The Saga of Two Centuries of Urea: Nontoxic Toxin or Vice Versa?

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Summary: In the early 1700s, a substance ultimately identified as urea was reported for the first time in urine. About a century later, in 1828, synthesis of this organic compound was achieved, thus giving rise to modern organic chemistry. In parallel, physicians showed that urine comes from the kidneys and contains large amounts of urea, which is produced outside of the kidneys, establishing the humoral approach of renal physiology. Urea was the first uremic retention solute to be identified and it has been used as a marker of renal disease ever since. However, progress in the knowledge of urea metabolism has shown that it is susceptible to many extrarenal variations and, therefore, it cannot be a reliable marker of renal function. It reflects protein intake in the stable patient and has been used to assess nutrition and dialysis efficacy in renal patients. Although it has been studied for almost 200 years, its toxicity has been largely debated. An indirect toxicity occurring through carbamylation of lysine residues is now well established and some evidence from recent work also supports direct toxicity of urea, offering additional rationale for interventional prevention of uremic complications.
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The discovery of urea is an example of collaboration between chemists and physicians. In the 16th century, Van Helmont (1577-1644) (Fig. 1) observed a "salt of urine that never occurs outside man's body which is bred in the course of digestion from a substance not a salt…It differs from sea-salt, also present in urine, by remaining unchanged in its course through the body and on putrefaction of urine… The sea-salt in its cooling, adheres to a wooden vessel even while it is separated from saltpeter, but the salt of urine grows together in the bottom of the liquor."[1] Thus, from the late 1500s and early 1600s the existence of a salt-like substance in urine, different from NaCl, and specific to living organisms, was known. This substance was isolated by Boerhaave[2] in Leiden, who called it "the native salt of the urine" in 1727, well before Rouelle the younger[3] in Lyon. Its purification was improved by Fourcroy and Vauquelin,[4] who named this substance *urée* because of its origin, and completed by Prout[5] in London, who in 1814 described its chemical composition with remarkable accuracy as compared with that previously reported by Fourcroy and Vauquelin[4] and later by Bérard,[6] in Montpellier. These efforts in urea purification prepared the field for what is considered the starting point of modern organic chemistry: the synthesis of urea, achieved by Wöhler[7] in 1828. He synthesized urea from silver cyanate and ammonium chloride, being the first to obtain it outside the body from inorganic substances. He wrote to Berzelius in Stockholm: "I can make urea without needing a kidney, whether of man or dog. The ammonium salt of cyanic acid is urea" ($HCON + NH_3 \rightarrow H_2N\text{-}CO\text{-}NH_2$).

In parallel to the contribution of chemistry in understanding kidney function, the physicians were making progress guided by their incredible observational capacities. The question of whether urine comes from the kidneys or locally accumulates in the bladder was answered in the doctoral thesis of Comhaire[8] (1778-1860), who observed that bi-nephrectomized dogs had no urine in the bladder. He failed to show urea retention in his model because the available determination method was not yet sensitive enough to detect actual changes. Urea retention was shown by Prevost (1790-1850) and Dumas (1800-1884),[9] who nephrectomized dogs, sampled blood under alcohol extraction, and, after precipitation with nitric acid, obtained the same crystals in blood as the ones that were observed in urine. Therefore, by improving the ability to determine the urea level in blood before clotting and with a proper study design, this approach showed that urea is produced elsewhere than in the kidneys, and that kidneys are responsible for removing the urea accumulated by extrarenal production. This set the basis for the *humoral* view of renal physiology, as opposed to the *morbid anatomy* theory, which was the dominant approach at that time in Europe (early 1800) as supported by Bright among others. This change of paradigm was described nicely by Richet.[10]

The removal capacity of the kidneys was shown by Picard[11] (1834-1896), who adapted a new method of urea measurement (from Liebig's method) and was successful in determining the level of urea in human blood. This also allowed him to determine levels of urea differentially from the renal artery and vein of

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Figure 1. Jean Baptiste van Helmont (1577–1644), alchemist (painting by Mary Beale, c1674). Jean Baptiste van Helmont, a Brussels-born chemist and physician, was the founder of the Iatrochemical School, which looked for chemical explanations of vital phenomena. He was a man of great intellectual curiosity and studied philosophy in Louvain. His description of the salt of urine offered the first evidence of the urinary content of urea (see text).

dogs, leading to the observation that there was a significant decrease in the urea level in renal veins compared with the arteries, showing a clearance capacity by the kidneys, in contrast to the carotid artery and jugular vein, where the gradient was in the opposite direction. Christison[12] and Gregory[13,14] suggested that retention of urea might be deleterious, and Frerichs[15] (1819-1885) introduced the concepts of retention solutes and uremia when commenting on Bright's reports.

In summary, progress in chemistry by the pioneers in renal medicine of the 18th and first half of the 19th centuries established the basis of renal physiology at the same time that urea, a substance of biological origin, was synthesized from inorganic compounds outside a living body. Urea has since then been a chief element in medicine that has helped to identify renal failure, changing the thinking of renal pathology. It is the most studied retention solute: its accumulation in renal failure is used to identify lack of removal from the body and it is used as a marker of metabolic stability and nutrition. Its toxicity, proposed in the 1800s, is still under debate. The present article focuses on the following: (1) the analysis of the chemistry and metabolism of urea by addressing the question: urea, a marker of uremia; (2) its use to guide renal replacement therapy by analyzing its clearance: urea, a marker of dialysis adequacy; and (3) a reassessment of its supposedly harmless characteristics by addressing the question: urea, a uremic toxin?

UREA, A MARKER OF UREMIA?

Amongst all uremic toxins, urea is the one which shows the highest concentrations in the blood of uremic patients.[16] It is a small water-soluble molecule of 60 daltons. It contains two nitrogen atoms and it is the end-product of protein and nitrogen metabolism. In nephrology, urea levels have been measured and interpreted for many purposes, such as estimating uremia severity, glomerular filtration rate (GFR), protein intake, protein catabolic rate, and dialysis adequacy. Initially introduced by Piorry[17] in 1847, the term *uremia*, meaning "urine in the blood," referred to a blood intoxication by urine, characterized by increased blood levels of urea.[15] Uremia, in its present significance, is the disease clinically characterized by manifestations of the uremic syndrome, which is caused by retention of many more solutes than urea. The question to be answered then is whether serum urea levels still are adequate markers of a disease characterized by an increase in serum urea levels, but also of many other compounds that might be pathophysiologically more important than urea itself.

Blood Urea Concentration and Uremia

The serum concentration of urea is easily measurable and is given as a molar or mass concentration in many countries. In others, including the United States and Germany, however, serum urea concentration is referred to indistinctly as blood urea nitrogen (BUN) or serum urea nitrogen (SUN), and is expressed as the mass concentration of nitrogen equivalents. The conversion between different units is shown in formula 1.

$$\begin{aligned} \mathrm{BUN}\ (\mathrm{mg/dL}) &= 0.47 \times [\text{urea in mg/dL}] \\ &= 2.8 \times [\text{urea in mmol/L}] \end{aligned} \quad (1)$$

The normal range of BUN that generally is accepted extends from 5 to 20 mg/dL, which corresponds to urea concentrations of 11 to 43 mg/dL or 1.8 to 7.2 mmol/L. BUN levels can greatly increase in uremic patients, reaching 10 times the upper limit of the normal range in patients with end-stage renal disease before dialysis. If there is at least some parallelism between urea serum concentration and the stage of uremia, the relevance of plasma urea levels as a diagnostic marker of chronic kidney disease (CKD) is much more debatable. As shown in Figure 2, urea levels increase exponentially with reduced estimated GFR (eGFR), but significant increases become observable only when eGFR levels are reduced to about half the normal value. Urea levels follow a similar trend as that of serum creatinine levels, although the latter are a much more reliable marker because they are less subject to modifications unrelated to glomerular filtration. Even at low eGFR rates, BUN levels do not perform well for screening or identifying CKD patients. In addition, blood urea

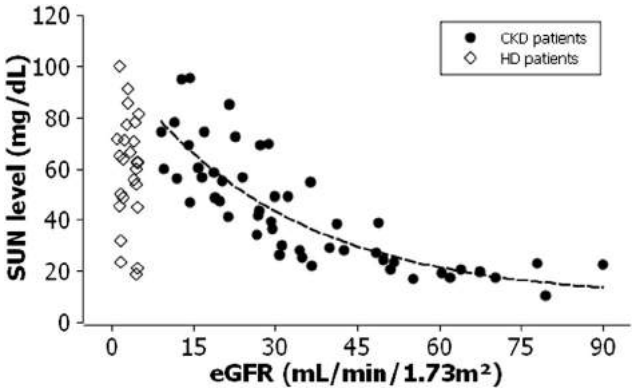

Figure 2. Serum urea Nitrogen (SUN) levels of CKD and HD patients depending on eGFR. For illustrative purposes, HD patients were given random eGFR values within 1 to 5 mL/min/1.73 m^2. Seventy-seven patients seen in the outpatient clinic who participated in a study assessing the proteomics as a predictive tool to identify renal failure progression[24] were included. It can be seen that SUN levels increased exponentially when eGFR decreased to less than 40 mL/min/1.73 m^2, and reached maximum levels of approximately 100 mg/dL ($R^2 = 0.68$). Patients treated by dialysis had a wide range of SUN values ranging from those observed in patients with a normal glomerular filtration rate (~20 mg/dL) to the highest values observed (~100 mg/dL). Thus, SUN does not allow differentiation of CKD stage.

levels may vary within short periods of time, from several hours in healthy patients to several days in CKD.[18] Changes in urea concentration can be the consequence of reduced urinary excretion, but also of changes in the patient's nutritional and metabolic states. Increased dietary nitrogen intake and catabolic states such as metabolic acidosis or fever are associated with a higher net protein catabolism and urea production, which potentially affect circulating levels.[19] Renal hemodynamics also influence urea blood levels: when renal perfusion decreases as in congestive heart failure or dehydration, there is an increase in blood urea levels.[20] Urinary tract obstruction, salt depletion, and/or use of diuretics also increase urea blood levels.[21] Because of the many factors influencing urea blood levels, they are not among the systematic laboratory examinations proposed by some regulatory agencies to evaluate renal function and screen disease,[22] and the French national authority even stated that urea blood level should not be used for CKD diagnosis.[23]

The high urea concentrations previously observed in end-stage renal disease are not frequently observed today thanks to renal replacement therapies. Thus, absolute values of serum urea levels are not good markers of uremia when comparing CKD patients with patients treated by dialysis. In a group of 77 CKD patients including 25 patients on hemodialysis seen for a study on proteomics, SUN levels scattered along a wide range of concentrations, from 20 to 100 mg/dL (Fig. 2).[24] As a result, the same value may be observed in a subject with near-normal eGFR or with an eGFR less than 15 mL/min/1.73 m^2. The dissociating capacity of SUN to differentiate a patient with a normal glomerular filtration rate from another being treated by dialysis thus is rather poor.

Data from the US Renal Data System reported an average BUN level of 88 ± 34 mg/dL in patients starting dialysis.[25] Urea freely diffuses through the dialyzer or peritoneal membrane. In hemodialysis, the urea reduction ratio is high, generally greater than 60%, and as a consequence the postdialysis BUN levels are often close to normal (eg, predialysis, 76 mg/dL; postdialysis, 31 mg/dL[26]). The National Cooperative Dialysis Study evaluated the influence of different targets of predialysis BUN level (70 or 120 mg/dL) on the frequency of hospitalization and of apparition or worsening of comorbidities.[27] There were no differences in mortality, but lower BUN levels were associated with less comorbidities or hospitalizations, but only in those patients with a sufficient protein catabolic rate (Fig. 3).[27,28] This suggests that urea blood levels alone are not good indicators of dialysis performance. In addition, other studies showed that maintaining high urea levels by adding urea to the dialysate had little side effects.[29] In conclusion, urea per se, at the concentrations observed in dialysis patients, does not appear to be toxic to the organism and it should not be used as a unique dialysis target or evaluation criterium of dialysis efficacy because it also can be related to the clinical state of the patient. A combined evaluation of urea levels along with the

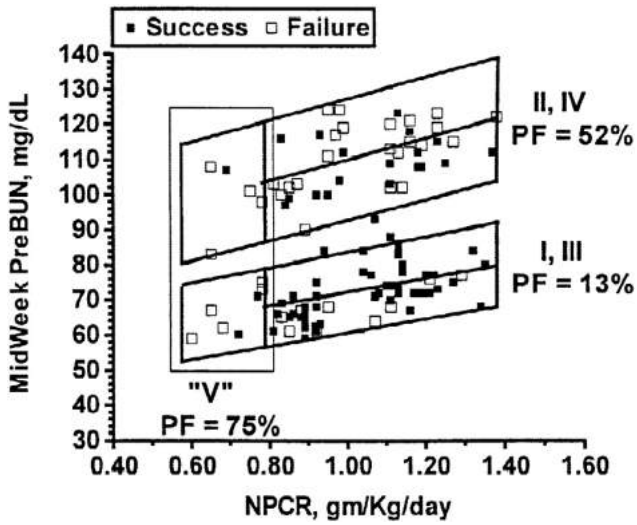

Figure 3. Patient outcome depending on predialysis midweek BUN level and normalized protein catabolic rate (nPCR). This plot from the National Cooperative Dialysis Study shows the original four treatment groups according to dialysis duration (long in groups I and II, and short in groups III and IV) and predialysis BUN targets (low in groups I and III, and high in groups II and IV). It shows that the failure rate was 52% in high BUN targets, and 13% in low BUN targets, arguing in favor of more intensive removal strategies. It also highlights the presence of a fifth group (V) in which patients had a low nPCR and a high failure rate (75%). Success and failure were defined as per the NCDS criteria. Reproduced with permission from Gotch.[30]

patient's protein catabolic rate has been proposed to estimate the patient's status and the adequacy of dialysis at the same time, as illustrated by results from the National Cooperative Dialysis Study study (Fig. 3).[30]

Urea Clearance and Kidney Function

Urea clearance has been used as a surrogate of renal function and CKD severity early in the study of nephrology.[31] At that time, it was thought to be an earlier marker of renal dysfunction than creatinine clearance. However, with the development of more precise GFR estimations, mainly based on serum creatinine level, it was possible to re-evaluate these performances. Using radioactive markers, investigators of the Modification of Diet in Renal Disease study showed that creatinine clearance provided a better estimation of glomerular filtration rate than urea clearance.[32] There are several reasons that explain why urea clearance is not a good candidate for GFR estimation. A good endogenous marker of GFR should be filtered by glomeruli and not secreted or reabsorbed by tubules. Although not secreted, 30% to 70% of filtered urea is passively reabsorbed by tubules. It has been known for long that tubular reabsorption of urea also depends on volumetric status, with increased reabsorption when urinary flow decreases.[33] In fact, volume depletion, salt depletion, use of diuretics, and urinary tract obstruction enhance tubular reabsorption of urea.[21] In addition, a good marker should be generated at a constant rate, whereas urea generation depends on liver function, protein metabolism, as well as dietary N intake.[18]

Nitrogen Balance and Protein Catabolism

Measurement of different nitrogen (N) appearance rates can be used to estimate protein intake. This method relies on the principle of conservation of mass applied to nitrogen. When the patient is considered a closed system, the N input (total N appearance [TNA]) is equal to the sum of the N output (N excretion) and the change in N in the system (N accumulation), which can be positive in anabolic patients or negative in catabolic patients (formula 2).

$$\text{TNA} = \text{N excretion} + \text{N accumulation} \quad (2)$$

Because dietary proteins are composed globally of 16% N,[34] estimations of TNA in grams can be converted into protein equivalents of total nitrogen appearance (PNA) using formula 3. In stable patients, PNA is mathematically identical to the protein catabolic rate (PCR).

$$\text{PNA} = 6.25 \times \text{TNA} \quad (3)$$

In stable patients who, by definition, have a neutral N metabolism, there is no N accumulation, and, consequently, TNA equals total N excretion. Nitrogen can be excreted as urea or nonurea compounds (amino acids, uric acid, creatinine, and so forth) through different routes, mainly urine, fecal material, and renal replacement therapy for those patients treated by dialysis. In CKD patients, including those on dialysis, nonurinary N excretion is stable and independent of dietary protein intake (DPI).[35,36] As a consequence, in nondialyzed patients, PNA can be estimated from the rate of urinary urea excretion, which reliably reflects protein intake.

The gold standard for measuring renal excretion of urea remains the 24-hour urine collection. It has been questioned whether bacterial ureases in the intestine, which break down urea into ammonium ions, influence the level of urinary N excretion because it could result in PNA understimation.[37] In renal patients, inhibiting intestinal urea metabolism with antibiotics was shown not to influence urinary urea output.[38] This suggests that in human beings, urea-derived N normally is reconverted to urea in the liver, whereas, in constrast, a significant recycling of urea N into proteins has been reported in hibernating bears.[39] The intestinal handling of urea is discussed further in the article by Jankowski et al of this issue.[40]

In dialysis patients, the approach to estimate urea N removal relies on urea kinetics modeling (UKM), which allows estimating the urea N appearance rate (UNA), formerly referred to as *urea generation rate (G)*, which is the amount of urea synthesized over a period of time. Because UNA directly is influenced by protein catabolism, it correlates nicely with TNA or its protein equivalents, PNA and the protein catabolic rate.[35,41,42] These observations lead to the establishment of directly applicable formulas to estimate PNA from UNA in dialyzed patients, but these values are accurate only in the steady-state patient in whom there is no positive or negative N accumulation.[43] The rate of protein metabolism is associated with body size, so that comparability across patients is possible only after normalization for body weight which depends on the chosen measure (eg, lean body mass, total body water mass, dry body weight).[44] Normalizing actual body weight can be misleading in malnourished, obese, or edematous patients, in whom using edema-free body weight therefore is preferred.[42] The main clinical use of normalized PNA (nPNA) is for DPI assessment and counseling, and European guidelines recommend values of nPNA greater than 1 g/kg/d.[45] However, in hemodialysis patients, estimated DPI was shown to be lower on average than true DPI, except for those patients with low DPI in whom it was overestimated.[46] This lack of accuracy could be explained either by methodologic limitations or by metabolic adaptations. Although not used widely, alternative methods exist to estimate PNA, based on quantitative determinations of

urea in spent dialysate.[47] In any approach, the presence of proteinuria or protein losses in the dialysate and their influence on nitrogen balance should be considered carefully and N excretion through residual kidney function should be included in calculations.

In conclusion, although increased urea circulating levels are frequently observed in CKD patients, these are not owing solely to uremic retention, but also are influenced greatly by protein and water metabolism, hepatic function, medications, dialysis treatment, and so forth. Current use of urea determinations rely on its close relationship with protein intake in the patient at a steady state. The Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines for nutrition do not comment on absolute BUN values but on minimal DPI and nPNA targets.[48] Still, estimations of DPI from UKM rely on empiric equations, the results of which can diverge from actual DPI. Positive N balance occurs in anabolic stages (growth, recovery) whereas a negative N balance is a sign of protein malnutrition or catabolic state. Estimating nitrogen balance and UNA provides an evaluation of the catabolic burden of nonsteady patients. Although limited to clinical research or to critically ill patients,[49] these approaches could be introduced in the monitoring of CKD patients who are at high risk of metabolic anomalies. Urea levels have been shown to perform at least as well as eGFR and creatinine to predict mortality in critically ill patients with acute kidney injury.[50] The BUN:creatinine ratio also has an important diagnostic value in prerenal (transient) acute kidney injury. Because its levels are associated with many relevant clinical parameters, urea can be an interesting marker of patient outcome in acute kidney injury, congestive heart failure, and other pathologies.

UREA: A MARKER OF DIALYSIS ADEQUACY?

As noted previously, uremia, the target of dialysis therapy, is caused by accumulation of exogenous and endogenous toxins normally excreted in the urine. In normal conditions these compounds are subject to different renal physiological mechanisms of selective retention and elimination to maintain the milieu intérieur: glomerular filtration, tubular reabsorption, and secretion. Filtration is the main mechanism for water-soluble and small molecular weight (MW) compounds, whereas tubular secretion may be the central way of removal for those compounds that are not filtered, such as protein-bound solutes. This differs from what was present in primordial sea organisms in which diffusion was the original and only excretory mechanism.[51]

Small Uremic Solutes

The glomerular filter is more permeable than most dialysis membranes, therefore it is likely that larger molecules are removed more effectively by the native kidney than by standard dialysis. However, the early success of hemodialysis using cellulose-based membranes with an absolute MW cut-off value of 10,000 daltons suggests that small MW substances with toxic, life-threatening effects are removed. The substantial prolongation of life in patients treated with dialysis using membranes that remove very few toxins in the molecular weight range greater than 3,000 daltons might suggest that there are no immediately lethal toxins among those not removed with these membranes; alternatively, it might be possible that their lethal activity is modified by dialysis with low-permeability membranes. The near-miraculous quick recovery from uremic coma that was observed by Kolff et al[52] when dialysis first was applied to patients dying from uremia suggests that the most acutely life-threatening aspects of uremia appear to be easily and quickly reversed by hemodialysis. Therefore, removing small easily dialyzable toxins and restoring the milieu intérieur are mandatory requisites for a dialysis system. The identity of such toxins remains elusive, even today after more than 50 years of studies involving highly sensitive separation and specific identification methods applied to dialysate, body fluids, and urine. Hundreds of candidate toxins have been identified but few have been linked to specific aspects of uremia, and most have shown little in vivo toxicity when given to animals.[16,53] The most abundant candidate found in both normal urine and in the blood of patients suffering from uremia is urea. Its molecular accumulation surpasses that of any other retained organic compound by a factor of 10 or more, placing it in obvious view of the chemist and difficult to ignore by the clinician. However, urea is also well tolerated even at the high levels commonly found in patients with CKD who have few symptoms of uremia, and, as noted previously, recovery from uremia has been shown when its concentration in the patient is held constant or actually increases.[29] Urea plays a role as a surrogate, not for uremic toxicity, but for dialyzer performance. The specific performance in this case is removal of small molecular weight retention solutes, and although urea is not very toxic, it dialyzes easily, similar to other small solutes that are more likely to be responsible for toxicity, such as potassium. We know that dialysis works: it transforms a patient certain to die in a matter of hours into a long-lived person who is capable of functioning in society, and it does so very quickly, in parallel with the quick removal of urea by dialyzers: urea clearance is a measure of what the dialyzer does best, removal of soluble low MW solutes.

Mathematic Models of Urea Kinetics

Urea clearance is conceptualized easily in patients dialyzed continuously but is difficult to measure. The

only way to determine the removal rate is to collect or monitor all of the dialysate.[54] Conversely, in patients dialyzed intermittently, clearance is difficult to conceptualize because the patient has long periods during which there is no dialytic clearance, but the measurement of urea clearance is facilitated greatly by the intermittence. The latter creates a high and a low concentration, respectively, at the beginning and at the end of the dialysis procedure, allowing mathematic models of urea kinetics to estimate the removal rate as an alternative method to dialysate collection. Because the urea distribution volume is unknown in each patient, the model is forced to express clearance as a fractional volume per dialysis (Kt/V). The obligatory but silent inclusion of V in the denominator satisfies the need for normalizing the clearance to body size, much like creatinine or inulin clearance is normalized to body surface area, and the obligatory inclusion of treatment time (t) in the numerator means that the modeled clearance is an average value integrated over the entire dialysis treatment. This measure of the dialysis dose often is referred to as the delivered clearance, a patient-specific parameter that is contrasted with the prescribed clearance calculated from known indices of dialyzer size, permeability, and the flow rates of blood and dialysate. Establishing the value of V is a sensitive point in urea kinetics because it cannot be measured easily in vivo and its calculation is prone to error. Nearly all of the mathematic models of urea kinetics in use today include terms for convective clearance as well as residual native kidney function, and are capable of calculating the patient's protein catabolic rate from the urea N appearance as noted earlier. Some models include terms for diffusion among body compartments, as discussed later.

Explicit Equations and Formulas for Calculating Kt/V

Urea kinetic modeling requires multiple iterations to resolve Kt/V, UNA, and other variables. Despite the ready availability of computers to accomplish this task, simplified equations have been developed to approximate results of the formal modeling process.[55,56] These may be useful for population studies and for batch processing but formal modeling remains the method to be compared with and has the widest use.[57] For intermittently dialyzed patients, the simplest correlate to urea clearance is the urea reduction ratio, calculated as follows: $(C_1 - C_2)/C_1$, where C_1 and C_2 are the predialysis and postdialysis urea concentrations, respectively. Although not as accurate as urea kinetic modeling–based calculations, the urea reduction ratio correlates well with Kt/V in population studies, and it is popular among dialysis regulators because of its simplicity and because there are no hidden calculations that could be used to falsely inflate its value.[58]

Solute Disequilibrium and Rebound

All solutes are compartmentalized within the body and urea is no exception. However, urea movement among compartments is rapid thanks to urea-specific transporters within cell membranes that facilitate urea diffusion, in particular across red cell membranes. The red cell is especially important because its short dwell time within the dialyzer requires rapid diffusion across both the red cell and the dialyzer membrane for effective removal from the body. Urea in blood cells exiting the dialyzer is near-completely equilibrated with plasma, whereas other solutes such as creatinine require several minutes to equilibrate, signifying incomplete removal from red cells.[59] Compared with other solutes, the clearance of urea is a more sensitive measure of dialyzer performance because the clearance of urea from red cells passing through the dialyzer is similar to plasma clearance.

However, equilibration with other body compartments is less than complete, causing a finite but relatively small rebound in urea concentration within the blood compartment when hemodialysis or hemofiltration is stopped,[60,61] particularly after shorter treatments, during which not enough time is allowed for equilibration between compartments. Recognition of this urea disequilibrium and the resulting rebound has caused some investigators to recommend waiting until equilibration is complete before measuring the postdialysis urea concentration. This maneuver and the mathematic shortcuts used to estimate the equilibrated postdialysis urea concentration provide a more realistic measure of effective patient clearance but still overestimate the clearance of other solutes.[62] The latter phenomenon has led to downgrading urea clearances to allow a closer match with the clearances of other solutes.[63,64] The resulting weekly standard clearance is the equivalent of a continuous clearance uninfluenced by intermittence, and as such allows a comparison of treatments given at different frequencies during the week. The specificities and pitfalls of kinetic modeling in dialysis are discussed further in the article by Masereeuw et al of this issue.[65]

Is Measuring Urea Removal Sufficient?

Although the assessment of urea clearance should be given priority as a pertinent and potent measure of dialysis and its adequacy, the treatment of end-stage renal disease patients cannot stop there. Patients may escape death from uremia but their quality of life on average leaves much to be desired, suggesting that the job is not done. Lingering symptoms, disability, and a high mortality rate have motivated the dialysis community to look for further improvements in therapy, by increasing removal of small solutes, dialysis time and frequency, and improving dialyzer efficacy (increasing surface, pore size, and reducing membrane

thickness).[66,67] The meager success of such efforts has led to an appropriate targeting of other solutes and, more specifically, other categories of solutes for removal.[68] Other unappreciated functions of the native kidney not replaced by dialysis also could contribute to this residual syndrome of disability.

UREA: A UREMIC TOXIN?

Urea toxicity has been suggested since the 19th century, as described by Christison[12] and Gregory.[13] However, demonstration of the toxic capacity of urea has remained elusive and it has been thought for a long time that the state of uremia was owing to associated uremic retention solutes but not to urea itself. Johnson et al[29] showed that adding urea to the dialysate of end-stage renal disease patients and maintaining a high blood urea level was well tolerated and had no acute toxic consequences. Analysis of the hemodialysis (HEMO) cohort and the Adequacy of PD in Mexico (ADEMEX) study showed no clear association between urea levels or removal and mortality.[66,69] Clinical studies related to uremia, including the HEMO study, are discussed more extensively in the article by Liabeuf et al of this issue.[70]

Evidence for Indirect Urea Toxicity

Although the direct toxicity of urea does not seem clinically evident, indirect toxicity through the modification of serum or tissue compounds by urea cannot be excluded. In addition to the putative toxicity caused by many of the uremic retention solutes, compounds modified by urea also may participate in the uremic syndrome. In this respect, an increasing body of evidence points to carbamylation as a central factor of uremic toxicity.

Urea in vivo spontaneously generates cyanate and ammonia ($H_2N\text{-}CO\text{-}NH_2 \leftrightarrows NCO^- + NH_4^+$). The active form of cyanate, isocyanic acid, irreversibly reacts with amino groups of free amino acids and with lysine residues of proteins, resulting in ε-amino-carbamoyl-lysine (homocitrulline), which may be present at several sites in the protein. Because urea and cyanate are in equilibrium, every time a cyanate molecule is converted by carbamylation, a new cyanate molecule is formed from urea.[71] Carbamylation may alter enzyme and hormone activity, or interfere with protein synthesis, thereby participating in a variety of metabolic disturbances observed in uremia.[71] The modifications induced by carbamylation of proteins are reminiscent of the glycosylation processes of the long-lived proteins, and of the oxidative modifications of different compounds also occurring in CKD.[72,73] All of these post-translational modifications may participate in the generation of uremic syndrome. However, glycosylation and oxidative modification of proteins are not induced directly by urea and therefore cannot be considered either direct or indirect urea-linked toxic effects.

There could a clinical interest in assessing carbamylated protein levels as suggested by recent work showing that protein-bound citrulline predicts mortality in end-stage renal disease patients[74] or that the more specific carbamylation of albumin is also a risk factor for mortality in patients with kidney failure (Fig. 4).[75] The latter study also reported that feeding mice with urea (67 mg/g of feed) increased the proportion of carbamylated albumin, with a more pronounced effect in animals on a very low protein diet, and that in vitro carbamylation could be prevented in the presence of particular amino acids in the milieu, especially glycyl-glycine, cysteine, cysteamine, and taurine.[75] Carbamylated low-density lipoprotein has been shown to have cytotoxic properties: it induces endothelial cell death[76] as well as oxidative stress and accelerated senescence in human endothelial progenitor cells.[77] Oral administration of urea also increased carbamylated low-density lipoprotein and resulted in a more severe form of atherosclerosis in apolipoprotein E–deficient mice.[78]

These results suggest that carbamylation displays toxic effects and has a relevant impact on mortality in

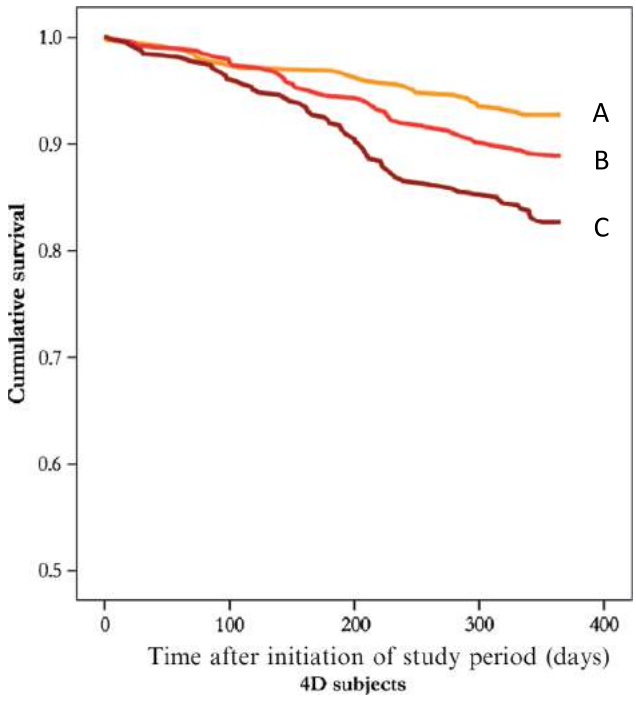

Figure 4. Kaplan-Meier curve estimates of the incidence of all-cause mortality in ESRD patients. Subjects were categorized into lower (A), middle (B), and upper (C) tertiles according to serum carbamylated albumin (%C-Alb) values measured at the outset of the study. The figure shows the twelve-month survival rates in patients from the Die Deutsche Diabetes Dialyze Studie (4D) trial. Similar findings were obtained in the Accelerated Mortality in Renal Replacement (ArMORR) cohort. Adapted from Berg et al[75] and reprinted with permission from the AAAS.

CKD patients. They also provide insight into new ways of treatment, such as supplementation with amino acids with carbamylation inhibiting capacities. Still, it is of note that urea serum levels and carbamylated product concentrations are correlated weakly, suggesting that other important sources or modulating factors may participate in the appearance of carbamylated protein.[74] This further supports the interest in determining carbamylated protein levels.

Evidence for Direct Toxicity of Urea

When factors predicting mortality are assessed in hemodialysis patients, serum albumin and low serum cholesterol were the best independent markers.[78] However, in the same observational study, the urea removal ratio (predialysis to postdialysis BUN level) was also a significant independent predictor of mortality, suggesting a link between urea metabolism or removal and mortality.[79]

D'Apolito et al[80] observed that urea reduces insulin-stimulated glucose transport in 3T3-L1 cells in a concentration-dependent manner, with a 76.4% reduction with 20 mmol/L urea concentration, showing a direct in vitro effect of urea in these cells at a disease-relevant concentration. This urea-induced insulin resistance was mediated by mitochondrial reactive oxygen species (ROS) synthesis and resulted in the synthesis of the adipokines retinol binding protein 4 and resistin. These modifications were observed in a mouse model of CKD as well as in normal mice after urea infusion, and were prevented by treatment with superoxide dismutase/catalase.[80] This work illustrates direct urea toxicity, whereas the efficacy of blockade of the involved pathways neutralizing these deleterious effects suggests that targeting urea-induced ROS might be beneficial in CKD.

A dose-dependent toxic effect of urea on monolayers of colonic epithelial cells T84 also was shown in vitro.[81] In this model, uremic-relevant concentrations of urea in the milieu reduced the transepithelial electrical resistance, as well as the abundance of proteins involved in tight junctions (zona occludens-1, occludin, and claudin-1). The addition of urease in the milieu to mimic intestinal bacterial activity induced a further reduction in the abundance of these proteins. These results suggest that urea may have a direct effect on the epithelial barrier integrity, which could lead to endotoxin absorption and chronic inflammation.

These results support the existence of the direct toxicity of urea potentially affecting different organs, among which are the adipose tissue and the intestinal epithelium. The increased blood urea levels observed in many CKD patients suggest that they are at high risk for urea endotoxicity, which could be responsible for some of the pathophysiological processes leading to uremic complications.

CONCLUSIONS

Urea has been a central compound in chemistry and medicine since its historical identification and purification. First believed an exclusively biological product, its synthesis from inorganic compounds gave birth to organic chemistry. Urea is the end-product of protein metabolism and its appearance in serum has been used as a marker of metabolic status and nutrition. Highly diffusible among the body compartments as well as through the dialysis membranes, it is the reference compound for evaluating the clearance capacities of dialyzers and has proven helpful in monitoring dialysis dose with the widely used urea kinetic modeling–based formulae, which allow a standardized evaluation of renal replacement therapy and comparisons between different treatments and across different patient groups.

Nevertheless, regardless of the level of toxicity that recent and future scientific work might allocate to urea, or the way we monitor urea metabolism status (estimated from UKM or determined by mass removal), physicians seek to integrate the urea-linked data into a larger data base of information with broader origins (clinical, biochemical, morphologic, as well as human [istic]) to obtain an as complete as possible view of the patient. The quality of a treatment and the prognosis of a patient cannot be reduced to a single number such as Kt/V_{urea}. Recent work showing the direct toxicity of urea may generate a stimulus and the rationale for interventional prevention of uremic complications, resetting the stage for new therapies such as antioxidant treatment or amino acid supplementation.

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