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The rebirth of interest in renal tubular function

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Lowenstein J, Grantham JJ. The rebirth of interest in renal tubular function. Am J Physiol Renal Physiol 310: F1351–F1355, 2016. First published March 2, 2016; doi:10.1152/ajprenal.00055.2016.—The measurement of glomerular filtration rate by the clearance of inulin or creatinine has evolved over the past 50 years into an estimated value based solely on plasma creatinine concentration. We have examined some of the misconceptions and misunderstandings of the classification of renal disease and its course, which have followed this evolution. Furthermore, renal plasma flow and tubular function, which in the past were estimated by the clearance of the exogenous aryl amine, para-aminohippurate, are no longer measured. Over the past decade, studies in experimental animals with reduced nephron mass and in patients with reduced renal function have identified small gut-derived, protein-bound uremic retention solutes (“uremic toxins”) that are poorly filtered but are secreted into the lumen by organic anion transporters (OATs) in the proximal renal tubule. These are not effectively removed by conventional hemo dialysis or peritoneal dialysis. Residual renal function, urine produced in patients with advanced renal failure or undergoing dialysis treatment, may represent, at least in part, secretion of fluid and uremic toxins, such as indoxyl sulfate, mediated by proximal tubule OATs and might serve as a useful survival function. In light of this new evidence of the physiologic role of proximal tubule OATs, we suggest that measurement of renal tubular function and renal plasma flow may be of considerable value in understanding and managing chronic kidney disease. Data obtained in normal subjects indicate that renal plasma flow and renal tubular function might be measured by the clearance of the endogenous aryl amine, hippurate.

renal plasma flow and tubular function; uremic retention solutes; residual renal function

when nephrology emerged in the period 1940–1960, its main attraction was the strong foundation of renal physiology that underlies the understanding of clinical disorders. Homer Smith, who had already achieved considerable fame studying kidney function in elasmobranchs, bony fish, and even the camel, became Professor of Physiology at New York University Medical School in 1930. He soon enlisted the collaboration of several members of the clinical faculty to apply clearance methods to the study of kidney diseases in humans (16, 45). The early history of renal physiology focused on renal tubular transport. This topic occupied three chapters (131 pages) in Homer Smith’s classic, The Kidney: Structure and Function in Health and Disease (47), and was based largely on measurement of infused substances that were transported by the renal tubule and could be measured in urine. The rate of glomerular filtration, measured by the clearance of inulin, was described in a mere seven pages by Smith (47).

Measurement of glomerular filtration rate (GFR), renal blood flow, and the relationship between glomerular filtration and renal blood flow, termed the filtration fraction, formed the basis for our current understanding of many nephrologic disorders. The observation that renal plasma flow was reduced, at times markedly, out of proportion to glomerular filtration is the basis for our understanding of essential hypertension as a disorder of renal vasoconstriction (16). Indicator dilution curves recorded across the renal vascular bed gave evidence that renal vasoconstriction was generalized rather than focal (36). Measurements of deep renal vein pressure (“wedged renal vein pressure”) suggested a mechanism for the exaggerated natriuresis seen in hypertensive subjects (35). Reduced glomerular filtration with normal or near normal renal plasma flow (low filtration fraction) was recognized as the mechanism responsible for avid sodium retention and edema in acute diffuse glomerulonephritis (15). The parallel reduction in glomerular filtration and renal plasma flow was seen as evidence of “whole nephron loss” in chronic renal disease (3).

The measurements of inulin and para-aminohippurate (PAH) in plasma and urine, which provided such valuable insights into renal pathophysiology, are precise, but not easily carried out in routine chemistry laboratories. Furthermore, these clearance measurements require infusion and maintenance of steady plasma concentrations of inulin and PAH during a timed period of urine collection, limiting application to clinical settings (45, 47). A reasonable approximation of inulin clearance (GFR) was introduced with measurement of the clearance of endogenous creatinine whose excretion is largely via glomerular filtration. As a consequence, the clearance of creatinine, which can be measured in routine automated chemistry laboratories, came to serve as a substitute, albeit not a perfect one, for estimation of GFR. However, this estimate also required collection of a carefully timed urine collection, sometimes a source of considerable error. The evolution that followed resembles a “slippery slope.” Since creatinine is produced in muscles at a fairly constant rate, it was reasoned that GFR could be estimated (eGFR) from measurement of creatinine, corrected for body size and age, in a single blood sample (32). It was assumed that renal tubular function (renal plasma flow and tubular secretion) declined in parallel with glomerular filtration (3). A lack of attention to renal blood flow and tubular function over the years followed this proven assumption.

The ability to obtain a quantitative assessment of GFR from a readily available blood test held the promise of retaining a

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focus on physiology in the care of patients with renal disease. However, we submit that the characterization of renal function solely by estimation of eGFR, whether by measurement of creatinine or its surrogate, cystatin (8), has presented a number of problems in our understanding of the underlying pathophysiology of clinical renal disease and its management.

First, utilization of eGFR as the basis for the definition of the stages of chronic kidney disease (CKD) (31), can be misleading as it equates all instances of reduced GFR as evidence of renal disease, ignoring the role of hemodynamics in determining the rate of glomerular filtration. However, we know that GFR can fluctuate in a normal individual between a daytime high of 122 ml·min$^{-1}$·1.73 m$^{-2}$ to a nighttime low of 86 ml·min$^{-1}$·1.73 m$^{-2}$ (29). The Kidney Disease Improving Global Outcomes guidelines clearly state that “the presence of CKD should be established, based on presence of kidney damage and level of kidney function (GFR), irrespective of diagnosis” (31). This implies that stage 1 eGFR is the beginning of renal disease. There is abundant evidence that many forms of progressive renal disease may be advanced before serum creatinine is elevated or eGFR reduced. Furthermore, this classification suggests that patients proceed from stage 1 to stage 5 CKD with time. This has proven to be an erroneous assumption, only partially corrected by the later inclusion of the presence or absence of proteinuria in the diagnosis (6). As stated by Couser (7): “Identification of stages of CKD implies, perhaps unintentionally, that CKD is a progressive process with those afflicted moving eventually from earlier to more advanced stages of disease. Although this clearly happens in many patients with defined forms of kidney disease like diabetes and glomerulonephritis [and we would add, polycystic kidney disease (14)], there is a paucity of data documenting such progression in patients with CKD defined only as GFR < 60 ml/min or GFR < 60 ml/min with microalbuminuria. Indeed, it is clear from several studies that many such patients do not progress over several years of follow-up.”

Second, eGFR is used to define “acute kidney injury” (AKI) replacing the older more physiologically based differentiation between “prerenal” or hemodynamically mediated reduction in glomerular filtration and intrinsic tubular injury. Furthermore, identifying AKI by an increase in serum creatinine or decline in eGFR has led to the belief that this injury occurs days after the insult, hemodynamic or toxic, since several days may pass before serum creatinine is significantly increased to the point that calculated eGFR is significantly reduced (43, 54). There is good evidence that the physiological changes in AKI or “tubular necrosis” occur very early in many instances (40). The search for biomarkers of AKI, many of which are proteins derived from the renal tubule, has not fully replaced an understanding of the hemodynamics.

Third, the shortcuts that allowed estimation of glomerular filtration from a routine blood test were introduced to increase awareness of renal function impairment in the general evaluation of patients by family physicians and surgeons so that they might institute measures to delay the progress of renal disease or control early manifestations of renal failure. To that end, they have been successful. However, recent studies (12) found that eGFR in patients with CKD stages 2–5, not on dialysis, was barely and inconsistently associated with plasma concentrations of protein-bound uremic toxins, which are now believed to be the major determinants of outcome in CKD. These data strongly suggest that factors other than GFR are more powerful determinants of uremic symptoms and outcome. These include renal tubular secretion of protein-bound uremic retention solutes (26, 33, 37, 52), diet content of toxin precursors, toxin production by gut bacteria, and gut transit time (1, 13, 39, 53, 55). Furthermore, impaired proximal tubular secretion is now thought to contribute to the increased plasma levels of trimethylamine oxide (23, 48, 50) and tissue calcification (25), which may influence the outcome in patients with chronic renal disease. There is increasing evidence that the most life-threatening consequences of advanced renal disease, particularly uremic cardiomyopathy characterized by heart failure, arrhythmias, and sudden death (36), are likely related to the accumulation of uremic solutes that are protein-bound and, therefore, not filtered or readily dialyzable, but rather are actively secreted by transporters in the proximal renal tubule (44). Organic anion transporters (OATs) in the kidney are exchangers located on the peritubular and luminal membranes of the proximal renal tubule (44). They serve to facilitate the secretion of protein-bound solutes, such as indoxyl sulfate and p-cresyl sulfate, considered to be important gut-derived uremic toxins (26, 33, 37, 52) now termed “uremic retention solutes.” The focus on eGFR (eGFR based on serum creatinine level) and $K_\text{V/urea}$ (the dialysance of urea divided by the volume of urea distribution, a measure of the efficiency of dialysis) have retarded recognition of the role of secreted, poorly dialyzable solutes in the pathogenesis of uremic cardiomyopathy and vascular disease in chronic renal failure.

Finally, the modification of drug dosage in patients with reduced renal function based on the value of eGFR is subject to two important limitations. Drugs that are secreted rather than filtered may compete with one of the OATs in the proximal tubule (34) and may require very different dosage modification than those excreted by glomerular filtration. The list of drugs that compete for transport is very long (5, 9). It seems reasonable that competition between excreted solutes should be considered in prescribing drugs, just as consideration is given to propensity for hemorrhage or thrombosis, glucose-6-phosphate dehydrogenase deficiency, or cytochrome $P-450$ status (9). Finally, adjusting drug dosage on the basis of eGFR may result in serious overdose in the case of drugs whose excretion is largely via secretion.

The conclusion of this critique is that measurement of serum creatinine and reporting of eGFR, although apparently simplifying diagnosis and classification, is undermined by potentially serious errors and cannot replace quantitative and qualitative measurements of renal tubular function.

In a recent review, Suchy-Dieye et al. (49) wrote, “Proximal tubule secretion represents an essential kidney function for rapidly clearing endogenous substances and administered medications from the circulation, including protein-bound molecules. Moreover, tubular secretion represents the primary renal mechanism responsible for the elimination of most administered drugs and their metabolites. Despite the biologic importance of proximal tubule secretion, this function is infrequently measured because of uncertainty regarding specific endogenous compounds that are cleared by secretion, lack of validated laboratory assays for secreted metabolites, and the cumbersome nature of timed urine collection.” There is increasing
evidence that the most life-threatening consequences of advanced renal disease, particularly uremic cardiomyopathy characterized by heart failure, arrhythmias, and sudden death (22), are very likely related to the accumulation of uremic solutes that are protein-bound and, therefore, not filtered or readily dialyzable, but rather are actively secreted by transporters in the proximal renal tubule (44). OATs in the kidney serve to facilitate the secretion of protein-bound solutes, such as indoxyl sulfate and p-cresyl sulfate, considered to be important gut-derived uremic toxins (2, 13).

Grantham et al. (18–21) reported that isolated rabbit proximal renal tubules were able to secrete fluid, i.e., to produce “urine,” and that the rate of fluid secretion was increased when PAH was added to the bathing medium. It seems likely that the driving force for osmotic secretion of water is the solute concentration attained in the proximal tubule segment. These studies revealed the enormous transport work proximal tubules can channel to raise urine PAH levels as high as 39 mM/l (20) and serve to direct our attention back to the functions of the renal tubule and tubular urine flow. When renal function declines, organic anions, e.g., hippurate and indoxyl sulfate, accumulate progressively in the plasma, achieving concentrations > 50 μM/l, sufficient to promote the secretion of fluid into proximal tubules (19, 20). The extent to which surviving proximal tubules in end-stage kidneys can secrete organic anions has yielded conflicting reports and deserves further study in newer models of CKD.

Most patients continue to produce urine even as they begin hemodialysis or peritoneal dialysis. This has been termed “residual renal function” (RRF). While it seems likely that small protein-bound solutes are normally removed by both glomerular filtration of the free or unbound fraction and tubular secretion of protein-bound solute by OATs, the reduction in glomerular filtration in chronic renal disease may increase the relative contribution of tubular secretion to the total renal removal of waste products. Given the evidence that protein-bound solutes are mainly secreted rather than filtered, it does not seem far-fetched to speculate that the urine that is produced by patients with advanced renal disease represents, wholly or in greater part, the product of tubular secretion of one or more metabolites, along with osmotically obligated fluid, as glomerular filtration declines. In effect, it may be that the patient with advanced or end-stage renal disease and RRF may excrete toxins in the same manner as the aglomerular anglerfish (33), namely by generating “urine” by tubular secretion rather than filtration. Jhawar et al. (26), Klammt et al. (28), and Marquez et al. (37) have observed that plasma indoxyl sulfate concentration is greater in anuric patients than in patients who retain RRF. Analysis of large data sets suggest that patients with RRF, i.e., continued urine production while undergoing hemodialysis or peritoneal dialysis, have better “quality of life” and less cardiovascular disease than patients who are anuric (2, 51).

Grantham had speculated years before that “under conditions of markedly reduced glomerular filtration, mammalian proximal tubules could secrete...some of the potentially toxic products normally excreted by the kidney”, which would “serve a useful survival function” (21).

Given the evidence that renal tubular function plays a critical role in maintaining fluid and solute homeostasis, there have been a number of attempts to quantify that function in clinical settings. Most of these are best termed radionuclide renography and employ detection of the renal uptake and disposition of radioactive tracers, including technetium, iodohippurate, and gadolinium (4), or the clearance from the kidney of fat-soluble tracers, such as xenon (24). The curves obtained by radioisotope renography are complex and reflect, in addition to renal blood flow, transport, cellular storage, tubular fluid flow, and exchange of isotope between at least three compartments, i.e., the peritubular capillary, the interstitium, and the renal tubule. These techniques provide valuable information regarding renal tubular function as seen in the study of Pathuri et al. (41), which demonstrated a predictive value of radiohippurate renography in a rat model of renal cystic disease.

Radioisotopic techniques, after mathematical deconvolution of the isotopic curves, can provide valuable information about renal tubular function, but do not provide a measure of renal blood flow per se.

Renal blood flow has been evaluated in humans by recording optical density curves during the passage of a protein-bound indicator (indocyanine green) across the renal vascular bed (10, 36). The transit time in normal humans, ~12 s, is far shorter than the “uptake phase” of any of the radionuclides that are used in radioisotope renography. Since we believe that there is a value in precise measurement of renal blood flow in renal disease, we here propose a possible new strategy, returning to the early studies of renal tubular physiology. The most widely accepted measure of renal blood flow is the clearance of the aryl amine, PAH (47), which is known to be secreted by the proximal renal tubule (18–21). While we recognize that PAH excretion requires active transport across the proximal renal tubule, estimation of renal blood flow from PAH clearance and simultaneous estimation from the transit of indocyanine green across the renal vascular bed agreed closely, evidence that PAH transport from peritubular capillary to the tubule lumen is rapid, with little evidence of accumulation of PAH in the renal interstitium (36).

Although measurement of renal blood flow traditionally required infusion of PAH, the past decade has recognized that many small protein-bound solutes are secreted by the same proximal renal tubular OAT as PAH (44). Hippuric acid (HA), another protein-bound aryl amine, possibly a uremic retention solute, is transported by the same OAT (44); we suggest the possibility that the clearance of this endogenous aryl amine might serve to measure renal plasma flow. Marquez et al. (37) reported hippurate clearance rates that averaged 270 ± 126 ml·min⁻¹·1.73 m⁻² and a hippurate/urea clearance ratio approaching 10 for endogenous hippurate in normal subjects, suggesting that hippurate may be cleared as efficiently as PAH. This raises the possibility that measurement of the renal clearance of endogenous hippurate could provide a measure of renal blood flow in humans.

If hippurate clearance is to provide a measure of renal plasma flow, several conditions need to be met. The secretion of PAH is highly efficient; at low plasma concentrations, the removal of PAH is virtually complete on a single pass through the kidney, allowing application of the Fick principle to the estimation of renal plasma flow without sampling renal venous blood (47). PAH and HA differ in their binding properties; HA is reported to exhibit roughly 65% protein binding compared with 25% binding of PAH (37). If the clearance of hippurate is to provide a measure of renal blood flow in humans, it will be necessary to establish that the removal of hippurate is virtually
protein-bound uremic solutes. The best studied of these uremic solutes, indoxyl sulfate, one of a very few poorly dialyzable uremic retention solutes identified in a metabolomic screen (42), appears to exert its adverse cardiovascular effect by binding to the cytoplasmic aryl hydrocarbon receptor, which acts as a nuclear transcription factor mediating macrophage migration, oxidative stress, and transforming growth factor-β signaling (17).

The management of renal failure, which, since the development of hemodialysis and peritoneal dialysis, has been guided by estimates of GFR and the tacit assumption that dialysis provides an efficient means of removing offensive solutes, will see important changes in strategy when the generation and secretion of protein-bound uremic solute are better defined. Current practices regarding the indications for and the time of institution of dialysis, as well as the frequency of dialysis treatments, may be revised when these decisions are based on knowledge of the concentration of uremic retention solutes (toxins), as well as hippurate. Renewed interest in and studies of the tubular transport and clearance of hippurate (i.e., renal plasma flow) and related toxic aryl acids could help move nephrology away from the glomerulocentric perspective that has stymied progress toward early treatment of progressive renal disorders.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
Author contributions: J.L. and J.J.G. drafted manuscript; J.L. and J.J.G. edited and revised manuscript; J.L. and J.J.G. approved final version of manuscript.

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