely water permeable and pump sodium chloride into the interstitium was soon proven to be incorrect.

The Chapel Hill results

In 1958–59 Margaret Mylle and I presented extensive data on the osmolality of fluid collected by micropuncture from proximal and distal convolutions, loops of Henle, collecting ducts and vasa recta from kidneys of various rodents [22, 23]. These results confirmed and extended the earlier studies of Walker et al and the more recent micropuncture results of Wirz, and, for the first time, provided the essential missing datum, direct measurement of the osmolality of fluid from the loop of Henle. All samples of proximal tubular fluid were isosmotic within the error of measurement. In the presence of antidiuretic hormone early distal fluid was hypo-osmotic and fluid from the late distal convolution isosmotic. The final hyperosmotic concentration of the urine occurred in the collecting ducts. The latter process presumably was a consequence of the earlier hyperosmotic reabsorption of sodium chloride in the loops of Henle. And, most importantly, fluid from the bend of loops of Henle and from collecting ducts and vasa recta at the same level were equally hyperosmotic (Fig. 3). We were also able to demonstrate directly by micropuncture in hamsters that the water permeability of thin descending limbs greatly exceeded that of thin ascending limbs of the loop [24]. In the presence of ADH both thin and thick ascending limbs of the loop of Henle had very restricted water permeability.

Shortly, we were able to confirm the limited data Wirz obtained during the elaboration of hypoosmotic urine and confirmed that the tubular fluid remained hypoosmotic throughout the distal convolution in the absence of ADH in animals with experimental diabetes insipidus [25]. Further, in hamsters with diabetes insipidus, we found that loop of Henle fluid and vasa recta blood from the tip of the papilla were equally hyperosmotic at a time when fluid from adjacent collecting ducts was distinctly hypo-osmotic (Fig. 3). Thus, water permeability of the distal convolution and collecting duct epithelium but not of the loop of Henle was profoundly influenced by ADH.

Lassiter, Gottschalk and Mylle [26], using isotope tracer techniques, provided quantitative measurements of net water, total solute, and urea transport in all major segments of the rat nephron. Solute loss exceeded water loss in the ascending limb of the loop, and urea diffusing out of the collecting ducts was trapped in the descending loop of Henle and was recirculated into the distal convolution. The results were confirmed by chemical determinations of urea and sodium concentration in loop fluid, made in collaboration with Karl Ullrich and Bodil Schmidt-Nielsen [27]. Urea recirculation contributes significantly to the maintenance of a high medullary interstitial solute concentration and is thus an important feature of the urinary concentrating mechanism.

Smith's acceptance

The accumulated data finally convinced Homer Smith that the countercurrent hypothesis had merit. In a revealing apologia presented at the New York Academy of Medicine in 1958, Smith reviewed the data and discussed his skepticism [28]. "In retrospect these criticisms are seen to be invalid: the micro-melting point method, inadequate as it then appeared, gave surprisingly good results, and a countercurrent hypothesis, in one form or another, is now an important chapter in renal physiology."

Smith was forthright:

> "I still do not like it: it seems extravagant and physiologically complicated—though so is the whole glomerular filtration–tubular reabsorption pattern. . . . Least of all, however, do I like to see the squamous epithelium of the thin segment freely permeable to water (if not to sodium also) in the descending limb, only to acquire water impermeability and active sodium transport at the tip of the loop for no better reason, apparently, than the circumstance that it has turned a corner. But I suppose that I can get used to that, too."

In his closure, Smith searched for alternative explanations consistent with the information then available. He believed that a countercurrent system—probably a multiplier—was operative in the loop of Henle, but "with respect to the fate of sodium and water in the renal tubules, I still oscillate between the poles of purgatorial doubt and heavenly certitude—between skepticism and dogmatism, the one always uncomfortable, the other, unprofitable." These comments could still be applied to the generation of the osmotic gradients in the inner medulla; much

of the present volume is concerned with the mechanism of their establishment.

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Reprint requests to Dr. C.W. Gottschalk, Department of Medicine (226H), School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27514, USA.

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