The first decade of *Kidney International*: treasure hunt for the kidney tubule

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The kidney tubule remains a source of wonder and of anxiety. Wonder in view of its level of specialization and differentiation, easily appreciable under the microscope, anxiety because of the intricate, highly regulated processes operating in the cells lining these segments.

The launch of *Kidney International* in January 1972 provided a fresh opportunity to communicate investigations related to the kidney tubule to a large, clinically oriented audience. Far-reaching contributions, based on modeling, cell and transport physiology, cell biology, and clinical descriptions graced our pages—shaping the view on the normal and diseased kidney tubule. Often, the molecular basis of these tubular processes has subsequently been discovered, for example, through genetic evidence, and they remain actively investigated.[1] Five seminal publications selected from the first years of *Kidney International* are briefly discussed below, arranged from the proximal to distal nephron segments.

Model for bicarbonate and fluid reabsorption in the proximal tubule

McKinney TD, Burg MB. Bicarbonate and fluid absorption by renal proximal straight tubules. Kidney Int. 1977;12:1–8. The proximal tubule plays an essential role in body fluid and acid-base homeostasis, through its capacity to reabsorb two-thirds of the filtered water and NaCl and most of the bicarbonate. The interactions between the transport of fluid, sodium, and bicarbonate in proximal tubules have long remained mysterious. A role for carbonic anhydrase was suggested, as acetazolamide decreased fluid absorption.[2] To solve the puzzle, McKinney and Burg[3] perfused isolated rabbit proximal straight tubules: they showed that both fluid and bicarbonate absorption were inhibited when sodium was replaced by other cations in the apical and basolateral medium, or when ouabain (an inhibitor of the Na^+–K^+–adenosine triphosphatase, Na^+/K^+-ATPase) was added to, or potassium removed from the basolateral side. They concluded that active sodium transport, driven by the Na^+/K^+-ATPase, drives fluid and bicarbonate absorption by the proximal tubule. Next, they showed that fluid transport is significantly lowered when apical bicarbonate is replaced by chloride or when acetazolamide is added. From these experiments, they proposed a model of integrated transport involving a sodium for hydrogen ion exchange located in the brush border (Figure 1). Sodium from the lumen enters the tubular cells down its gradient, to be pumped out by the basolateral Na^+/K^+-ATPase. The hydrogen ions alimenting the apical sodium for hydrogen exchanger result from the carbonic anhydrase activity in the cells. Blocking carbonic anhydrase activity by acetazolamide inhibits in parallel bicarbonate and fluid (presumably sodium) reabsorption. These predictions have been validated by the discovery of the apical Na^+–hydrogen exchanger (SLC9A3)[4] and basolateral Na^+–bicarbonate cotransporter (SLC4A4)[5] in the proximal tubule. Recessive inactivating mutations in *SLC4A4* cause proximal renal tubular acidosis with ocular abnormalities.[6]

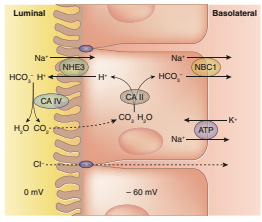

Figure 1 |

Figure 1 shows the interdependence between sodium, bicarbonate and proton transport and

Editor's Note

This article is part of the *KI* 60th anniversary series. This month's topic is related to seminal contributions related to various tubular segments in the normal and diseased kidney.

carbonic anhydrase activity in the cells lining the proximal tubule of the kidney. Derived with permission from McKinney TD, Burg MB. Bicarbonate and fluid absorption by renal proximal straight tubules. *Kidney Int.* 1977;12:1–8.[3] Copyright © 1977 International Society of Nephrology.

Intralysosomal digestion of endocytosed proteins in proximal tubule cells

Christensen EI, Maunsbach AB. Intralysosomal digestion of lysozyme in renal proximal tubule cells. Kidney Int. 1974;6:396–407. The proximal tubule reabsorbs and processes a large amount of albumin and low-molecular-weight (LMW) plasma proteins that are filtered through the glomerular basement membrane. These LMW proteins are reabsorbed through receptor-mediated endocytosis, a process that requires the multiligand receptors megalin and cubilin.[7] After internalization, receptor–ligand complexes progress along the clathrin-dependent endocytic pathway. The endosomes undergo a progressive acidification that dissociates the receptor–ligand complexes, with receptors being recycled to the apical membrane, whereas ligands are directed to acidic lysosomes.[8] While it was assumed that endocytosed LMW proteins are digested in the proximal tubule lysosomes, the evidence remained indirect.

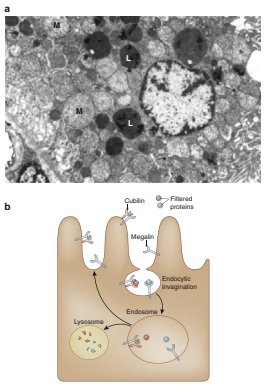

Figure 2|

The first direct evidence for intralysosomal digestion of protein in intact proximal tubule cells was provided by Christensen and Maunsbach.[9] They injected ^{125}I-labeled LMW lysozyme (14 kD) into rats, removed the kidney after 1 hour, and combined autoradiographic and chemical analysis of kidney cortex slices to test whether the labeled protein was digested within the lysosomes. Most (about 90%) of the initial radiolabeled lysozyme was located in lysosomes and endocytic vesicles in proximal tubule cells (Figure 2). The degradation of the ^{125}I-labeled protein was followed *ex vivo*: after 2-hour incubation, about 37% of the total radioactive label was trichloroacetic acid –soluble, identifiable mainly as small monoiodotyrosine metabolite, indicating that the labeled protein initially located in lysosomes was degraded. The concept that endocytosed LMW proteins can be digested in lysosomes (Figure 2) has been validated for multiple ligands in proximal tubule cells.[10,11] This critical function is defective in congenital and acquired lysosomal storage disorders,[1,12] potentially leading to defective autophagy and proximal tubule dysfunction.[13]

Figure 2 shows receptor-mediated endocytosis in the proximal tubule of the kidney. Panel (a) is adapted with permission from Christensen EI, Maunsbach AB. Intralysosomal digestion of lysozyme in renal proximal tubule cells. *Kidney Int.* 1974;6:396–407.[9] Copyright © 1974 International Society of Nephrology.

The countercurrent multiplication system in the medulla

Kokko JP, Rector FC Jr. Countercurrent multiplication system without active transport in inner medulla. Kidney Int. 1972;2:214–223. The kidney is able to concentrate or dilute the urine over a large range, which is critical for maintaining water homeostasis. In the early 1970s, it was accepted that the final urine concentration reflected osmotic equilibration between the lumen of the collecting duct and the hypertonic interstitium in the inner medulla.[14] Microperfusion studies demonstrated that the thick ascending limb (TAL) is water impermeable while actively transporting sodium, driving the system.[15] Passive transport pathways operating in the inner medulla and papilla, which do not contain TAL, were unclear.

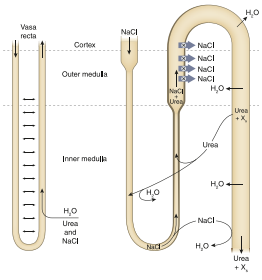

Figure 3|

Kokko and Rector[16] microperfused isolated segments of the rabbit nephron to develop a model of the countercurrent multiplication system (Figure 3). The model postulates that the TAL, which actively transports sodium outward but is impermeable to water, is the osmotic driver of the system, whereas both thin limbs of the loop operate by passive equilibration due to their specific properties: the descending thin limb is only permeable to water, whereas the thin ascending limb is permeable to sodium and urea but not to water. Urea plays a key role in the system, as its concentration in the tubular fluid entering the

collecting duct reflects the action of the TAL, being progressively increased by the reabsorption of sodium and water. Accordingly, urea diffuses out of the inner medullary collecting ducts, contributing to the interstitium hypertonicity. The latter drives water out of the descending limbs and the collecting ducts, diluting the interstitial concentration of NaCl. As a result, NaCl will tend to diffuse out of the ascending thin limbs whereas urea will recycle through the ascending thin limbs. The vasa recta serve as countercurrent exchangers, preserving the longitudinal gradients and providing an exit for the solute and water reabsorbed from the loops of Henle and the collecting ducts.

The passive mechanism hypothesis of Kokko and Rector[16] has been substantiated by the identification of the water channels AQP1 in the descending thin limbs[17] and the urea transporters in the ascending thin limbs and collecting ducts[18] and is found in most textbooks. Recent studies, based on measurements on the thin limbs (rat kidney), 3-dimensional reconstructions of tubules and vessels in the inner medulla and mathematical modeling have refined the model.[19]

Figure 3 shows the model of the countercurrent multiplication system without active transport in the renal medulla. Adapted with permission from Kokko JP, Rector FC Jr. Countercurrent multiplication system without active transport in inner medulla. *Kidney Int.* 1972;2:214–223.[16] Copyright © 1972 International Society of Nephrology.

Treatment of Bartter syndrome with indomethacin

Verberckmoes R, van Damme BB, Clement J, et al. Bartter's syndrome with hyperplasia of renomedullary cells: successful treatment with indomethacin. Kidney Int. 1976;9:302–307. In 1962, Bartter *et al.*[20] reported a syndrome characterized by hypokalemic metabolic alkalosis, inappropriate potassium wasting, hypertrophy of the juxtaglomerular apparatus, normotensive hyperaldosteronism, and increased urinary excretion of prostaglandins. Phenotypic variants forming a group of salt-losing tubulopathies ("Bartter-like syndromes") have been reported subsequently, their molecular basis being established by Lifton.[21] The only treatment of these disorders was liberal NaCl intake, substitution with potassium and, often, potassium-sparing diuretics.

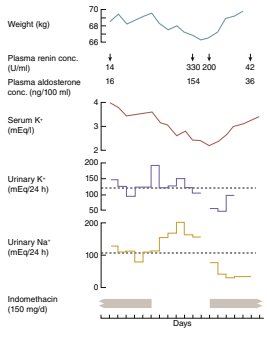

Figure 4|

In 1976, Verberckmoes *et al.*[22] reported a 25-year-old man diagnosed with Bartter syndrome who presented marked hypokalemia with muscular weakness while being treated with potassium and spironolactone. The introduction of indomethacin resulted in a decrease in sodium excretion, weight gain, and normalization of potassium levels with decreased loss in urine. Potassium levels remained within normal limits under treatment with indomethacin alone, replacing spironolactone and supplements, for more than 8 months at time of reporting. Studies under fixed sodium and potassium intake indicated that interruption of indomethacin resulted in rapid and pronounced changes in sodium balance, stimulated plasma renin and aldosterone, potassium depletion and hypokalemia; all the changes resolved after resuming indomethacin (Figure 4). The investigators concluded that Bartter syndrome results from a tubular defect in sodium handling and that prostaglandins could play a role.

Nowadays, elevated prostaglandin E2 levels are considered instrumental for most clinical abnormalities in Bartter patients. Prostaglandins increase potassium waste by activating the renin-angiotensin-aldosterone system, and decrease sodium reabsorption in the TAL, likely via inhibition of NKCC2.[23] Blocking the prostaglandin production with indomethacin enhances the urinary concentrating ability by acting on TAL (NKCC2) and collecting ducts (AQP2).[24,25] Treatment with indomethacin (or other nonsteroidal anti-inflammatory drugs) is often started in infants 4 to 6 weeks old to reduce polyuria and hypercalciuria, improve hypokalemia, and normalize plasma renin. Generally, a combination of nonsteroidal anti-inflammatory drugs and potassium supplementation controls the disease and stimulates development of growth and intellectual ability.[26,27] However, care should be taken with newborns and prenatal nonsteroidal anti-inflammatory drug treatment as it might result in gastrointestinal complications, kidney failure, and potentially in hyperkalemia. This study illustrates how a clinical observation can yield both mechanistic and therapeutic insights in rare diseases.

Figure 4 shows the influence of indomethacin (single treatment and interruption) on clinical and biological parameters in a patient with Bartter syndrome. Adapted with permission from Verberckmoes R, van Damme BB, Clement J, et al. Bartter's syndrome with hyperplasia of renomedullary cells: successful treatment with indomethacin. *Kidney Int.* 1976;9:302–307.[22] Copyright © 1976 International Society of Nephrology.

Plasticity in the distal nephron following acid-base modifications

Hagége J, Gabe M, Richet G. Scanning of the apical pole of distal tubular cells under differing acid-base conditions. Kidney Int. 1974;5:137–146. Although most tubular segments of the nephron contain only one type of cell, the collecting ducts consist of at least two: intercalated and principal cells. These cells are highly differentiated and have specific properties, allowing them to interact and to regulate acid–base and sodium, potassium, and water homeostasis.[28]

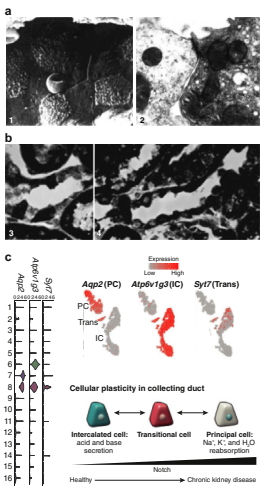

Figure 5 |

From the late 1960s, Richet and colleagues studied the different cell types they could identify in the collecting ducts. By combining light and electron microscopy examinations of the rat kidney, they identified two main types of cells, light and dark, as well as intermediates. The light cells are more frequent, with light cytosol, cuboidal shape, and relatively few microvilli on their apical pole. The dark cells are less frequent, intercalated between the light cells; their cytosol contain many mitochondria and free ribosomes and their apical pole shows numerous microvilli and deep infoldings. In the 1974 paper, Hagége *et al.*[29] applied scanning electron microscopy to analyze the cell types in response to respiratory acidosis or bicarbonate loading. They discovered that the proportion of dark cells, protruding in the lumen, increased when animals were exposed to acid–base challenges (Figure 5). The investigators concluded that light and dark cells do not represent two different cell types; instead they reflect different functional responses of a single and unique kind of cell. They linked this capacity of interconversion to the role played by the distal nephron in acid–base regulation.

Multiple studies have now established the properties of the intercalated cells and the pathways involved in their differentiation versus that of the principal cells of the collecting ducts.[30,31] Critical regulators (e.g., the transcription factor Tfcp2l1) of cell progenitors in the mouse collecting duct, involving Notch signaling, have been identified.[32] A transitional cell type expressing markers of both the principal and intercalated cells has been identified using single-cell RNA sequencing of the mouse kidney (Figure 5). Fluorescent lineage tracing confirmed transitions of principal cells and intercalated cells in the adult kidney, through this transitional cell type. An abnormal transition from intercalated to principal cells could play a role in metabolic acidosis associated with chronic kidney disease.[33]

Figure 5 shows cellular plasticity in the collecting duct. Panels (a) and (b) are adapted with permission from Hagége J, Gabe M, Richet G. Scanning of the apical pole of distal tubular cells under differing acid-base conditions. *Kidney Int.* 1974;5:137–146.[29] Copyright © 1974 International Society of Nephrology. Panel (c) is adapted with permission from Park J, Shrestha R, Qiu C, et al. Single-cell transcriptomics of the mouse kidney reveals potential cellular targets of kidney disease. *Science.* 2018;360:758–763,[33] and Humphreys BD. Mapping kidney cellular complexity. *Science.* 2018;360:709–710.[34] Copyright © 2018, The Authors, some rights reserved. Adapted with permission from the American Association for the Advancement of Science.

DISCLOSURE

The author declares no conflicts of interest.

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