Homeostasis, the Milieu Intérieur, and the Wisdom of the Nephron

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Abstract
The concept of homeostasis has been inextricably linked to the function of the kidneys for more than a century when it was recognized that the kidneys had the ability to maintain the “internal milieu” and allow organisms the “physiologic freedom” to move into varying environments and take in varying diets and fluids. Early ingenious, albeit rudimentary, experiments unlocked a wealth of secrets on the mechanisms involved in the formation of urine and renal handling of the gamut of electrolytes, as well as that of water, acid, and protein. Recent scientific advances have confirmed these prescient postulates such that the modern clinician is the beneficiary of a rich understanding of the nephron and the kidney’s critical role in homeostasis down to the molecular level. This review summarizes those early achievements and provides a framework and introduction for the new CJASN series on renal physiology.

Introduction
Critical advances in our understanding of renal physiology are unfolding at a rapid pace. Yet, remarkably, the lessons learned from early crude measurements and careful study still hold true; indeed, classic articles still serve as the basis for introductory textbooks on renal physiology and provide a solid working knowledge to clinicians. Drawings with just a handful of transporters at each nephron segment, known for more than half a century, are sufficient to understand basic mechanisms of autoregulation, clearance, and the effects of diuretics—the tools needed to care for patients. Yet we clinicians also benefit from a treasure trove of subsequent scientific advances, which have given us a detailed and comprehensive understanding of how the kidney maintains stable body chemistries and volume balance.

The layers of complexity and the mysteries that continue to unravel make it difficult to stay abreast of current research. Still, the modern nephrologist is in good company. In 1959, a medical student wrote to Homer Smith, the uncontested patriarch of modern nephrology at the time, to inquire about his rectilinear depiction of the nephron (Figure 1) and why he failed to mention the counter current theory in his famous 1956 textbook, the Principles of Renal Physiology (1,2). Indeed, the structure of the loop of Henle had been well known since the mid-1800s, but the importance of that eponymous structure, the gradient that it generated, and its role in the final product urine was only just elucidated at the time of the student’s correspondence. Before this, Homer Smith felt that the hairpin turn was just a vestige of embryology. This student’s missed was a curiosity, rather than a criticism. Carefully framed questions have always served to advance our understanding. In this overview, we will describe, all too briefly, the ingenious methods used by early investigators and the secrets they unlocked to help create the in-depth understanding of renal physiology and pathophysiology that we enjoy today. Additional details will follow in the new CJASN series of review articles on the physiology of the kidney.

The Milieu Intérieur and the Kidney’s Essential Role
In the early 1800s, Darwin’s Theory of Evolution combined with the recognition that the body chemistries of many disparate species were remarkably similar led Claude Bernard to develop his theory of the Milieu Intérieur: “The constancy of the internal environment is the condition of a free and independent existence” (3). By this, he meant that the ability of our ancestor organisms to leave the oceans required that they develop the ability to “carry the ocean with them” in the form of an internal ocean, bathing their cells constantly in fluids that resemble the very seas from which they evolved. This concept, although reminiscent of the notion of bodily humors (4,5), marked an enormous advance because Bernard described both the features of bodily fluids and the need to maintain that internal milieu. Maintenance of the internal milieu was first called the “wisdom of the body” by Starling (6), who recognized that organisms must maintain the constancy of this internal ocean despite great fluctuations in diet, fluid intake, and other environmental conditions. The term homeostasis was later coined by behaviorist Walter Cannon to describe the physiologic processes that, in aggregate, maintain the constancy of the internal
chemistries, as well as BP, body temperature, and energy balance (7).

The earliest insights into renal physiology came from the assiduous study of anatomy because, to a large degree, renal function follows structure. Meticulous drawings and histologic study of the animal and human kidney from William Bowman (8,9), Jacob Henle (10), and others, complete with capsule, capillaries, and convoluted tubules were available in Bernard’s time, yet the mechanisms for the formation of urine and the kidney’s role in homeostasis were not embraced until the next century. Until the 1920s, a debate raged on the mechanism of urine formation. Some researchers championed a filtration doctrine and others ascribed secretory power to both the glomerulus and tubules (11,12). The secretory theory was more popular, however, because the sheer volume of blood that would first need to be filtered and then reabsorbed by the kidney was enormous. Homer Smith noted that the filter-reabsorption strategy “seemed extravagant and physiologically complicated” (2).

**Early Investigations Form the Framework**

An interest in comparative physiology and the advent of the marine biologic laboratories that studded the Atlantic coast at the turn of the century helped frame an early understanding of the kidney and its role in evolution. Remarkably, increasingly complex fish with salty interiors adapted to fresh water, whereas amphibians rose from the sea to face the challenges mandated by scarce water. In his famed opus, Smith summarized the observations to date and waxed poetic (and philosophic), declaring, “Superficially, it might be said that the function of the kidneys is to make urine; but in a more considered view, one can say that the kidneys make the stuff of philosophy itself” (13). This impression, the sense of wonder at the intricate dealings of the kidney, permeates the scientific writings from that time to this day.

Studies performed in frogs, rabbits, and dogs in the early 1900s showed that the constituents of blood and urine differed because urine contained urea, potassium, and sodium salts, whereas blood contains protein, glucose, and very little urea. Furthermore, balance studies suggested that the volume and constituents of urine changed depending on changing intake or experimental infusions. In his “The Secretion of Urine” monograph published in 1917, Cushny summarized the available literature to date and described the brisk diuresis that followed sodium chloride infusions. He also reported findings that showed that the kidney produced acid urine in humans and the carnivora, whereas the herbivora had alkaline urine unless fed a protein diet (14). Despite this careful review and his presentation of the “modern view” that acknowledged that both filtration and secretion could exist, Cushny struggled with the data and felt that the theories were “diametrically opposed” because secretion and reabsorption would result in opposing currents along the renal epithelium. Clearly, methods were needed that could allow direct measurement of the filtrate and its modification along the nephron.

When Wearn and Richards introduced the micropuncture technique to the study of the kidney (first in amphibia, which had large renal structures amenable to manipulation), the debate on the formation of urine was resolved (11,15). By sampling the fluid elaborated from the glomerular capsule of a frog, the team demonstrated a protein-free filtrate that was otherwise similar to blood. By contrast, the frog bladder urine had a different composition from the blood and was free of glucose. These findings, in light of earlier data, supported the notion that urine is formed by glomerular filtration, and the urine is then modified in the tubules, by a combination of reabsorption and secretion. Subsequent studies by Walker and others inserted oil “plugs” or “blocks” in various segments of the nephron and distal to the sampling pipette so that the investigators could avoid contamination but still study urine from...
different segments of the nephron and, therefore, characterize each segment’s function, the ions absorbed, and the osmolarity of the fluid (16). These investigators then developed the “stop flow” technique in which they placed a pipette distal to the oil droplet, infused fluid into that segment, and then sampled the fluid at the end of that segment to determine how the fluid had been altered (Figure 2A) (17).

This meticulous work was confirmed in mammals by extension of the micropuncture technique to rat and guinea pig kidneys (Figure 2B). However, despite the ingenious use of oil blocks to prevent upstream tubular fluid from reaching downstream segments and then substituting artificial perfusates, micropuncture studies did not permit control of the composition of fluids on both sides of the tubular epithelium. This limitation was remedied after World War II, when Hans Ussing developed his famed chamber methods (Figure 3). With this strategy, transport across isolated epithelia could be studied quantitatively by systematically altering the ionic composition and voltages of the solutions on either side of the epithelium (18). Careful transport studies using model epithelia from nonmammals, including the toad bladder, the turtle bladder, and the flounder bladder, which anatomically and functionally model collecting duct principal cells, collecting duct intercalated cells, and distal tubule cells, respectively, gave important insights into transport mechanisms in these segments. In the late 1960s, Burg and colleagues developed methods for isolating and perfusing individual mammalian nephron segments, first from rabbits and then from mice. These preparations, along with the ability to measure minute quantities of ions and volumes from these tubules with ion-specific electrodes, including the picapnotherm (which measures minute quantities of carbon dioxide), permitted investigators to examine in detail the mechanisms, driving forces, and regulation of transport across individual nephron segments (19). With painstaking effort, investigators dissected tubules, perfused the segment with fluid of specific ion concentrations, and collected the “waste” fluid from the other end of the segment (Figure 4).

Figure 2. | Micropuncture and “stop flow” techniques were used to help define the role of each segment of the nephron. (A) The proximal tubule from the kidney of the aquatic salamander is illustrated here. A micropipette removes the filtrate at a point just proximal to a “plug” of mineral oil. To determine the role of the tubule in handling of individual constituents (reabsorption, secretion, or diffusion), fluid was injected into the tubule at different locations and then collected distally. This “artificial” fluid could be altered to differ from the normal filtrate by one or more constituents. (B) A sketch of a camara lucida drawing of a guinea pig nephron after microdissection these drawings were created with the aid of a light projector because photomicrographs were not readily available at the time). Oil or mercury blocks could be inserted at various points along the nephron and fluid from the lumen could be collected and studied. A is modified from reference 17, with permission; B is modified from reference 16, with permission.
This arrangement allowed investigation of individual segments of the nephron to better characterize the features of transport, electrochemical gradients, coupling with other ions, active versus passive transport, the threshold for reabsorption, and the permeability to water (1). The resulting flurry of studies, spanning nearly 2 decades, defined the phenomenology and regulation of transport, and identified, at least functionally, the transporter proteins responsible for homeostasis (20).

Meanwhile, in the clinical realm, the flame photometer became available in the late 1940s and this innovation made it possible to measure more than a dozen samples of blood for both sodium and potassium in under an hour. Before this time, electrolyte measurements were onerous and involved both chemical extractions and precipitations (21). Studies of electrolytes and the metabolic derangements were now possible; when this process was linked to an autoanalyzer that also provided chloride and total CO₂ interest in acid-base disorders soared and the concept of “Gamblegrams” flourished (22). Dr. Gamble, a disciple of Henderson, studied a range of different insults from gastrointestinal losses to advanced CKD and their effect on electrolytes, and described the kidney as the “remarkable organ of regulation, the kidney sustains the chemical structure of extracellular fluid” (23).

Later, availability of the automatic analyzers also spurred a large literature using metabolic balance studies to characterize everything from bed rest or water immersion to the effects of pharmacologic agents like chlorothiazide (24,25). In these detailed studies, investigators characterized vital signs, weight, intake, excretion, electrolytes, clearance, plasma volume, and hormonal levels. These data helped solidify the concepts of the steady state in homeostasis (26).

The stage was now set to identify specific renal transporters, describe how they function, and characterize how they are regulated. The remarkable reabsorptive task of the nephron tubules requires energy and active transport. It was not long after Nobel laureate Jens Skou’s 1957 discovery of the Na-K-ATPase in crab nerve microsomes (27,28) that this critical transporter was identified in the kidney. Because of its abundance, the enzyme was identified in crude homogenates of the renal cortex and medulla and Na-K-ATPase activity was later measured in individual segments of the nephron. The highest activity was in the thick ascending limb and distal convoluted tubule (DCT) and considerable activity was also observed in the proximal tubule. Further study helped identify the polarity of the renal epithelial cells with the Na-K-ATPase at the basolateral membrane. This finding helped solidify the concept that energy generated from this housekeeping enzyme, which maintains the normal cellular ion concentration, is harnessed by the kidney to reabsorb the bulk of the filtered sodium along with a host of other substances (29).

With a map in place for the role of each segment of the nephron and a solid understanding of factors that influence homeostasis, the next step was to identify specific renal transporters.
Study of isolated perfused tubular segments allowed study of each of the different nephron segments independently. (A) A photomicrograph of a portion of a rabbit proximal convoluted tubule during perfusion. (B) A schematic diagram of the technique. One end of the dissected tubule was connected to a micropipette, which was used to perfuse the lumen, and the other end was connected to a collection micropipette. Both the luminal fluid and the peritubular fluid could be controlled to assess tubular transport characteristics. A is reprinted with permission from Burg MB: Perfusion of isolated renal tubules. Yale J Biol Med 45: 321–326, 1972.
Sodium and Water Homeostasis

Sodium, the major extracellular cation, plays a pivotal role in the maintenance of extracellular fluid volume and perfusion of vital organs and capillary beds. The kidney has an elaborate array of sodium transporters throughout the nephron (36). In the proximal tubule, it is linked to an elegant mechanism to reabsorb the filtered bicarbonate load by excreting $\mathrm{H^+}$ ions with the electroneutral antiporers or $\mathrm{Na^+/H^+}$ exchangers. Reabsorption of the ample filtered sodium also plays an important role in the reabsorption of glucose, sulfate, phosphate, and several amino acids. The remaining fraction of filtered sodium is reabsorbed with unique transporters in each of the subsequent nephron segments in which apical reabsorption of sodium is rate limiting. These transporters include the furosemide-sensitive channel in the loop of Henle, the thiazide-sensitive sodium chloride cotransporter that is primarily in the DCT, and the epithelial sodium channel transporter that is located primarily in the collecting tubules (Figure 5).

Our understanding of the mechanisms of sodium transport along the nephron comes from disparate sources. The advent of sulfonamides, investigated initially as much-needed antibiotics after World War II, were promptly recognized for their saluretic effects and soon revealed a wealth of secrets regarding the transport of sodium throughout the nephron (37). Genetic disorders also provided important clues. Endocrinologist Frederick Bartter and others described a set of youths afflicted with growth and mental retardation, muscle cramps, salt craving, polyuria, and polydipsia. Bartter initially attributed his eponymous syndrome to a state of aldosterone excess with angiotensin resistance but when three-fourths adrenalectomy did not resolve the defect, he focused on the loop of Henle. Subsequent contributions from physiologists helped distinguish this disorder from Gitelman’s syndrome and helped define the interplay of transporters in the loop of Henle and the DCT. However, it was the stunning characterizations by geneticists that helped identify mutations in several genes; these discoveries explained the subtle differences in the phenotype of these disorders and will be carefully considered within this series (38,39).

An endocrinopathy was also the initial theory that Dr. Liddle invoked to describe a family with early onset severe hypertension and hypokalemia that was notable for suppressed renin and aldosterone. As soon as the epithelial sodium channel was characterized, investigators demonstrated complete linkage in affected individuals with a defect in this transporter that resulted in a constitutively active sodium channel (40). Insights into the molecular biology, structure, function, and regulation of each of these sodium transporters has clearly enriched our grasp of renal physiology and complemented earlier predictions.

Hormonal and sympathetic nervous input can greatly augment sodium reabsorption, particularly by angiotensin II in the proximal tubule and aldosterone in the distal nephron, whereas the effect of atrial natriuretic peptide in the medullary collecting duct was found to be the opposite (41). Knowledge of these transporters is critical to the understanding of the

Figure 5. | The unique transporters and cell structure of each segment of the nephron work in concert to maintain homeostasis. ENaC, epithelial sodium channel; NKCC2, Na⁺-K⁺-2Cl⁻ cotransporter; ROMK, renal outer medullary potassium.
clinical use of diuretics and the care of patients with a wide variation of issues, from the patient with essential hypertension to the complex patient with cirrhosis.

In the 1960s, Guyton proposed that all hypertension, ultimately, is a result of the failure of the kidney to excrete the excess of total body sodium with a normal pressure natriuresis (42). Although this theory has been disputed over the years, it is notable that monogenic defects that lead to hypertension or hypotension are found exclusively in genes that encode either renal transporter proteins or proteins that regulate the function of renal transporter proteins and ultimately renal sodium handling.

Despite mounting evidence on the importance of sodium in the kidney’s role in homeostasis, investigators in the 1950s soon recognized that the measured serum sodium correlated poorly with the total body sodium by comparing these values in heterogeneous patients with a variety of chronic conditions. Instead, the serum sodium correlated well with the serum osmolality (particularly when corrections were made for the osmotic contributions of glucose and nonprotein nitrogen) (43). (Of note, the same investigators also recognized that the Na\(^{+}\)-K\(^{+}\)/total body water ratio correlated closely with “corrected” serum sodium and explained the importance for accounting of potassium repletion during the treatment of hyponatremia.) Maintenance of the plasma osmolality was noted to be tightly regulated by both the release of vasopressin and the kidneys’ response (44). This interplay between the two is essential for water homeostasis, a critical factor in the maintenance of cell volume. Although cells have developed strategies to deal with excess or insufficient water, these volume regulatory changes require extrusion or inclusion of electrolytes, which alters the cellular interior milieu and wrecks havoc on normal cellular function. Later adaptions allow cells to return toward normalcy but only within a small range. Water reabsorption requires the ability to both establish an osmotic gradient in the kidney and to reabsorb water from the urinary filtrate. The kidney has an elegant strategy to concentrate or dilute the urine by its response to vasopressin and the ability to deploy aquaporins to the luminal membrane (45). At least seven aquaporin isofoms are expressed in the kidney and play important roles at different sites. In the proximal tubule and thin descending limb, aquaporin 1 appears to serve as the dominant gateway for water reabsorption, whereas trafficking of aquaporin 2 along cytoskeletal elements in the collecting duct cells allows reabsorption of water and urine concentration in the principal cells of the collecting ducts (46). Detailed study of the molecular structure and cell physiology of these transporters has allowed insight into the rare genetic diseases that affect aquaporins, such as congenital nephrogenic diabetes insipidus and the common acquired defects related to lithium, calcium, and even urinary obstruction. Similarly, study of the vasopressin receptor has resulted in new strategies and pharmacologic agents for the treatment of states of excess antidiuretic hormone and polycystic kidney disease.

**Acid-Base Homeostasis**

Maintenance of pH is a critical activity of the kidney and is essential for normal cellular function because the pH dictates the charged state of proteins that affects conformational shape, enzymatic activity, binding, and cellular transport and, thus, allows proteins to perform essential metabolic functions. Although some acid-base enthusiasts enjoy consideration of the “strong ion difference” to reconcile data, the normal kidney’s remarkable response to subtle differences in pH or, more likely, intracellular CO\(_2\) does not take these differences into account. Although the exact mechanisms used to sense pH are still not yet understood, it is well known that the kidney plays a dominant role in the regulation of the acid-base balance (47).

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**Divalent Cations and Phosphate Homeostasis**

The kidney plays a critical role in maintaining both normal extracellular calcium ion levels and the vast repositories of
calcium needed for normal intracellular function and maintenance of the skeleton. Integrated control by parathyroid hormone and 1,25-dihydroxyvitamin D helps achieve this end (59). Calcium is reabsorbed through a paracellular route in the proximal tubule and the thick ascending limb, whereas there are unique, well characterized transient receptor potential ion channels in the DCT. Each of these regions is controlled by local effects prescribed by the calcium sensing receptor and modulated by the pH (60). There is also an interesting pas de deux between sodium and calcium handling. Volume depletion or decreased sodium delivery decreases urinary calcium either by increasing proximal reabsorption or by promoting reabsorption in the DCT with changes in the activity of the basolateral Na+/Ca2+ exchanger or hyperpolarization of the luminal plasma membrane (61,62). The fate of magnesium homeostasis is intimately linked to that of other cations. In parallel to calcium, the majority of filtered magnesium is reabsorbed in the proximal tubule and thick ascending limb by paracellular movement mediated, in part, by the claudin proteins, which govern ion movement through the otherwise tight junctions in those regions (63). In addition, the action of the renal outer medullary potassium transporter to supply more potassium in the lumen for the NaKC2 transporter is thought to create a lumen-positive transepithelial potential difference that can favor cation reabsorption in this segment (64). By contrast, in the DCT, movement of Mg2+ is an active transcellular process. At this segment, cation channels from the melastin transient receptor potential subfamily play a major role in this endeavor, evidenced by Mg2+ wasting seen in the rare genetic disorders with defects in this channel (65).

Emerging details on phosphate metabolism have identified a family of sodium phosphate transporters that help reabsorb the bulk of the filtered phosphate in the proximal tubule (66). These transporters appear to be affected by a series of factors, including the recently characterized fibroblast growth factor 23 and its obligate coreceptor Klotho, which together, via the fibroblast growth factor receptor, inhibit the reabsorption of sodium-dependent phosphate reabsorption and lower vitamin D levels by downregulating the gene for 1α-hydroxylase (67). Phosphate that escapes proximal reabsorption and is delivered distally is available to bind H+ as an important source of “titratable acid” (68).

Protein Metabolism

Even Richard Bright (69) in the 1820s recognized that in “dropsy” (or edema) of renal origin, urea was increased in the blood and decreased in the urine such that urea could serve as a marker of kidney failure. One hundred years later, Thomas Addis tried to assess renal function using urea clearance and “rest” the kidneys from the “work” of clearing proteins by prescribing a low-protein diet (70). Landmark studies by Brenner’s group suggested that this strategy was correct because high-protein diets fed to laboratory animals can be shown to increase renal blood flow and glomerular filtration and subsequently contribute to the progression of CKD (71). Nevertheless, the specific mediators that lead to hyperfiltration and contribute to the changes seen with a high-protein diet are yet to be determined.

Renal Physiology Has Significant Clinical Relevance

One of the considerable gifts to the field of nephrology is that there is a deep and growing understanding of the intricacies of kidney function and the ingenious methods that the kidney uses to govern homeostasis. These discoveries complement observations made with careful consideration and primitive measurements in the past. Predictions on the movement of ions have been translated by detailed characterization, on the molecular level, of ion transporters and provide insight into the integrated responses of the kidney to the maintenance of the internal milieu. Those of us who are privileged enough to care for patients with disorders of the kidney can utilize knowledge gleaned in the laboratory to understand real clinical concerns.

Because it is difficult for any practicing nephrologist to stay abreast of the rapidly unfolding revelations, this new renal physiology series will serve as an update to the current understanding of the nephron. CJASN will provide careful reviews of the nephron, sequentially, from the glomerulus to the collecting duct, followed by a review on the control of urinary drainage and bladder function. Next, there will be a series of cohesive reviews that will address how the kidney factors in the integrated response to sodium and water homeostasis, potassium handling, acid-base homeostasis, excretion of organic cations and anions, and divalent cations and phosphate homeostasis. Protein metabolism and control of renal nitrogen excretion as well as sensory functions of the kidney will follow. Finally, the role of the interstitium and hormonal function of the kidney will be considered.

In jingoistic banter that many nephrologists can echo with sincerity, Homer Smith asserted, “The responsibility for maintaining the composition of [the internal milieu] ... devolves to the kidneys. It is no exaggeration to say that the composition of the body fluids is determined not by what the mouth takes in but by what the kidneys keep; they are the master chemists of our internal environment” (13). With this series in hand, the CJASN reader will be privy to the cutting-edge science of nephrology; knowledge of the dramatic advances in our understanding will likely turn any nephrologist into a philosopher.

Authors’ Note

The landmark works described in this article are freely accessible on the Internet for those who would like to indulge in the primary sources. These texts and manuscripts have been made available as part of Google Scholar and the Internet Archive, nonprofit digital libraries with the mission to allow universal access to all knowledge. These archival texts include Homer Smith’s The Principals of Renal Physiology and From Fish to Philosopher, Bowman’s treatise On the Structure and Use of the Malpighian Bodies of the Kidney, and texts by Starling, Cushing, Cannon, and Bernard.

Disclosures

None.

References


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