THE MECHANISMS OF URINARY CONCENTRATION AND DILUTION AND THE ACTION OF THE ANTIDIURETIC HORMONE

A Review of the Literature and Modification of the Countercurrent Hypothesis Based on Electron Microscopic Studies

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MORE than a century ago, Claude Bernard first pointed out that the true medium in which we live is neither air nor water, but the fluid that bathes all the tissue cells. The osmotic pressure of this extracellular fluid, and presumably of the cells they bathe, is carefully guarded at values of 280 to 300 mOsm./kg. water. The cellular milieu of the human kidney, however, must somehow cope with the fluid contents within its tubules, the osmolality of which ranges from under 50 to over 1,300 mOsm./kg. water, between the extremes of maximum diuresis and antidiuresis that is from roughly one-sixth to over four times the osmotic pressure of the plasma.

The flexibility of renal function has understandably been subjected to considerable experimental scrutiny and various conclusions have been deduced and formulated into hypotheses to explain the mechanisms of dilution and concentration of the urine. In reviewing the historical development of the various theories it is interesting to note how often the concentrating function of the kidney has been linked to the loop of Henle. Such a relationship was suggested in 1909 by Peter who noted a correlation between the maximal concentrations of the urine achieved by various mammals and the lengths of the thin segment of the loop of Henle in their kidneys. In 1927 Crane pointed out that only mammals and birds can form concentrated urine and that it is only in these phyla that thin segments of the loops of Henle occur. Closely related to this observation were the experiments of Burgess, Harvey and Marshall in 1933 which showed that it was only in birds and mammals that antidiuretic hormone increased the tubular reabsorption of water. In fact, on the basis of these experiments Burgess and his co-workers were led to formulate the almost prophetic hypothesis that the urine was concentrated

in the loop of Henle and that the antidiuretic hormone acted upon this segment of the nephron.

This hypothesis, however, seemed to be invalidated by the classic studies of Walker, Bott, Oliver and MacDowell in 1941, which demonstrated that the tubular urine obtained by micropuncture from the distal convolution of the nephron of the rat was at most isosmotic and was certainly not hyperosmotic as would be expected if the final concentrating operation occurred in the loop of Henle. An alternative mechanism for concentrating the urine was then suggested by Homer Smith and his co-workers Wesson and Anslow (Smith, 1951, 1952; Wesson and Anslow, 1952; Wesson, Anslow and Smith, 1948); until quite recently this was the most popular and the most plausible theory of the mechanisms of urinary concentration and dilution.

Utilizing micropuncture and microanalytic techniques, Walker and his colleagues (1941) established that the fluid in Bowman's capsule was an ultrafiltrate with respect to osmotic pressure, electrical conductivity, pH, glucose, creatinine and electrolytes. At the end of the proximal tubule:

- (a) All the glucose and phosphate of the glomerular filtrate had been reabsorbed.
- (b) The volume of the glomerular filtrate had been reduced by 80%.
- (c) The creatinine concentration had increased almost fivefold.
- (d) The *total* osmotic pressure remained equal to that of the plasma.

Since creatinine is neither secreted nor reabsorbed from the tubules, intraluminal concentration of this substance can be achieved only by removal of water. Net osmotic activity, however, did not change; reabsorption of water, therefore, must have been accompanied by an amount of solute in such proportion as to maintain isotonicity between the reabsorbate, the tubular fluid and the plasma. Smith and his co-workers (Wesson and

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Anslow, 1952; Wesson, Anslow and Smith, 1948; Smith, 1947; Wesson and Anslow, 1948) by a series of ingenious experiments, proved that sodium chloride is the main solute which is actively reabsorbed by the proximal tubules, carrying with it an isosmotic amount of water. The net accomplishment of operations within the proximal tubules is to return to the circulation the bulk of the filtered water and solutes. This process of 'routine conservation' reduces the volume of the glomerular filtrate by 80 to 85%.

Smith and his co-workers discarded the view that the loop of Henle was the site of urinary concentration. The previous finding by Walker and others (1941) in three punctures of the distal tubule of concentrating rat kidneys that the fluid therein may be somewhat hypotonic to plasma was thought to indicate that the filtrate that has already traversed the loop of Henle is not concentrated within the loop. According to Smith's theory, the active process of primary movement of sodium in the proximal tubule might slightly outstrip the passive reabsorption of water, rendering the fluid hypotonic; the thin limb composed of epithelium permeable to water would allow diffusion of water so that isotonicity could be re-established.

The processes of glomerular filtration, proximal reabsorption and equilibration in the loop of Henle, resulting in an isosmotic fluid, seem to be fixed and, in an osmotic sense, unselective. To the distal tubule and collecting duct, therefore, in Smith's hypothesis, are detailed the discriminatory mechanisms which determine the ultimate concentration or dilution of the urine, an operation which Smith calls 'facultative reabsorption', mediated largely by the influence of antidiuretic hormone (ADH) of the neurohypophysis; and implying, unlike the 'obligatory reabsorption' in the proximal system, that movements of solute and water are not always isosmotic. The isosmotic fluid in the distal system can be rendered hypotonic by the active removal of solute without water from the tubular fluid. The epithelium of the distal tubule in this theory, in the absence of any activity of ADH, i.e. in water diuresis or in diabetes insipidus, is virtually waterproof and so water is restrained from following in the wake of the actively transported sodium. For the first time, then, water is retained in the tubular lumen without being completely obligated by an equivalent amount of an osmotically-active solute.

Under conditions of dehydration, or under the influence of ADH, to elaborate a hypertonic urine from an isotonic filtrate there must be an abstraction of water without solute. Smith preferred to locate the concentrating site in the collecting ducts. Production of hypertonic urine, therefore, may be conceived as occurring in two phases. The first,

an 'isosmotic-making phase', is the passive diffusion of osmotically-free water liberated by the active reabsorption of sodium, under direct control of ADH, to raise its concentration to that of the plasma. The second phase, the 'hyperosmotic-making process', is the further reabsorption of an additional amount of pure water from this residual isotonic fluid, rendering the tubular contents hypertonic to plasma. It is apparent that the movement of water during this latter operation must take place against an osmotic gradient. Since there seemed to be no reasonable alternative, this final abstraction of water necessary to produce hyperosmotic urine was thought to involve an active transport of water.

This hypothesis of Smith and his colleagues was widely accepted and stimulated a great deal of clinical and physiological work. The hypothesis, however, postulated the active transport of water and this posed considerable conceptual problems. The favourite model for active transport processes generally consists of a biochemical pump which handles molecules or ions of the transported substances one by one and which is driven by metabolic forces. With water, the energy consumption of the hypothetical pump would exceed by several orders of magnitude that predicted from the rate of oxygen consumption.

In 1955, Brodsky, Rehm, Dennis and Miller demonstrated the thermodynamic impossibility of the theory of active water reabsorption. This group concluded that the energy required to produce a concentrated urine by active water reabsorption would be 1,000 times the capability of the tubular cells. Clearly it was time to search for another mechanism to concentrate the urine; fortunately, such a mechanism had already been suggested.

The Counter-current Hypothesis

In 1951, a bold new theory had been suggested by Wirz, Hargitay and Kuhn, all of the University of Basel to explain the mechanism whereby the kidney concentrates the urine. These investigators saw in the structural arrangement of the loop of Henle the possibility of the operation of a counter-current multiplier system.

The basic feature of a counter-current system is that the juxtaposition of two streams moving in opposite directions multiplies small exchanges of energy or material between them. The renal medulla has a unique spatial configuration as a result of the anatomical orientation of the structures within it, which provides a virtual battery of counter-current systems arranged in parallel (Fig. 1). The descending and ascending limbs of the loops of Henle constitute one such system, and the arterial and venous limbs of the vasa recta,

FIG. 1.—Diagram showing a superficial cortical, a juxtamedullary and an intermediate nephron. Note that the medulla consists of a battery of counter-current systems derived from the loops of Henle and the vasa recta of the juxtamedullary nephrons.

intermingling with and parallel to the loops of Henle, form another. Hargitay and Kuhn (1951) suggested that the mechanism of concentration of the urine is based on the presence of a long tube that bends back on itself, so that flows in the two limbs are opposite in direction (hairpin-counter-current), combined with some active process that creates a small concentration-difference between the two limbs, the descending being slightly hypertonic to the ascending.

This initial concentration-difference between the two limbs could be created if sodium were actively transported from the lumen of the *ascending* limb to the interstitial tissue by the tubular cells, and water were to move passively from the lumen of the *descending* limb as the result of the slight hypertonicity of the interstitial tissue maintained in this way. These movements of sodium and water will maintain the concentration of the contents of the descending limb higher than those of the ascending: as the former move round to replace the latter this effect will be continuously

multiplied until it produces high concentrations in both limbs.

Continuous action of such a mechanism will ultimately result in a steady state; the fluid within the system will become progressively more concentrated towards the hairpin-bend and rediluted on its way back up the ascending limb, resulting in an osmotic stratification along the long axis of Henle's loop. The result of the counter-current mechanism in the loops would be a zone of increasing hypertonicity towards the tip of the renal papilla. Concentration of the final urine was then considered to occur in the collecting ducts through a passive movement of water from the fluid in them as they traverse the progressively more hypertonic medullary interstitial fluid. That the final concentration of the urine occurs in the collecting ducts is in accord with the classical theory of Smith: the disagreement concerns the mechanism by which water is removed and the role played by the loop of Henle.

Experimental evidence for this theory was

FIG. 2.—Diagram representing the most widely accepted version of the counter-current mechanism as it is believed to operate in a nephron with a long loop during the processes of concentration and dilution of the urine; this is based largely on the views of Wirz (1957) and Gottschalk and Mylle (1959). The numbers represent hypothetical osmolality values. No quantitative significance is to be attached to the number or thickness of the arrows and only net movements are indicated. The active sodium transport by the epithelium of the collecting tubule is based on the work of Hilger, Klumper and Ullrich (1958), and the urea transport on the work of Lassiter, Gottschalk and Mylle (1961). Note that in this hypothesis, the distal convoluted and collecting tubules are considered to be impermeable to the passive movement of water in the absence of the antidiuretic hormone while the counter-current mechanism proceeds equally in both the concentrating and the diluting nephron.

presented in 1951 by Wirz, Hargitay and Kuhn. They showed by microcryoscopy on 30 μ -thick tissue slices cut perpendicularly to the axis of the papilla, that the osmotic pressure of the fluid in the cortical tubules is the same as that of the blood and that it increases in the medulla continuously to the tip of the papilla. This approach revealed the kidney to be composed of concentric shells, each of uniform osmotic concentration.

Another valuable approach has been afforded by microanalysis of cortical and medullary slices for specific solutes. Ullrich and co-workers (Ullrich, Drenckhahn and Jarausch, 1955; Ullrich and Jarausch, 1956) have shown in hydropenic dogs that the concentration of sodium increases progressively from the cortex to the tip of the papilla.

A third line of evidence consists of measurement of the osmolality of fluid obtained by micropuncture. It was demonstrated that fluid obtained by micropuncture from the proximal convoluted tubule was isosmotic with peripheral plasma, that the fluid obtained from the loop bend (Gottschalk and Mylle, 1959) or superficial capillaries of the papilla (Wirz, 1953) in hydropenic animals was markedly hyperosmotic, while the fluid leaving

the loop, in the early part of the distal convoluted tubule, was hyposmotic (Gottschalk and Mylle, 1959; Wirz, 1956).

The most attractive feature of the counter-current hypothesis is that it dispenses with the active transport of water molecules, the entire operation being mediated by the active transport of sodium, a process that to one degree or another is going on throughout the length of the nephron. Another attractive feature is that for the same mechanism (sodium reabsorption) to serve either to concentrate or to dilute the urine, only a redefinition of the locus of action of the antidiuretic hormone may be required.

It is widely accepted that the action of ADH is to increase the permeability of the tubular epithelium to water. This action was attributed to the opening of hypothetical 'pores' which facilitate the diffusion of water (Koefoed-Johnsen and Ussing, 1953; Sawyer, 1957). 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 (Gottschalk and Mylle, 1959). Hence, in the absence of ADH, all, or nearly all, the osmotically-free water generated

by the reabsorption of sodium 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. No direct proof has been presented by these workers for this assumption about the site and mode of action of ADH (Smith, 1959).

Renal physiologists seem to have largely exhausted their methods and special techniques of investigation of this problem and they are unlikely to get much more data. It seems probable that the present methods of microcryoscopy and microanalysis of thin renal slices or micropuncture of the renal tubules and capillaries, however ingenious and accurate, are unlikely to yield much more information. The renal tubules in the outer zone of the medulla, for instance, are deeply seated and inaccessible to micropuncture. Similarly, microcryoscopy and microanalysis of thin slices of renal tissue, though very valuable in giving an overall picture of the osmolality and chemical composition at a particular level, are unable to discriminate between the minor differences in the adjacent tubules at that particular level.

One of the oldest methods adopted by biologists and research workers for the study of physiology is to correlate function and morphology. However, the artifacts encountered in such forms of study were so great that they obviated the use of this method of approach using light microscopic studies. However, at the molecular level attained by electron microscopy, chemistry and morphology become one and the histologist and chemist meet on common ground.

Approaching the problem of the mechanisms of urinary concentration and dilution and the site of action of the antidiuretic hormone by electron microscopic study, Sabour, MacDonald, Lambie and Robson (1964) examined the kidneys of the following groups of rats by the electron microscope at the height of diuresis or antidiuresis:

1. Hydrated rats, forcibly given 5 or 20 ml. water.
2. Dehydration experiment where rats were dehydrated for 24 or 48 hours.
3. Hydration followed by dehydration experiment.
4. Pitressin experiment where rats were given 50 milliunits aqueous pitressin i.v. and were killed when the urine passed showed an osmolality above 2,000 mOsm./kg.

The most striking change found in this study was marked thickening of the basement membrane of the descending limb of the loop of Henle in water diuresis whether this limb is the pars recta

FIG. 3.—The basal parts of two pars recta of proximal tubules with an intervening capillary. The basement membranes are very markedly swollen. From a forcibly hydrated rat at the height of the water diuresis. ($\times 5,700$).

FIG. 4.—Markedly thickened basement membrane of a thin segment of a loop of Henle in the inner medulla of a hydrated rat at the height of the diuresis. The adjacent efferent capillary looks normal and its basement membrane is of average thickness. ($\times 30,000$).

of the proximal tubule (Fig. 3) or the thin descending segment (Fig. 4). On the other hand, this basement membrane was found to be very thin, as

FIG. 5.—Thin segment of a loop of Henle from a dehydrated animal. The basement membrane is of normal thickness and the basal cell foldings are deep and complex. ($\times 15,500$).

FIG. 6.—The basement membrane of this thin segment from a pitressin experiment is of normal thickness. ($\times 6,500$).

thin as the basement membrane of the rest of the nephron, in dehydrated animals and in animals to whom pitressin has been administered (Figs. 5 and 6). The collecting tubules did not show opening up of 'pores' or disappearance of the intercellular cement in dehydrated animals or those given ADH as claimed by Ginetsinskii from light-microscopic studies (Ginetsinskii, 1958). On the contrary, at the height of the water diuresis lateral separation between adjacent collecting tubule cells in the inner zone of the medulla was quite apparent (Fig. 7).

From the data obtained by micropuncture,

FIG. 7.—Two adjacent cells in an inner medullary collecting tubule showing lateral separation, but they are still adherent at the terminal bar. At the height of water diuresis. ($\times 11,000$).

microanalysis and microcryoscopy techniques, one can propose numerous variations of the counter-current hypothesis that will explain the facts that are now available. However, the electron microscopic studies referred to above have shown that the main morphological difference in the nephron between water diuresis and dehydration is in the basement membrane of the descending limb of the loop of Henle. These results do not conflict with the suggestion that the loop of Henle functions as a counter-current multiplier system. They must, however, modify the present views in the following way:—

In water diuresis, in the absence of the antidiuretic hormone, the basement membrane of the descending limb of the loop of Henle is swollen and may become relatively impermeable to water. In this way it prevents the outward movement of water from the lumen into the interstitium in response to the increased amount of sodium pumped out actively from the contents of the ascending limb into the interstitial space. By preventing osmotic equilibrium taking place in this manner, the fluid in the descending limb remains isosmotic with the plasma, and the loop will no longer multiply the osmotic difference created by active sodium transport out of the ascending limb. The interstitium at the papilla will be only very slightly hypertonic and very little water will diffuse back from the fluid flowing down the collecting ducts. In dehydration, in the presence of ADH, the basement membrane of the descending limb of Henle's loop becomes very thin and permeable to water and allows the loop to multiply the original small osmotic difference created by the activity of the cells of the ascending limb.

This modified hypothesis differs from the accepted version of the counter-current hypothesis in the following important points (Fig. 8):

1. In the hypothesis suggested here, the loop of Henle acts as a counter-current multiplier

FIG. 8.—Diagram representing the proposed hypothesis explaining the mechanisms of urinary concentration and dilution. The numbers represent hypothetical osmolality values. A semi-quantitative significance is attempted in the number and thickness of the arrows. Note that the state of the concentrating nephron is similar to that seen in the accepted version of the counter-current hypothesis. In this new hypothesis, the descending limb of the loop of Henle is considered to be impermeable to water (and possibly to urea) in the absence of the anti-diuretic hormone and therefore the loop does not function as a counter-current multiplier in the diluting nephron.

the concentrating kidney only. In the currently accepted hypothesis, the loop acts as a counter-current multiplier both in the concentrating and diluting kidney.

2. The site of action of ADH in the current hypothesis is on the distal and collecting tubules. In the one suggested here, ADH acts on the descending limb of the loop of Henle.

In addition to the morphological support given by electron microscopy to this modified hypothesis, the following physiological data, which were difficult to explain by the current hypothesis, strongly support the modification suggested:

1. Gottschalk and Mylle (1960) by puncturing the loops of Henle and vasa recta of rats with diabetes insipidus have found that the fluid there has an osmolality of about 500 mOsm./kg. water. The same investigators (Gottschalk, Lassiter, Mylle, Ullrich, Schmidt-Nielsen, Pehling and O'Dell, 1960) and others (Wirz, 1953) have found the osmolality in the loops of Henle and vasa recta blood in the concentrating kidney to be about 2,200 mOsm./kg. water. If the loop of Henle was acting as a counter-current multiplier all the time and ADH acted on the collecting tubules, the osmolality of the fluid at the tip of the loop should be about 2,200 mOsm./kg. water whether ADH was present or absent. On the other hand, if the loop acted as a counter-current multiplier in the presence of ADH only, the osmolality at the tip of

the papilla should be just above isotonicity in absence of ADH, which is what has been found in diabetes-insipidus rats.

2. Ullrich and Jarausch (1956) have shown that during water diuresis sodium and chloride are concentrated slightly in the papilla, urea very little, and creatinine not at all. This indicates that during water diuresis no water diffuses out of the descending limb of the loop of Henle into the interstitium and so creatinine is not concentrated at all. The system does not work as a counter-current multiplier and the slight increase in the concentration of sodium and chloride is due to the initial effect of active sodium transport by the ascending limb into the interstitium, an effect which is not multiplied in water diuresis. This may explain the results of Berliner and Davidson (1957), and del Greco and de Wardener (1956), who were able to produce urine of very slight hypertonicity (in the region of 500 mOsm./kg.) in the absence of ADH by slowing the rate of flow through the system, when the urine flow in the collecting tubules might be slow enough to allow equilibration with the medullary interstitium to occur.

The role of ADH in the current hypothesis is to open up 'pores' in the walls of the distal and collecting tubules. No pores have been seen to open up in these segments of the nephron in hydropenic animals nor in those to which ADH has been

FIG. 9.—Thin segment of a loop of Henle in chronic potassium depletion. The basement membrane is thick and fibrillar. ($\times 9,700$).

administered, even by electron microscopy. On the contrary, 'pores' were seen to open up by lateral separation of adjacent cells in the papillary collecting tubules at the height of water diuresis (Fig. 7). In the absence of ADH the basement membrane of the descending limb of the loop of Henle was seen to be much swollen (Figs. 3 and 4). It is suggested that in absence of ADH this basement membrane imbibes water, holds it intimately, swells, and thereby withstands a much higher pressure and hinders the further flow of water out of it. This suggestion as to what occurs in the basement membrane of the descending limb of the loop of Henle is analogous to what has been proved for structures of similar composition. Fessler (1960) has shown that Wharton's jelly, the Ranvier bulla and a synthetic complex of water and a high polymer of open structure and fibres, will imbibe water, swell, hold the water intimately in no discernible space and thereafter withstand a much higher pressure and become resistant to further flow of water. The basement membrane consists of polysaccharides, proteins, salts and water in a gel-form in a mesh of fine fibrils (Ham, 1962), and so can be expected to behave in a similar manner to the above-mentioned structures, in water diuresis, in the absence of ADH. In the presence of ADH, these changes in the basement membrane do not occur or are reversed. ADH probably affects depolymerization of the polysaccharide macromolecules in the basement membrane of the descending limb of the loop.

FIG. 10.—Thin segment of a loop of Henle in hypercalcaemia due to hyperparathyroidism. The basement membrane looks much thickened and fibrillar. ($\times 8,250$).

The experiment of dehydration after forcible hydration has shown that the changes which occur in the nephron after a physiological dose of water are completely reversible on subsequent dehydration. On the other hand, the changes that follow an unusually large water load are incompletely reversible. This might explain the clinical observation that prolonged and excessive drinking in primary polydipsia as well as in diabetes insipidus will gradually diminish the sensitivity of the kidney to ADH and will eventually lead to a permanent loss of the power of the kidney to conserve water (Barlow and de Wardener, 1959; Kleeman, Maxwell and Witlin, 1958).

The study of the mechanism of urinary concentration must not seem too detached from clinical medicine. The introduction of electron microscopy in the approach to this problem will add a very useful new tool that might clarify many obscure findings. It has already contributed in this respect. In potassium deficiency, a lesion in the basement membrane of the descending limb of Henle's loop has been reported (MacDonald, Sabour, Lambie and Robson, 1962) (Fig. 9); and in hypercalcaemia, due to hyperparathyroidism, a similar lesion in the same area was observed (Fig. 10). The investigation of the other causes of impairment of the power of concentration of the urine by the electron microscope can be expected to yield rewarding results in the near future.

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