THE FATE OF SODIUM AND WATER IN THE RENAL TUBULES*

The Sixth Harlow Brooks Memorial Lecture

HOMER W. SMITH, SC. D.

Professor of Physiology, New York University College of Medicine, New York, N. Y.

ON MARCH 30, 1950—back in the Dark Ages, so to speak, of our present subject—some of our British colleagues banded together in what they called the Renal Association**. They referred to themselves facetiously as the "renologists". After listening to formal presentations in the comfortable quarters of the Ciba Foundation at 41 Portland Place, they ofttimes dined together in one of the better restaurants near Piccadilly, and on many such occasions we may accept that the cross-chop of conversation which rippled over the long table contained frequent references to salt and water.

The Renal Association is still active scientifically and, I hope, gastronomically. To my knowledge not one of its members has come to an untimely end, despite their preoccupation with a technically intricate subject and a consequent measure of what the uninformed frequently call absent-mindedness. Nor do I wish a single one of them any misadventure. I cannot, however, avoid a perverse temptation to speculate: What would have happened had a renologist, at about this time, suffered rude juxtaposition with a bus or taxi while on his way from Gower Street to dinner?

I construct the following sequence from the known habits of renologists. As the victim of this hypothetic traffic accident took his place before the Gates of Heaven, St. Peter would, I anticipate, hand him a piece of chalk, perhaps fluorescent red, and request him to draw upon the golden pavement a "typical mammalian nephron". Peering over the

---

* Delivered at the Annual Postgraduate Week, *Research Contributions to Clinical Practice,* of The New York Academy of Medicine, October 17, 1958.

Studies related to this problem, carried out by the writer and his colleagues, have been aided by a grant (H-1172) from the National Heart Institute of the National Institutes of Health.

**The moving spirits in the Renal Association, which met quarterly, were Professor Kenneth Franklin, Dr. A. A. Osman, Dr. W. W. Payne and Mr. John Sophian.

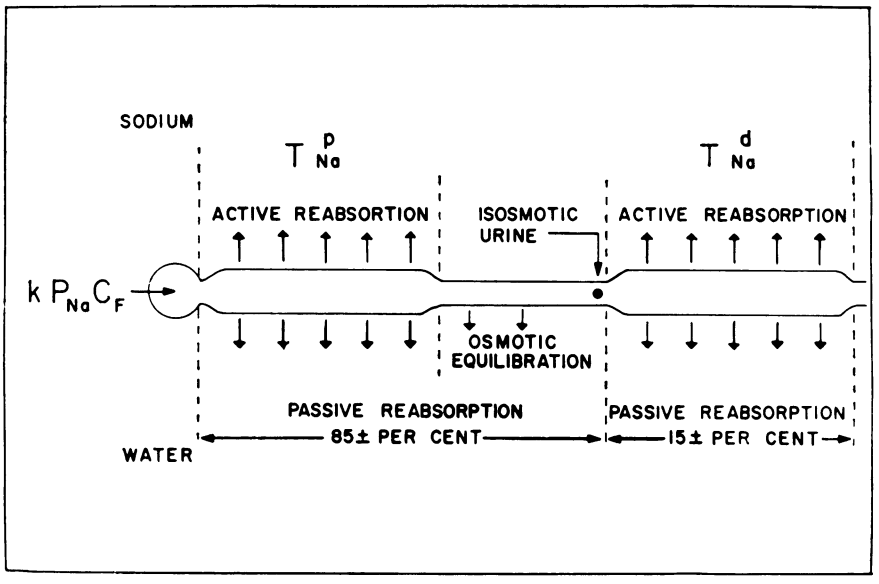

Figure 1—The rectilinear nephron, as previously drawn by the writer on several occasions with minor modifications.

renologist's shoulder as, on one knee, he begins his diagram, we follow the chalk as it inscribes a partially closed circle representing the glomerulus; and, extending to the right in a manner best described by Euclid's definition of a straight line, a tubule with a long, constricted part in the middle indicating the thin segment interposed between the so-called proximal and distal segments; and, lastly, a collecting duct (Fig. 1). Neither the ureter nor the bladder, nor, as a superior substitute, a ureteral catheter, is included, presumably because at that time these appendices appeared to possess no functional significance*.

Scarcely is the diagram completed, however, before its fluorescence is eclipsed in shadow as St. Peter also leans forward, his brow wrinkled, his eyes suggesting a venerable anatomist who is seeing something absolutely new, absolutely for the first time.

"And where in the world did you get that?"

The pavement artist puts the other knee on Heaven's floor and

* At low urine flows significant quantities of water and solutes diffuse across the ureter and bladder along their respective concentration gradients. Some diffusion may be predicated at unilateral flows in the dog at 1 ml/min., and diffusion becomes substantial at flows below 0.2 ml/min. Diffusion is most marked with respect to urea and water and requires revision of data based on bladder urine collected during oliguria[22].

fingers his chalk as he struggles to save what he divines is already lost.

"Why," he says with a show of confidence, "it's in the text-book—it's on page 732, or perhaps it's page 327—it's been in the text-book, at least in some of them, for a long time and—"

"No doubt, young man! There are text-books and text-books. But perhaps you should go back to some other text, say volume *ii* of that monumental work entitled *Handbuch der systematischen Anatomie des Menschen*, published in 1862 by the great anatomist Friedrich Gustav Jakob Henle*; or, if you do not read German, you might look into the 7th edition of the great English text, *Anatomy, Descriptive and Surgical*, published by Henry Gray in 1874."

"Oh, yes Sir! I know about Henle's loop, but *Karl* Peter, I think it was, in his monograph on the kidney implied, in effect, that Henle's loop is only an incident of organogenesis because—"

"And so! Did Herr Peter imply that an 'incident of organogenesis' need have no functional significance? Your cerebral cortex is also an incident of organogenesis!"

"No, but—"

The authority of the nacreous gates raised his hand. "I'm sorry, lad. Go back to the mammalian kidney and leave reductionist diagrams to—to the nuclear physicist. The chalk, please. And do come again."

And so it was that our renologist reappeared in London, perhaps never having been missed, certainly never knowing how narrowly he had missed out on Heaven.

Heaven for a renologist, I suppose, is a psychologic state characterized chiefly by certitude, of such a degree at least that he can say, "of this I am confident, so now I can go on to that". I have enjoyed few such ecstatic moments because of the skepticism of my colleagues, most of whom are veritable plagues in this respect. Indeed, my existence should perhaps be described as a sort of purgatorial nexus spun at the nodal points of doubt and certainty, its phasic interferences reminding me of the multiangular dinner conversation of the renologists. Which circumstance explains in part why, viewed objectively, I may seem to oscillate between dogmatism and disbelief, or to be seeking both poles at once.

* Henle[12, 13] (1862) incorrectly believed that the medullary loop was a U-shaped extension of a single convolution and thus comprised a closed system. Schweigger-Seidel[97] (1865) corrected this error by showing that the ascending limb, after additional convolutions, is connected with the collecting duct. von Kölliker was the first to use the expression "loop of Henle."

Perhaps I sat at dinner with our renologist in London in July of 1950, and perhaps I impressed him as a little too much in the heavenly confident mood. But I was at the opposite pole of doubt when, in August of that year, I drank several glasses of Carlsberg beer, at the XVIIIth International Physiological Congress in Copenhagen, with Heinrich Wirz of the University of Basle.

Henry had spent the academic year of 1946-47 with us, and now he had some exciting observations to show me. These observations concerned the mechanism by which the mammalian kidney elaborates a urine which is osmotically more concentrated than the blood. This operation is chiefly important in water balance when the available supply of free water is small. All vertebrates can excrete urine isosmotic with, or osmotically more dilute than the blood, but only the mammals (and to a lesser extent the birds) can excrete a hyperosmotic urine and thus compensate for a substantial deficit of water. Elsewhere I have suggested that the concentrating capacity of the mammalian kidney may have played an important role in our evolution, and specifically in the ultimate domination of the mammals over the dinosaurs and other reptiles at the end of the Mesozoic Era[38].

The elaboration of an osmotically concentrated urine requires that pure water, i.e., without any solute whatsoever, be removed from the isosmotic glomerular filtrate at some point along the nephron. In 1950 it was suspected that this operation may occur in the collecting ducts, but how it could be effected was a moot question—the only notion then available was that the epithelial cells could transport water molecules *per se* from the urine back into the blood. Since this must occur against a steep concentration gradient some sort of active transport must be involved, and no one was very happy with an hypothesis that invoked the active transport of water molecules[2].

The observations which Henry Wirz showed me consisted of measurements of the osmotic pressure at various levels in the rat kidney, determined in frozen sections by the melting point of ice crystals. He had used a micromelting-point method which is based on the fact that ice crystals are birefringent and light, while the water is dark, when examined by polarized light[11]. He had found that the fluids in the cortex were isosmotic with plasma, but the medulla showed a continuous increase in osmotic pressure from the cortical-medullary junction to the tip of the papilla (Figure 2). At any one level, no differences were

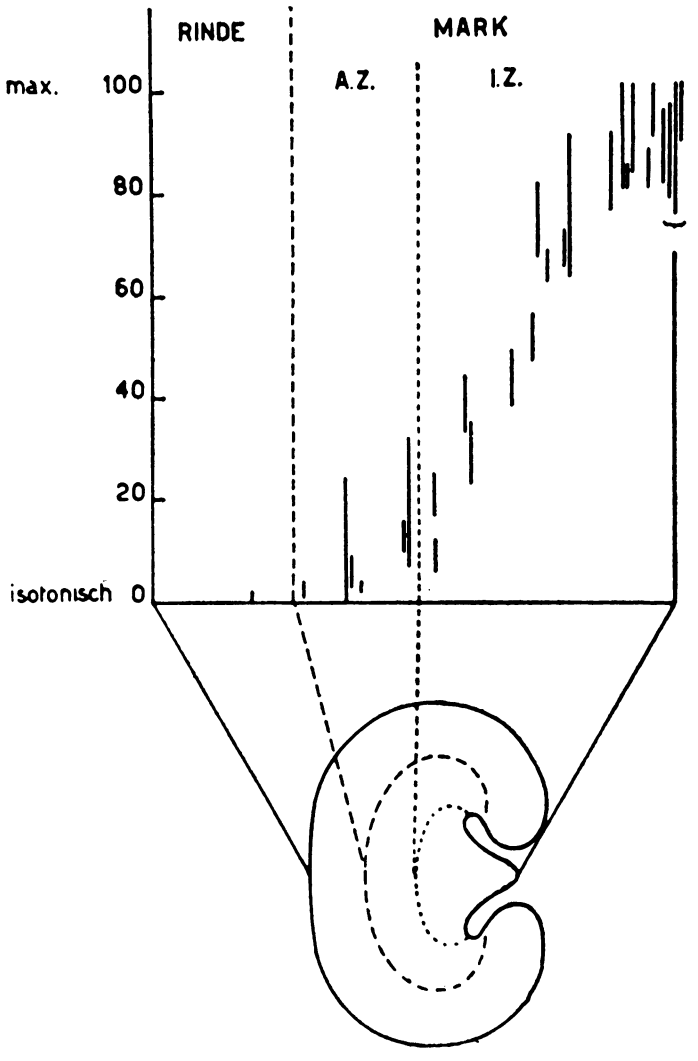

Figure 2—Osmotic pressure of the fluids in the kidney of the hydropenic rat, increasing from the isosmotic state in the cortex to reach a maximal value at the tip of the papilla. A. Z., outer zone, I. Z., inner zone of the medulla. This and Figures 3 and 4 are reproduced by permission of the authors and publisher[50].

discernible between the tubular urine, the capillary blood and the urine in the collecting ducts. This approach revealed the kidney to be composed of concentric shells, each of uniform osmotic concentration (Figure 3). To explain this surprising result, Wirz and his colleagues, Bartholomaüs Hargitay and Werner Kuhn of the University of Basle, had invoked a principle known as a countercurrent multiplier, wherein

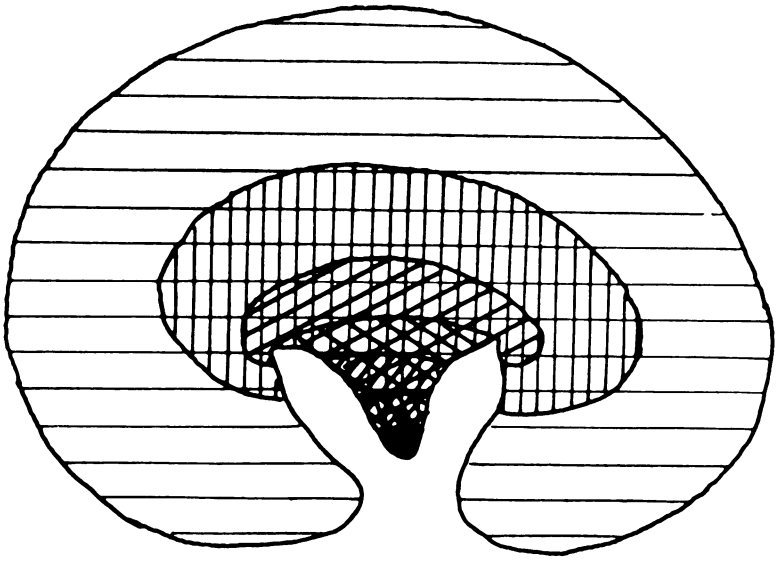

Figure 3—Regions of approximately equal osmotic pressure in the hydropenic rat kidney comprise concentric shells parallel to the corticomedullary boundary and medullary zones. As described by Wirz, Hargitay and Kuhn[50].

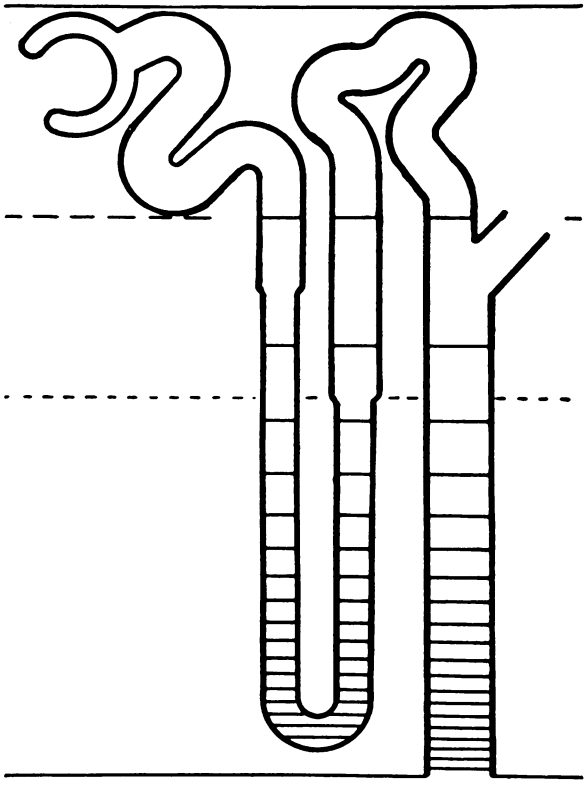

Figure 4—Variation of osmotic pressure in a single nephron and collecting duct. As described by Wirz, Hargitay and Kuhn[50].

the opposing streams in the descending and ascending limbs of the loop of Henle play an important role[50].

This was, at the time and for some time thereafter, very confusing. The countercurrent hypothesis placed the burden of concentrating the urine on the thin segment (which comprises the greater part of the nephron in the medulla), and by its structure the thin segment seemed scarcely able to support this burden. The hypothesis required that the collecting ducts be freely permeable to water and that the final urine be concentrated by the passive diffusion of water into the hyperosmotic interstitium established by the thin segments. It required that the tubular urine be concentrated at some point along the thin segment, only to be diluted again in the distal convolution, which teleologically seemed wasteful. And lastly, it required that all the blood traversing the medullary capillaries (*vasa recta*) be concentrated to exactly the same extent as the urine in the collecting ducts at any transverse level of the medulla, which meant more wasted energy (Figure 4). All this was difficult to believe, and I, for one, was inclined to question the micromelting-point method on technical grounds*.

In retrospect these criticisms are seen to be invalid: the micromelting-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.

The basic principle of a countercurrent system is that two streams, moving in opposite directions, are so juxtaposed as to facilitate the mutual exchange of energy or substance in one form or another—what form is immaterial to the principle. Countercurrent systems may operate in one of two ways, as simple countercurrent exchangers, or as countercurrent multipliers. Countercurrent exchange systems (Figure 5) are widely used in engineering, but I doubt that the basic principle is patentable because nature invented it many millions of years ago. One example is presented by certain arctic birds which walk about on snow

* The fact that Hargitay and Kuhn[10] had developed the theory of the countercurrent multiplier in terms of an osmotic model which operated by means of hydrostatic pressure, and that no substantial hydrostatic pressure was available for this purpose in the loop of Henle, also handicapped the idea—though unfairly, as we now see.

Perhaps the major criticism in the writer's mind lay in the fact that ice has a substantial vapor pressure (3.013 mm. Hg at -5°C., 3.568 mm. Hg at -3°C., 3.880 mm. Hg at -2°C., 4.217 mm. Hg at -1°C., and 4.579 mm. Hg at 0°C., as compared with the vapor pressure of water of 12.788 mm. Hg at 15°C.), and diffusion, or more properly, sublimation of vapor across microscopic dimensions could lead to the equalization of osmotic pressure between the fluid in the collecting ducts and that in the medullary tubules and capillaries. Wirz[47] fully recognized the validity of this criticism as regards small distances, and it was perhaps because of sublimation that he failed to discover the dilute urine in the ascending limb of the loop of Henle.

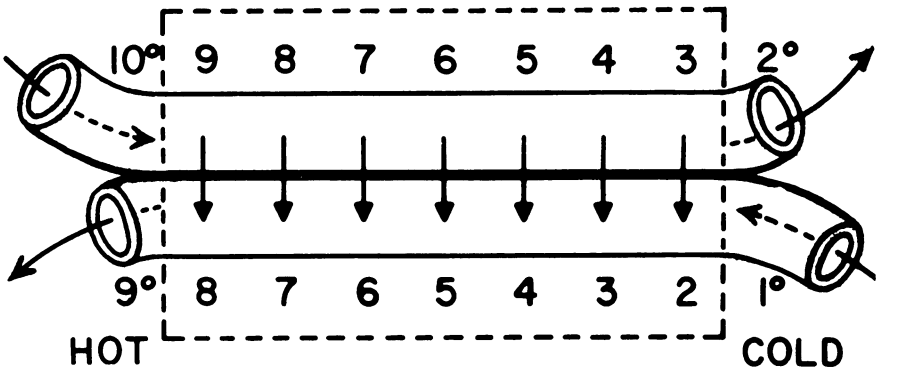

Figure 5.—A simple example of a countercurrent exchange system permitting the exchange of heat between two opposing streams initially at different temperatures. The system is conceived to be insulated externally. Note that in consequence of heat exchange both streams are warm at one end and cold at the other. Adapted from Scholander[31].

or wade in cold water. If the feet were near body temperature the heat loss would be considerable, but actually this loss is negligible because the feet may have a temperature close to that of the snow or water. Where the arteries and veins emerge from the body to enter the thigh they are intertwined complexly in a *rete mirabile*, this close juxtaposition of vessels facilitating the exchange of heat from the arterial to the venous blood; the arterial blood is thereby cooled and carries little heat into the foot, while the venous blood is thereby warmed and carries no large heat deficit into the body. A neat solution to the problem of how, at a trifling cost in calories, to maintain the body temperature and yet keep the feet refrigerated.

Claude Bernard in 1876 recognized the importance of heat exchange between the large arteries and veins in mammals, and many studies have recently been made by Irving, Scholander and others on thermal exchange systems in the limbs of various terrestrial and aquatic mammals (arctic dogs, porcupines, reindeer, caribou, beaver, muskrat, etc.), in birds and in arctic whales and seals*. The countercurrent exchange principle is utilized in the respiratory function of the gills as well as in the oxygen-filled swim bladder of fishes[15, 32-36, **].

* The surprising feature of this thermal exchange mechanism is its sporadic occurrence: a well-developed *rete* is present in the tropical sloth, manatee and whales, but absent in the cold water ducks, geese, sea gulls and swans; when present it may be related as much to heat dissipation as to heat loss.

**The swim bladder of the pelagic fishes contains oxygen at nearly 1 atmosphere pressure, as opposed to a partial pressure of one fifth of an atmosphere in the air and water, but in the deep sea fishes the oxygen pressure may reach 200 to 300 atmospheres, a 1000- to 1500-fold increase over the partial pressure of oxygen in the water.

The countercurrent multiplier differs from the countercurrent exchanger only in the fact that, besides adding and subtracting, it also multiplies, but the basic principle is not greatly different—you will recall from your ordinary calculating machine, if not from your elementary arithmetic, that you can multiply (or divide) by the simple expedient of adding (or subtracting) repetitively. The principle of the countercurrent multiplier has been applied in several studies since 1938 by Professor Werner Kuhn, Director of the Physical-Chemical Institute at the University of Basle. The principle operates in the rectification column of a distillation apparatus, and it has been applied by Kuhn and Martin to the development of the gas centrifuge and the separation of optical isomers from solution[28, 29].

It was one of Kuhn's graduate students, Bartholomaüs Hargitay, who first conceived of a countercurrent osmotic multiplier and its possible application to the kidney[10], though the final achievement represented the collaborative efforts of Kuhn, Hargitay and Wirz.

In the classical osmometer, hydrostatic pressure is used to equalize the vapor pressure (or activity) of a solvent across a semipermeable membrane. Any solution, however, may be further concentrated by dividing it between the two compartments of an osmometer (Figure 6) and applying hydrostatic pressure to one compartment (C_1), when solvent will be forced through the semipermeable membrane to dilute the fluid on the other side (C_2). The degree of concentration which can be effected in a single such operation by a given hydrostatic pressure is, of course, strictly limited in theory, but the process can be repeated indefinitely (in the ideal case) by removing the more concentrated solution (from C_2) and redistributing it between the compartments of a smaller osmometer and then reapplying the same hydrostatic pressure. Hargitay and Kuhn[10] calculated that after 20 such transfers, in each of which the volume is reduced by one half, one cubic meter of solution subjected to a hydrostatic pressure of only one tenth of its osmotic pressure will yield 0.48 ml. of a solution having twice the original concentration.

The novel feature in Hargitay and Kuhn's countercurrent system is the substitution, for this discontinuous step-wise procedure, of a continuous series of infinitesimal increments. Their basic theoretical model consists of an osmometer designed in the shape of a hair-pin or U-tube with a common wall consisting of a semipermeable membrane, the two

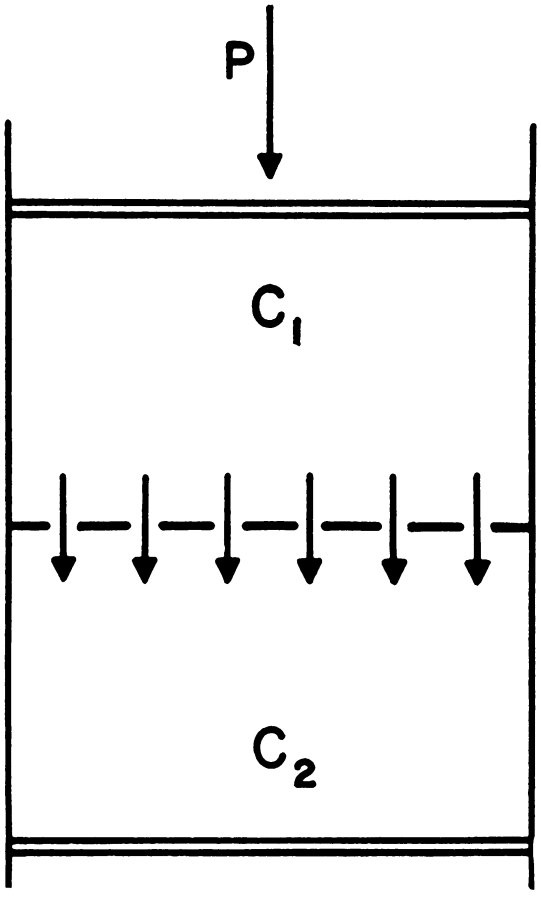

Figure 6—A conventional osmometer in which a hydrostatic pressure, P, increases the osmotic concentration of a solution in the compressed compartment, C_1, and dilutes it in a second compartment, C_2, beyond the semipermeable membrane.

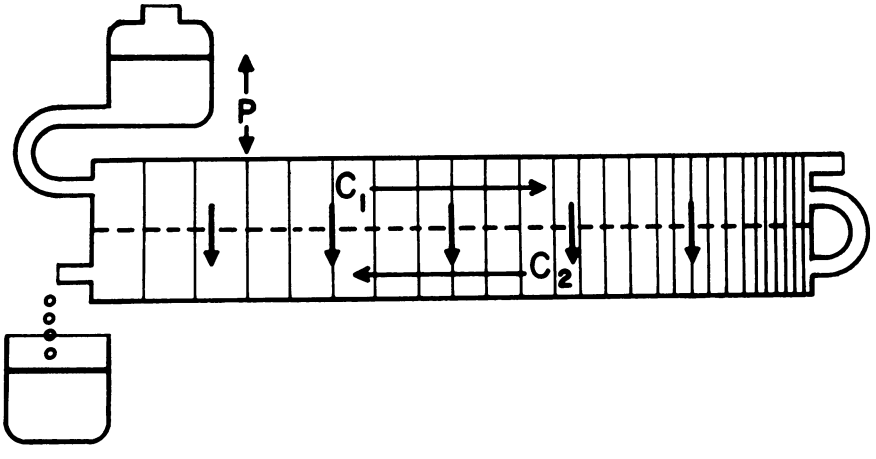

Figure 7—The hair-pin, continuously operating countercurrent multiplier as designed by Hargitay and Kuhn[10]. At the right are shown the capillary "reversal point" and an outlet for bleeding off small quantities of highly concentrated solution.

compartments being connected to each other at the far end by a fine capillary tube (Figure 7). After the compartments are filled with a suitable colloidal solution, circulation through the system is maintained by applying a constant hydrostatic pressure to one compartment (C_1), the other being left open to drain freely. The hydrostatic pressure in the first compartment is transmitted uniformly up to the capillary, or "reversal point", where in consequence of viscous resistance this pressure is abruptly reduced to a low value.

In consequence of the difference in hydrostatic pressure water will move from the upper to the lower compartment across the semipermeable membrane, but at any one point along the membrane equilibrium will be approached and the opposing fluids will have nearly the same osmotic pressure. Hence a fixed, limited hydrostatic pressure in C_1 will bring about additional filtration at each successive point, with the consequence that the osmotic pressure on both sides will increase along the length of the hair-pin, to reach its maximal value at the reversal point. In the eventual steady state this maximal osmotic pressure will greatly exceed that attainable by the same hydrostatic pressure in a static osmometer. The concentrated fluid can be bled off in small quantities at the reversal point without disrupting the process. Other things being equal, the narrower the compartments and the longer the apparatus, the more efficient will be its operation. (In the theoretical model developed by Hargitay and Kuhn (assuming certain finite dimensions), a 0.1 Osm solution could be concentrated to 2.0 Osm by a hydrostatic pressure of 3.75 atm.; to accomplish this in a single step would require 50 atm.).

In brief, the countercurrent multiplier effects only a small, theoretically limited change in concentration at any one point, but by additively imposing a new increment on the accumulated change at successive points, a final concentration can be attained at the reversal point which may be arbitrarily large and substantially greater than the theoretically limited local change. The operation is analogous to the compound interest law except that in the countercurrent multiplier the rate of increase is proportional to the *square* of the accumulated change.

Hargitay and Kuhn brought to the countercurrent osmotic multiplier some mathematics not customarily used by renologists, whose mathematics do not often go beyond the arithmetic required to keep a bank account. They also brought to the problem considerable mechanical

ingenuity in the construction of a physical model which consisted basically of two circular brass plates, each inscribed with a spiral groove 1 mm. deep, 5 mm. wide and 4 m. long; for a semipermeable membrane they used a sheet of commercial cellophane, and for a colloidal solution, sodium polyacrylate. This model converted a net filtration pressure into osmotic pressure with a multiplication factor of two-fold, a surprisingly good result considering the recognizable departures in the apparatus from ideal conditions*.

The countercurrent principle is applicable to any change of state, in the physical-chemical sense, and for the principle to apply to the kidney it is first required that the osmotic pressure of the interstitium of the medulla be increased. Hargitay and Kuhn recognized that this might be effected by the electro-osmotic movement of water into the tubular urine in the ascending limb, or conversely by the active reabsorption of salt (which for quantitative reasons must be sodium chloride) from the tubular urine in the ascending limb and the deposition of this sodium chloride in the interstitium—the change of emphasis from water to osmotic solute requiring only a change of sign in the basic equations[10, p. 554].

The evidence now available is convincing that a medullary countercurrent mechanism is involved in concentrating the urine, and that this same mechanism is involved in part in elaborating the dilute urine of water diuresis. This evidence can be most conveniently summarized by reference, in the first instance, to maximal antidiuresis in the hydropenic state.

Some of the most valuable of this evidence has been obtained by the exquisite and difficult micropuncture method which A. Newton Richards and his colleagues developed thirty years ago, and which investigators in Philadelphia and elsewhere have applied so resourcefully to the study of renal function.

In 1953 Wirz showed that renal papillary blood, collected by micropuncture of the superficial capillaries of the papilla in the golden hamster (which, like the kangaroo rat and some other rodents, has a long papilla extending well into the ureter) is essentially isosmotic with the bladder urine[46, 47], as is required by the countercurrent theory.

* It cannot be too strongly emphasized that in conceiving the application of the countercurrent multiplier to the loop of Henle, Wirz, Hargitay and Kuhn[10, 50] recognized that the *vis a tergo* in the kidney could not be hydrostatic pressure, and any tendency to impute to them a misconception on this point should be corrected.

However, information on the tubular urine was until recently confined to the convolutions near the surface of the cortex. The convolutions of the proximal segment represent about two-thirds of this segment: the last third is represented by the *pars recta* descending into the medulla and is not accessible to puncture. The distal tubule is accessible only in the convoluted portion beginning at the macula densa, where the straight ascending limb of Henle's loop makes contact with the vascular pole of the glomerulus of origin, and ending at its junction with the descending collecting duct. The collecting duct is accessible only in the tip of the papilla.

The studies of Walker, Bott, Oliver and MacDowell[44], reported in 1941, had shown that in the rat, guinea pig and opossum the fluid in Bowman's capsule and throughout the first half of the proximal tubule is isosmotic with the blood. It is clear that a considerable fraction of filtered sodium is reabsorbed in the proximal segment by an active transport process, osmotic equilibration being effected by passive diffusion of water[45]. (The argument that sodium and water are both reabsorbed proximally by diffusion along an osmotic gradient established by the colloid osmotic pressure of the plasma has been effectively answered by Berliner, Levinsky, Davidson and Eden[2].)

In the studies of Walker and his collaborators the evidence on the distal tubule was ambiguous. In 1956, however, Wirz[48] showed that at the beginning of the distal convolution (i.e., near the macula densa) the urine is distinctly hypo-osmotic to the blood even in hydropenic animals (the osmotic U/P ratio ranges from 0.5 to 0.8, and during NaCl infusion may decrease to 0.3)[9]; it attains the isosmotic state about half way along the distal convolution, but invariably remains isosmotic until it enters the collecting duct. It follows that the hyperosmotic state must be attained in the collecting duct itself.

These observations on proximal and distal urine have been fully confirmed in the rat and golden hamster by Carl Gottschalk and Margaret Mylle[8, 9] of the University of North Carolina. More importantly, Gottschalk and Mylle have shown in the hamster and kangaroo rat that during antidiuresis both the capillary blood (confirming Wirz) and the tubular urine collected near the tip of the loop of Henle have essentially the same osmotic pressure as the urine in adjacent collecting ducts at the same level*.

* Limited observations on *Psammomys obesus*, a North African rodent in which all nephrons have long loops, conform with the observations in the rat, which has both short and long loops[9].

Another valuable approach has been afforded by the analysis of cortical and medullary slices for specific solutes. That the chloride content of medullary tissue in the rabbit increases from the outer medullary zone (cortico-medullary junction) to the tip of the papilla has been known for many years[7, 26]. More recently Ullrich and Jarausch[42], of the University of Göttingen, have shown in hydropenic dogs that the concentrations of sodium, urea, exogenous creatinine and amino acids increase in a similar manner, though not all to the same extent: urea undergoes the greatest concentration and then, in decreasing degree, sodium, chloride, creatinine and amino acids*. Potassium (perhaps because it is not actively reabsorbed in the ascending limb), magnesium, calcium, and inorganic phosphate (perhaps because of their intracellular inclusion) do not show any concentration gradient between the outer medulla and the tip of the papilla.

These observations have been confirmed for sodium in studies using Na^{22} in the rat kidney by Krakusin and Jennings[20] of Northwestern University, and by direct analysis of sodium, chloride and urea in the dog kidney by Levinsky, Davidson and Berliner[23, 24] of the National Heart Institute. The rapid accumulation and the ultimate axial gradient of I^{131}-tagged albumin in the dog medulla, recently demonstrated by Lassen (then with the Institute of Mental Health), Longley (Institute of Arthritis and Metabolic Diseases) and Lilienfield (Georgetown University)[21, 25], conforms with this pattern; it is not known, however, how much of this albumin is contained in the capillaries and how much escapes into the interstitial fluid**.

---

* During water diuresis sodium and chloride are concentrated slightly, urea very little, and creatinine not at all. These changes are hard to interpret because of the difficulty of attaining a steady state. Moreover the concentrations are expressed per ml. of tissue water—and in the diuretic kidney an unknown quantity of dilute urine is included in the distal tubule and collecting ducts. It is improbable that changes in sodium and chloride concentration reflect changes in the concentration in the tubular urine, but this circumstance may explain the behavior of creatinine. The interpretation with respect to amino acids, which are only slightly concentrated in the hydropenic animal, is even more difficult. The situation with respect to urea is discussed later.

Ulrich and Jarausch's statement that in hydropenia the urea concentration in the papilla (μmols/ml.) is equal to that in the urine must be qualified in light of the more recent demonstration by Levinsky and Berliner[22] that at low urine flows the composition of bladder urine is markedly altered by diffusion of water into, and of urea out of the urine across the ureters and bladder.

Ulrich, Drenckhahn and Jarausch[40] have concluded from the osmometric (swelling) method that the osmotic pressure of kidney slices follows the pattern described by Wirz, Hargitay and Kuhn, and which is to be expected from their analyses. It is well known, however, that the determination of the osmotic pressure of tissues by this method is unreliable; moreover, in this particular instance Berliner, Levinsky, Davidson and Eden[2] have found that fresh slices of medulla swell even in very hypertonic sucrose solution. The only seemingly reliable method for measuring the osmotic pressure of a tissue en masse is that of Maffly and Leaf[27]; this method reveals the medulla to be grossly hyperosmotic to the blood (skeletal muscle, liver, heart and brain are isosmotic), but quantitative interpretation is still complicated, even in hydropenic animals, by the presence of dilute urine in the ascending limb.

**Albumin reaches 85 per cent of its 60 minute concentration in 3 minutes, and the fact that I^{131}-tagged γ-globulin is concentrated much more slowly suggests that albumin escapes more rapidly from the capillaries.

So much for evidence. As for theory, at the time (1951) when Hargitay and Kuhn[10] published their paper on the countercurrent theory, and Wirz, Hargitay and Kuhn[50] on the kidney, nothing was known about the composition of the urine between the proximal convoluted tubule and the renal pelvis, and the physiological characteristics of the loop were not spelled out. It was posited that the "single" or initial effect, or *vis a tergo*, of the countercurrent system in the medulla consisted either of the movement of water from the descending into the ascending limb (which virtually amounts to active water transport), or conversely of salt (sodium chloride) from the ascending to the descending limb; they failed, however, to recognize that the latter mechanism could not operate as an osmotic multiplier unless the permeability of the ascending limb to water is restricted*.

After Wirz in 1956 had demonstrated the osmotically dilute nature of the urine in the early distal convolution in hydropenic animals, he recognized that the ascending limb of the loop must be relatively impermeable to water and he favored the belief that this dilution was effected by the reabsorption of sodium chloride in this segment (he did not distinguish between the ascending thin segment and the thick segment which extends from the inner zone of the medulla up to the macula densa). The permeability characteristics of the descending limb were not specified, though it was clear that sodium chloride must gain access to (and probably water lost from) this limb if the system is to work as a countercurrent multiplier. As in the original paper, it was posited that the progressive accumulation of sodium chloride along the long axis of the loop increased the osmotic pressure of the interstitium and concentrated the urine by passive abstraction of water through the collecting ducts[48,49].

Recognizing the theoretical difficulties of the 1951 description, and discounting Wirz' necessarily tentative suggestions of 1956, Robert Berliner and his colleagues[2] in 1958 proposed that sodium chloride is actively reabsorbed throughout the length (descending and ascending limbs) of the thin segment, and, as a necessary corollary, that this segment is everywhere essentially impermeable to water. Here the loop does not operate as a countercurrent mechanism in any sense, though

* Wirz, Hargitay and Kuhn[50] said that they did not observe an osmotic pressure difference between the structures at any given level, but that there does exist a *small* osmotic pressure difference (presumably too small to measure), underlining the word "small" for emphasis. This statement could imply some water impermeability in the ascending limb.

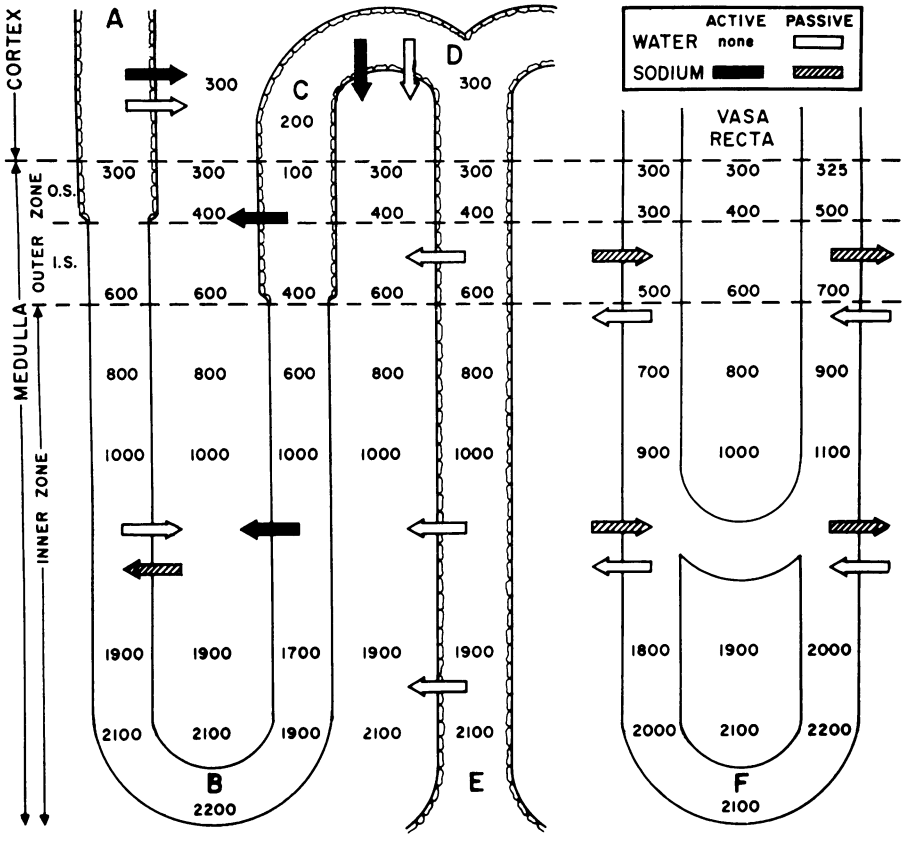

Figure 8—The loop of Henle as a countercurrent osmotic multiplier, basically as conceived by Gottschalk and Mylle[9]. Sodium chloride is actively reabsorbed in the ascending limb of the loop of Henle; the hyperosmotic interstitium abstracts water from the water-permeable descending limb (and possibly sodium chloride diffuses into this limb), thus increasing the concentration of sodium in the tubular urine delivered to the ascending limb and converting the system to a countercurrent multiplier. Since an osmotically dilute fluid is delivered to the distal convoluted tubule, the ascending limb must be relatively impermeable to water. (Reprinted by permission, Amer. J. Physiol.)

The system operates to establish an osmotic concentration gradient in the interstitium, increasing from the corticomedullary junction to the tip of the loop, and to concentrate the urine by the passive abstraction of water through the water-permeable collecting ducts. The *vasa recta* (right) serve as countercurrent exchangers, promoting the overall efficiency and carrying away in the ascending capillaries the water abstracted from the urine.

Sites for which data on the osmotic pressure of the tubular urine are now available: A, proximal convoluted tubule (cortex); B, tip of loop of Henle in papilla; C, distal convoluted tubule near macula densa, and D, near entry to collecting duct; E, papillary urine; and F, blood in the capillaries near tip of the papilla. O. S. outer stripe, I. S. inner stripe of the outer zone of the medulla. The figures are nominal estimates of osmotic pressure (in mOsm/kg. water) given by Gottschalk and Mylle.

the *vasa recta* continue to serve as contercurrent exchangers. In this hypothesis, however, the urine at all points around the loop should be hypo-osmotic to the blood in consequence of the removal of sodium, and the recent and contrary demonstration by Gottschalk and Mylle[9] that the urine at the tip of the loop is actually isosmotic with the urine negates this otherwise attractive theory.

The interpretation proposed by Gottschalk and Mylle[9], which fits the facts recited to this point, is that sodium chloride reabsorption begins in the ascending limb of the thin segment (Figure 8) and continues in the thick segment of the ascending limb and throughout the distal convolution. The entire ascending limb (thin and thick segments) is conceived to be relatively impermeable to water, thus assuring delivery of a dilute urine to the distal convolution. (The fact that infusion of NaCl increases the degree of hypotonicity of the distal urine is strong evidence that the loop does in fact reabsorb sodium at some more proximal region, and that it operates on sodium rather than on water *per se*.) As a working assumption, the descending thin segment is conceived to be permeable to water (and possibly sodium chloride), as was implied by Wirz[49]*; by this postulate the descending urine will come into osmotic (and sodium?) equilibrium with the interstitium, the system will serve as a true countercurrent multiplier, and the osmotic pressure of the interstitium will increase along the axis of the loop to reach a maximal value at the tip, or the "reversal point", which we may take to be the point where the water- and sodium-permeable descending limb gives way to water impermeability and active sodium reabsorption in the ascending limb. Again, of course, passive diffusion of water out of the collecting ducts serves to concentrate the urine, and the *vasa recta* (right of Figure 8), being in free interchange with the interstitium, serve as countercurrent exchangers, promoting the efficiency of the system and carrying away the sodium chloride reabsorbed locally and the water abstracted from the urine**.

This is essentially the hypothesis formulated by Wirz in 1956[48, 49]

---

* The question whether the descending and ascending thin segments are permeable to urea raises some difficult quantitative problems which can perhaps be answered only by micropuncture and direct analysis.

**Maximal concentrating effect will be achieved when flow through the collecting ducts is small relative to flow through the loop, when local sodium reabsorption is relatively large, and when blood flow through the *vasa recta* is relatively small.

It may be noted that though anastomoses never connect the descending and ascending limbs of the nephron, lateral anastomotic channels frequently connect the two limbs of the *vasa recta*[9].

but now spelled out in more detail and supported by direct analysis of the urine at the tip of the loop of Henle—the all-important datum required to substantiate a countercurrent hypothesis. Neither here nor in previous theories is there any requirement that the tubular or vascular channels be physiologically juxtaposed because lateral diffusion over microscopic distances can effect sodium and water equilibration.

I still do not like it: it seems extravagant and physiologically complicated—though so is the whole glomerular filtration-tubular reabsorption pattern. Only about one in seven nephrons in the human kidney have long loops, and not all of these have an extended thin segment in the inner medulla because many turn in the outer zone; the remainder are so-called cortical nephrons which at best reach the outer zone of the medulla, while many, perhaps most, turn in the cortex and in these the thin segment is very short or entirely absent[30]. In the dog, however, all the nephrons have long loops and long thin medullary segments[39]; yet the difference in concentrating power between dog and man subsisting on a mixed diet is not equally impressive. 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.

But the problem may be more—or less—complicated than this.

Jarausch and Ullrich[16] have inserted microcatheters up the collecting ducts in the hamster kidney and examined the urine at two different levels in these ducts. Hilger, Klümper and Ullrich[14] confirm that the osmotic concentration increases along the length of the ducts; but more importantly, by reference to the concentration of inulin, they conclude that the ducts also actively absorb sodium from the urine*. These and other observations[43] lead these investigators to dismiss the thin segment as playing only a passive role in the concentrating mechanism—in effect they treat it as freely permeable to water, sodium chloride, urea, and presumably other solutes in the interstitium. They suggest that the "initial concentrating effect" in the concentrating operation is the reabsorption of sodium chloride in the thick segment of the ascend-

* The ducts also participate in K^+:Na^+ ion exchange, according to Hilger, Klümper and Ullrich's observations, and in NH_4^+ secretion[11].

ing limb (outer medulla) whereby the urine is diluted and the interstitium is enriched osmotically. Sodium reabsorption by the collecting ducts also enriches the sodium content of the inner medulla, a process which they call the "terminal concentrating effect". These two operations, they believe, suffice to concentrate the urine.

This interpretation has the advantage of shifting active sodium reabsorption out of the thin segment, but it presents several difficulties. If we assume that, at any specified level, the urine and interstitium are approximately in osmotic equilibrium, relatively more sodium than water must be removed from the urine in the collecting ducts if the interstitial osmotic concentration is to be increased, and this operation alone would serve to dilute rather than concentrate the urine inside the duct; it is difficult to see how transportation of sodium and/or water along the medullary axis in either direction, whether in the permeable thin segment or the *vasa recta*, could change the algebraic sign of this net operation. Second, it is not clear how sodium reabsorption by the thick segment and the collecting duct together could lead to an osmotic (and sodium) gradient which increases along the axis of the loop from the outer medulla to the papilla unless sodium reabsorption by the collecting duct is of some very large order of magnitude.

Assuming that the progressive increase in sodium (and chloride) concentration down the long axis of Henle's loop finds a satisfactory explanation in a countercurrent system, some subsidiary postulates may be required to explain the roughly parallel progression in concentration of exogenous creatinine, albumin, and perhaps amino acids. Inasmuch as the hair-pin pattern of the *vasa recta* affords a countercurrent exchanger, it is conceivable that the more rapid diffusion of water molecules between the tops of the capillary loop might create a sump in which more slowly diffusing solutes are dynamically trapped and held by continuous recirculation*. But this is very speculative, and in any

* Gottschalk and Mylle[9] suggest dynamic trapping to explain the increase in red cells observed in the *vasa recta* at the tip of the papilla; their reference here, however, is to the movement of protein-free fluid between the capillaries in compliance with the difference in plasma protein osmotic pressure, rather than to water molecules. But when they speak of the trapping of sodium, urea, and other diffusible solutes in the medulla, differential molecular diffusion seems to be implied.

All assumptions concerning permeability of the descending limb of the loop of Henle (or of the collecting ducts) to exogenous creatinine come into conflict with the observed osmotic U/P ratio in the papilla, the observed fraction of filtered water (*ca.* 1/8th) normally available for water diuresis in the dog (and man), and the observed constancy of the creatinine/inulin clearance ratio (1.0) in the dog, and are best avoided at this stage of the theory. This presents only one of several unresolved obscurities.

case it will not explain the fact that, relative to the cortex, urea is concentrated in the medulla to a much greater extent than the other substances studied.

This high papillary concentration of urea finds its explanation in the recent evidence presented independently by Levinsky, Davidson and Berliner[23, 24] and Klümper, Ullrich and Hilger[18] that in the hydropenic animal the collecting ducts are relatively permeable not only to water but also to urea. When urine is collected from the renal pelvis in the dog (avoiding diffusion across the ureters and bladder [see footnote p. 306*] the urea concentration (μmol/ml.) in the inner medulla ranges from 60 to 90 per cent of the concentration in the urine, and changes in the latter are invariably reflected by changes in the former in a variety of conditions (reduced filtration rate, low protein diet, mannitol and urea diuresis). This has the extraordinary consequence that the medullary urea and urine urea almost balance each other osmotically; in assessing the intrinsic osmotic operation of the loop of Henle some 60 to 90 per cent of the urine urea (and the osmotic pressure attributable thereto) must be ignored—in other words, correspondingly less osmotic work is required for the excretion of urea, and a given osmotic load of urea entails a correspondingly smaller excretion of water in the urine than does an equal osmotic load of sodium chloride, mannitol, etc., to which the collecting ducts are impermeable. Hence it was (as Berliner and his colleagues emphasize) that Gamble, McKhann, Butler and Tuthill[4] in 1934 noted the "remarkable economy in renal function referable to urea".

Three attractive features of the countercurrent hypothesis have been noted by all investigators. First is the fact that the urine is concentrated by an osmotic gradient down the long axis of the loop, with only a small gradient across any one cell layer in the loop. Second, the hypothesis dispenses at long last with the active transport of water molecules—the entire operation is mediated by the active transport of sodium chloride, a process that to one degree or another is going on throughout the length of the nephron. The third attractive feature is that, for the same mechanism (sodium chloride reabsorption) to serve either to concentrate or to dilute the urine, only a redefinition of the locus of action of the antidiuretic hormone (ADH) may be required.

It is widely accepted that the action of ADH is "permissive", in that

it simply promotes passive osmotic equilibration between the tubular urine and the blood by increasing the permeability of the "tubular" epithelium to water. Sawyer[31] and Wirz[49] have independently attributed this action to the opening of hypothetical "pores" which facilitate the diffusion of water, in a manner analogous to the action of the neurohypophysial water-balance principle on amphibian skin, as demonstrated in 1952 by Koefoed-Johnsen and Ussing[19]. Without specific reference to this "pore" theory, Ginetsinsky, Ivanova, Sax and Titova[5, 6] of the Institute of Evolutionary Physiology, Academy of Sciences, Leningrad, USSR, have suggested that ADH liberates hyaluronidase in the tubules and that this enzyme depolymerizes the hyaluronic acid cement between the epithelial cells and lets water pass through.

That ADH has no action on water reabsorption in the proximal convoluted tubule has been demonstrated by Wirz[49] and Gottschalk and Mylle[9]. These investigators have also demonstrated that in the early distal convoluted tubule the urine remains osmotically dilute (osmotic U/P ratio = 0.3 to 0.6) even in the hydropenic state, showing that ADH has no major action on the permeability to water of the ascending limb of Henle's loop or on the early distal convoluted tubule itself*. (Action on the descending limb of Henle's loop, a possibility suggested by Wirz, has not been excluded.)

It has been noted that during antidiuresis osmotic equilibration between urine and blood is attained from the middle of the distal convoluted tubule on, the urine remaining isosmotic until it enters the collecting duct. In water diuresis, however, the urine remains dilute throughout the distal convolution; and, in fact, both Wirz[49] and Gottschalk and Mylle[9] find that it appears to be further diluted between the end of the distal tubule and the bladder. Though these observations comprise "spot" anatomical tests and cannot exclude the mixing of urine of different composition from various nephrons, further dilution in the collecting ducts may reflect active sodium reabsorption in these ducts. One fact is clear, however, and that is that in water diuresis the collecting ducts are relatively impermeable to water.

The simplest interpretation of these data is to suppose that the locus of (the "pore") action of ADH extends from the early part of the distal convoluted tubule all the way down the collecting ducts[2, 9, 14, 31, 49].

* del Greco and de Wardener,[3] Berliner and Davidson[1] and Kleeman, Maxwell and Rockney[17] have shown that the concentrating mechanism continues to operate (though at an undetermined level) in the absence of ADH. However, Gottschalk and Mylle[9] suggest that ADH may somehow promote the activity of this mechanism.

Hence, in the absence of ADH all (or nearly all) the osmotically free water generated by the reabsorption of solutes – primarily sodium chloride – in the loop of Henle and the distal segment remains to be delivered to the collecting ducts; since these are relatively impermeable to water in the absence of ADH and therefore isolated from the hyperosmotic medulla, this free water emerges from the kidney as a copious volume of dilute urine.

Here I must pause to express my admiration for the painstaking and resourceful investigations that have bridged the gulf between 1950 and the present position of renal physiology. And I happily add that in this presentation I have had the good fortune of correspondence with Henry Wirz and access to unpublished manuscripts by Dr. Gottschalk, Dr. Berliner and their colleagues, as well as the opportunity for numerous personal discussions, for all of which I am deeply grateful.

I was not in so favorable a position last spring when I received a letter which started about as follows:

"Dear Dr. Smith: I am one of a handful of students in the first year class of Harvard Medical School who are confused about the Wirz, Hargitay and Kuhn countercurrent theory, and wonder why you fail to mention it in your *Principles* [of 1956]. . . ."

In a brief, elementary work one hews as narrowly as possible to the line of close-to-heavenly certitude, and I could have best replied:

"Dear Mr. Davidson: I am also confused. . . ."

Today I would answer that I believe that a countercurrent system—probably a multiplier—operative in the loop of Henle is here to stay; but the basic postulations with respect to the physiological activities of various segments, not to mention issues that arise from comparative anatomy, pathological physiology, embryology, and what-not, are still a little confusing . . . and so 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.

When St. Peter interrupted him, our renologist was about to say, "—but in 1956 Smith drew the nephron in a straight line!" Of course this weak appeal to authority would have availed nothing before the Great Empiricist, but I promise you that Smith will not draw it that way again—even if he has only the foggiest notion of how it should be drawn!

REFERENCES

1. Berliner, R. W. and Davidson, D. G. Production of hypertonic urine in the absence of pituitary antidiuretic hormone, *J. clin. Invest. 36:*1416-27, 1957.
2. Berliner, R. W., Levinsky, N. G., Davidson, D. G. and Eden, M. Dilution and concentration of the urine and the action of antidiuretic hormone, *Amer. J. Med. 24:*730-44, 1958.
3. del Greco, F. and de Wardener, H. E. The effect on urine osmolarity of a transient reduction in glomerular filtration rate and solute output during a "water" diuresis, *J. Physiol. 131:*307-16, 1956.
4. Gamble, J. L., McKhann, C. F., Butler, A. M. and Tuthill, E. An economy of water in renal function referable to urea, *Amer. J. Physiol. 109:*139-54, 1934.
5. Ginetsinsky, A. G. and Ivanova, L. N. The role of system hyaluronic acid—hyaluronidase in the process of water reabsorption in the renal tubules, *Doklady Akademii Nauk SSSR 119:* 1043-45, 1958.
6. Ginetsinsky, A. G., Sax, M. G. and Titova, L. K. The mechanism of the action of the antidiuretic hormone, *Doklady Akademii Nauk SSSR 120:* 216, 1958. (See also *Nature 182:*1218-19, 1958.)
7. Glimstedt, G. Quantitatif histotopochemische Untersuchungen über die Nieren, *Z. mikr.-anat. Forsch. 52:*335-58, 1942.
8. Gottschalk, C. W. and Mylle, M. Evidence that the mammalian nephron functions as a countercurrent multiplier system, *Science 128:*594, 1958.
9. Gottschalk, C. W. and Mylle, M. Micropuncture study of the mammalian urinary concentrating mechanism: evidence for the countercurrent hypothesis, *Amer. J. Physiol. 196:*927, 1959.
10. Hargitay, B. and Kuhn, W. Das Multiplikationsprinzip als Grundlage der Harnkonzentrierung in der Niere, *Z. Elektrochem. 55:*539-58, 1951.
11. Hargitay, B., Kuhn, W. and Wirz, H. Eine mikrokryoskopische Methode für sehr kleine Lösungsmengen (0,1 -1γ), *Experientia 7:*276-78, 1951.
12. Henle, F. G. J. *Handbuch der systematischen Anatomie des Menschen,* 3 vols., F. Vieweg u. Sohn, Braunschweig, 1855-71. (See vol. 2, 1862, pp. 300-305).
13. Henle, F. G. J. Zur Anatomie der Niere, *Abhandl. d. Königl. Gesellsch. d Wissensch. zu Göttingen 10:*223, 1862. Bound copy, B P 579, N. Y. Acad. Med. Library. pp. 1-34.
14. Hilger, H. H., Klümper, J. D. and Ullrich, K. J. Wasserrückresorption und Ionentransport durch die Sammelrohrzellen der Säugetierniere, *Pflüg. Arch. ges. Physiol. 267:*218-37, 1958.
15. Irving, L. and Krog, J. Temperature of skin in the arctic as a regulator of heat, *J. appl. Physiol. 7:*355-64, 1955.
16. Jarausch, K. H. and Ullrich, K. J. Zur Technik der Entnahme von Harnproben aus einzelnen Sammelrohren der Säugetierniere mittels Polyäthylen-Capillaren, *Pflüg. Arch. ges. Physiol. 264:*88-94, 1957.
17. Kleeman, C. R., Maxwell, M. H. and Rockney, R. Production of hypertonic urine in humans in the probable absence of antidiuretic hormone (ADH), *Proc. Soc. exp. Biol. Med. 96:*189-91, 1957.
18. Klümper, J. D., Ullrich, K. J. and Hilger, H. H. Das Verhalten des Harnstoffs in den Sammelrohren der Säugetierniere, *Pflüg. Arch. ges. Physiol. 267:*238-43, 1958.
19. Koefoed-Johnsen, V. and Ussing, H. H. The contributions of diffusion and flow to the passage of D_2O through living membranes. Effect of neurohypophyseal hormone on isolated anuran skin, *Acta physiol. scand. 28:*60-76, 1953.
20. Krakusin, J. S. and Jennings, R. B. Radioautographic localization of Na^{22} in the rat kidney, *A.M.A. Arch. Path. 59:*471-86, 1955.
21. Lassen, N. A., Longley, J. B. and Lilienfield, L. S. Concentration of albumin in renal papilla, *Science 128:*720-21, 1958.
22. Levinsky, N. G. and Berliner, R. W. Changes in composition of the urine in ureter and bladder at low urine flow,

Amer. J. Physiol. In press.

23. Levinsky, N. G. and Berliner, R. W. The role of urea in the urine concentrating mechanism, *J. clin. Invest. In press.*
24. Levinsky, N. G., Davidson, D. G. and Berliner, R. W. Effects of reduced glomerular filtration on urine concentration in the presence of antidiuretic hormone, *J. clin. Invest. 37*:910-11, 1958.
25. Lilienfield, L. S., Rose, J. C. and Lassen, N. A. Diverse distribution of red cells and albumin in the dog kidney, *Circulat. Res. 6*:810-15, 1958.
26. Ljungberg, E. On the reabsorption of chlorides in the kidney of the rabbit, *Acta med. scand., suppl. 186*, 1947.
27. Maffly, L. H. and Leaf, A. Water activity of mammalian tissues, *Nature 182*:60-61, 1958.
28. Martin, H. and Kuhn, W. Multiplikationsverfahren zur Spaltung von Racematen, *Z. Elektrochem. 47*:216, 1941.
29. Martin, H. and Kuhn, W. Multiplikationsverfahren zur Trennung von Gasgemischen, insbesondere bei Anwendung von Schwerefeldern, *Z. phys. Chem. 189*:219-316, 1941.
30. Peter, K. *Untersuchungen über Bau und Entwickelung der Niere*, Jena, Gustav Fischer, I. 1909, II. 1927.
31. Sawyer, W. H. The antidiuretic action of neurohypophysial hormones in Amphibia. *The Neurohypophysis* (Colston Papers), H. Heller, editor. London, Butterworth's Scientific Publications, 1957, pp. 171-82.
32. Scholander, P. F. Secretion of gases against high pressures in the swimbladder of deep sea fishes. II. The rete mirabile. *Biol. Bull., Wood's Hole, 107*: 260-77, 1954.
33. Scholander, P. F. Evolution of climatic adaptation in homeotherms, *Evolution 9*:15-26, 1955.
34. Scholander, P. F. "The wonderful net," *Sci. Amer. 196*:96-107, April, 1957.
35. Scholander, P. F. and Krog, J. Countercurrent heat exchange and vascular bundles in sloths, *J. appl. Physiol. 10*: 405-11, 1957.
36. Scholander, P. F. and Schevill, W. E. Counter-current vascular heat exchange in the fins of whales, *J. appl. Physiol. 8*:279-82, 1955.
37. Schweigger-Seidel, F. *Die Nieren des Menschen und der Säugethiere in ihrem feineren Baue*, Halle, 1865.
38. Smith, H. W. *From fish to philosopher.* Boston, Little, Brown and Co., 1953.
39. Sperber, I. Studies on the mammalian kidney, *Zool. Bidr. Uppsala 22*:249, 1944.
40. Ullrich, K. J., Drenckhahn, F. O. and Jarausch, K. H. Untersuchungen zum Problem der Harnkonzentrierung und -verdünnung, *Pflüg. Arch. ges. Physiol. 261*:62-77, 1955.
41. Ullrich, K. J., Hilger, H. H. and Klümper, J. D. Sekretion von Ammoniumionen in den Sammelrohren der Säugetierniere, *Pflüg. Arch. ges. Physiol. 267*:244-50, 1958.
42. Ullrich, K. J. and Jarausch, K. H. Untersuchungen zum Problem der Harnkonzentrierung und Harnverdünnung, *Pflüg. Arch. ges. Physiol. 262*: 537-50, 1956.
43. Ullrich, K. J. and Pehling, G. Aktiver Natriumtransport und Sauerstoffverbrauch in der äuszeren Markzone der Niere, *Pflüg. Arch. ges. Physiol. 267*: 207-17, 1958.
44. Walker, A. M., Bott, P. A., Oliver, J. and MacDowell, M. C. The collection and analysis of fluid from single nephrons of the mammalian kidney, *Amer. J. Physiol. 134*:580-95, 1941.
45. Wesson, L. G., Jr., Anslow, W. P., Jr. and Smith, H. W. The excretion of strong electrolytes, *Bull. N. Y. Acad. Med. 24*:586-606, 1948.
46. Wirz, H. Der osmotische Druck des Blutes in der Nierenpapille, *Helv. physiol. acta 11*:20-29, 1953.
47. Wirz, H. The production of hypertonic urine by the mammalian kidney, *The Kidney. Ciba Foundation Symposium.* Boston, Little, Brown and Co., 1954, pp. 38-49.
48. Wirz, H. Der osmotische Druck in den corticalen Tubuli der Rattenniere, *Helv. physiol. acta 14*:353-62, 1956.
49. Wirz, H. The location of antidiuretic action in the mammalian kidney. *The Neurohypophysis* (Colston Papers), H. Heller, editor. London, Butterworth's Scientific Publications, 1957, pp. 157-69.
50. Wirz, H., Hargitay, B. and Kuhn, W. Lokalisation des Konzentrierungsprozesses in der Niere durch direkte Kryoskopie, *Helv. physiol. acta 9*:196-207, 1951.