Review

Clinical review: Renal tubular acidosis – a physicochemical approach

Troels Ring[1], Sebastian Frische[2] and Søren Nielsen[3]

[1]Consultant, Department of Nephrology, Aalborg Hospital, Aalborg, Denmark
[2]Assistant Professor, The Water and Salt Research Center, Institute of Anatomy, University of Aarhus, Aarhus, Denmark
[3]Professor of Cell Biology and Pathophysiology, Director, The Water and Salt Research Center, Institute of Anatomy, University of Aarhus, Aarhus, Denmark

Corresponding author: Troels Ring, tring@gvdnet.dk

Published online: 25 August 2005
This article is online at http://ccforum.com/content/9/6/573

Critical Care 2005, **9**:573-580 (DOI 10.1186/cc3802)

Abstract

The Canadian physiologist PA Stewart advanced the theory that the proton concentration, and hence pH, in any compartment is dependent on the charges of fully ionized and partly ionized species, and on the prevailing CO_2 tension, all of which he dubbed independent variables. Because the kidneys regulate the concentrations of the most important fully ionized species ($[K^+]$, $[Na^+]$, and $[Cl^-]$) but neither CO_2 nor weak acids, the implication is that it should be possible to ascertain the renal contribution to acid–base homeostasis based on the excretion of these ions. One further corollary of Stewart's theory is that, because pH is solely dependent on the named independent variables, transport of protons to and from a compartment by itself will not influence pH. This is apparently in great contrast to models of proton pumps and bicarbonate transporters currently being examined in great molecular detail. Failure of these pumps and cotransporters is at the root of disorders called renal tubular acidoses. The unquestionable relation between malfunction of proton transporters and renal tubular acidosis represents a problem for Stewart theory. This review shows that the dilemma for Stewart theory is only apparent because transport of acid–base equivalents is accompanied by electrolytes. We suggest that Stewart theory may lead to new questions that must be investigated experimentally. Also, recent evidence from physiology that pH may not regulate acid–base transport is in accordance with the concepts presented by Stewart.

Introduction

Renal tubular acidoses (RTAs) are forms of metabolic acidoses that are thought to arise from a lack of urine excretion of protons or loss of bicarbonate (HCO_3^-) due to a variety of tubular disorders. Characteristically, this causes a hyperchloraemic (non-anion gap) acidosis without impaired glomerular filtration. Molecular studies have identified genetic or acquired defects in transporters of protons and HCO_3^- in many forms of RTA. However, at the same time these transporters have been found also to be involved in transport of Cl^- and Na^+. Furthermore, in a few cases RTA has been associated with primary defects in electrolyte transporters alone.

The core of Stewart theory is that transport of protons as such is unimportant to regulation of pH. In contrast, the theory states that acid–base homeostasis is directly regulated by electrolyte transport in the renal tubules. H^+ is effectively a balancing requirement imposed by physical chemistry. Accounting for how this occurs will probably lead to an improved understanding of homeostasis.

We begin the review by describing the classical formulation of the renal regulation of acid–base homeostasis. We then describe the quantitative physical chemistry notion of acid–base as described by Stewart (henceforth called the 'physicochemical approach'). On this basis we analyze some of the mechanisms that are active in RTA. We show that the physicochemical approach may lead to new questions that can be pursued experimentally to supplement insights already gained with classical theory. Several authors have suggested that the physicochemical approach could be used to the benefit of our understanding of RTA [1,2].

The kidney as regulator of acid–base balance

According to traditional concepts [3], daily acid production is calculated as the combined excretion of sulphate anion (SO_4^{2-}) and organic anions in the urine, whereas renal elimination of acid equivalents is computed as the combined titrable acidity + ammonium – excreted HCO_3^-, called net acid excretion (NAE). Cohen and coworkers [4] reviewed

A_{TOT} = total concentration of weak acids; CA = carbonic anhydrase; CD = collecting duct; DCT = distal convoluted tubule; dRTA = distal renal tubular acidosis; kNBC = kidney Na^+/HCO_3^- cotransporter; NAE = net acid excretion; P_{CO_2} = partial CO_2 tension; PHA = pseudohypoaldosteronism; ROMK = renal outer medullar K^+ channel; RTA = renal tubular acidosis; SID = strong ion difference; SLC = solute carrier; TSC = thiazide-sensitive cotransporter.

evidence indicating that the traditional view may be inconsistent with observations in patients in renal failure and in a number of experimental studies. In one of the studies assessed, Halperin and coworkers [5] examined rats loaded with extra alkali on top of already basic ordinary rat chow. Amazingly, increasing unmeasured organic anions had a 10-fold greater effect on alkali disposal than did changes in NAE, as traditionally computed. Similar findings had already been reported by Knepper and coworkers [6] in 1989. That acid–base balance is always accounted for by standard measurements may therefore be disputed. Although fervently rejected [3], this has given rise to a proposal of a new classification system for NAE that includes the regulation of loss of organic anions or potential HCO_3^- [7].

Difficulties in measuring titrable acidity and organic anions are one main source of disagreement with regard to acid–base homeostasis [4] both in normal persons and in those with renal impairment [8]. A recent Danish study [9] reinforced the concept from studies of healthy humans exposed to acid loads that nonmetabolizable base excretion is important to renal regulation of acid–base homeostasis.

Central to renal acid–base physiology is excretion of ammonium. One view [10] is that ammonium is produced as NH_4^+ in large quantities from hydrolysis of peptide bonds, and its excretion in urine has no bearing on acid–base chemistry except for the fact that for nitrogen balance it would otherwise have to be converted to urea – a process seen to consume bicarbonate. Exactly this argument was used again by Nagami [11] in an authoritative review of renal ammonia production and excretion. Most recently a study of normal individuals [12] showed that ureagenesis increased during experimental acidosis produced by $CaCl_2$. This contrasted with the authors' expectations because ureagenesis was supposed to cost alkali.

However, the traditional view is that NH_4^+ excretion is one of the most important mechanisms for eliminating metabolic acid equivalents because the leftover from deamination of glutamine is effectively bicarbonate and the process comes to a halt if NH_4^+ is not eliminated [13]. As stated in recent accounts, this view also accounts for the bicarbonate toll of ureagenesis [14] but the details of regulation and overall stoichiometry are still debated. However, it seems that the handling of NH_4^+ in the kidney is of great importance because a complicated network of transport mechanisms have evolved [11]. Most recently, a new group of putative NH_4^+ (and NH_3?) transporters related to the rhesus group of proteins has been described [15]. As far as we know, the result of missing one or more of these transporters on acid–base balance is not yet known, and because of redundancy it could be limited. Finally, apart from being a transported quantity that is of importance *per se*, NH_4^+ has also been found to influence a number of other tubular processes that are involved in acid–base regulation [16,17]. Hence, although there can be no doubt that excretion of NH_4^+ is important to acid–base homeostasis, it is not entirely clear why this is so. We suggest that the physicochemical approach to acid–base provides a more coherent picture of the role played by NH_4^+.

The Stewart approach to acid–base chemistry

Here we consider the approach to acid–base chemistry proposed by PA Stewart [18,19]. Biological fluids are dominated by a high concentration of water, approximately 55 mol/l. Physical chemistry determines the dissociation of water into protons and hydroxyl ions. If the determinants of that equilibrium are unchanged, then concentration of protons, and therefore pH, will be as well.

A number of important substances (e.g. many salts) dissociate completely to ions, when dissolved in water, whereas water itself dissociates to a very minor degree. Nonetheless, the dissociation of water into H^+ and OH^- provides an inexhaustible source and sink of acid–base equivalents. The proton concentration, and hence pH, is determined by the requirement that positive and negative charges must balance and by the combined equations that govern dissociations of involved species. The approach is formally based on analysis of separate compartments and leads to the result that $[H^+]$ in a compartment of physiological fluid is determined by the concentrations of fully ionized substances (strong ion difference [SID]), partial CO_2 tension (P_{CO_2}) and partly dissociated substances termed 'weak acids' in that compartment.

In a solution containing only fully dissociated salt (e.g. NaCl) the requirement for electrical neutrality leads to the following relation:

$$(Na^+ + H^+) - (Cl^- + OH^-) = 0 \quad (1)$$

The water dissociation equilibrium must also be obeyed:

$$[H^+] \times [OH^-] = K_w \times [H_2O] \approx K_w' \quad (2)$$

The SID is defined as the difference between fully dissociated cations and anions, and in the NaCl solution it is calculated as follows:

$$SID = [Na^+] - [Cl^-] \quad (3)$$

Combining Eqns 1, 2 and 3 leads to the following relation:

$$[H^+]^2 + SID \times [H^+] - K_w' = 0 \quad (4)$$

The positive solution to this second-degree polynomial yields:

$$[H^+] = -\frac{SID}{2} + \sqrt{[K_w' + (SID/2)^2]} \quad (5)$$

And from Eqn 2:

$$[OH^-] = -\frac{SID}{2} + \sqrt{[K_w' + (SID/2)^2]} \quad (6)$$

Hence, in a compartment/solution containing NaCl or similar salt solution, the proton concentration is simply determined by SID and the water ion product (K_w). Addition or removal of protons or hydroxyl ions may or may not be possible but will not change pH [20].

It is possible that the development of Stewart concepts to this extent will suffice for analysis of renal influences on acid–base homeostasis from a whole body or balance perspective. However, to present the theory of Stewart in a more complete form, we may also add weak acids and CO_2 to this framework. A full account of the Stewart approach with some later adaptations is available in a previous issue of this journal (see the report by Corey [21]).

Adding a weak acid, specifically a substance that participates in proton exchanges and hence that has a charge that is dependent on pH, Stewart showed that Eqn 7 had to be satisfied.

$$[H^+]^3 + (KA + SID) \times [H^+]^2 + (KA \times [SID - A_{TOT}] - Kw) \times [H^+] - KA \times Kw' = 0 \quad (7)$$

Where KA is the equilibrium constant and A_{TOT} is the total concentration of weak acids. To arrive at a satisfactory explanation for acid–base homeostasis from the whole body perspective, the pervasive effect of continuing production and transport and pulmonary excretion of CO_2 evidently must be taken into account. To do this, two more equations were needed:

$$[H^+] \times [HCO_3^-] = KC \times P_{CO_2} \quad (8)$$

$$[H^+] \times [CO_3^{2-}] = K3 \times [HCO_3^-] \quad (9)$$

Solving these together, Stewart's model in its most integrative form is now given by Eqn 10:

$$[H^+]^4 + ([SID] + KA) \times [H^+]^3 + (KA \times [[SID] - [A_{TOT}]] - KW - KC \times P_{CO_2}) \times [H^+]^2 - (KA \times [KW + KC \times P_{CO_2}] - K3 \times KC \times P_{CO_2}) \times [H^+] - KA \times K3 \times KC \times P_{CO_2} = 0 \quad (10)$$

These equations have explicit entries of constants and concentrations or tensions, but the practical use of the framework must be developed with detail sufficient to deal with the problem at hand. In plasma, other strong ions (e.g. Ca^{2+} and lactate) and weak acids are frequently found but they are treated on an equal footing.

A number of studies have shown that this algebra yields an accurate description or prediction of acid–base measurements. More importantly, however, the physicochemical approach may lead to a better understanding of mechanisms that are active in disease and treatment. An example of what may be accomplished is the successful application of the physicochemical approach to exercise physiology. Here, the ability of the independent variables to predict measured pH has been proven (correlation 0.985), but more importantly changes over time and between the different body compartments in these independent variables explain how a range of interventions influence acid–base as a part of muscle physiology [22].

CO_2 is transported in the body as a number of species and because the processes involved have variable latency (e.g. the Cl^-/HCO_3^- exchanger band3 in red blood cells [23]), widely differing values of P_{CO_2} are found in the body [24]. The physicochemical approach, focusing as it does on each compartment separately and having no special interest in the quantitatively lesser compartment of arterial blood, is at no disadvantage relative to conventional concepts in elucidating this difficult area. Although this is less of a problem when overall renal regulation of acid–base homeostasis is considered, notwithstanding that urine CO_2 may be of utility when diagnosing variants of RTA [25], it is a major problem with respect to understanding the underlying cellular transport processes. Further, recent results showing the complicated organization of transporters together in physically connected complexes indicate that much work will be needed if we are to understand the integrated molecular details of anion transport and CO_2 metabolism in renal tubules [26].

Whereas the physicochemical approach explains how pH is determined from independent variables, when applying this to urine the focus is not on regulation of urine pH but on the renal regulation of the independent variables that determine plasma and whole body acid–base balance. These independent variables are the SID, weak acids, and P_{CO_2}. Hence, from the point of view of the physicochemical approach, assessing urine with the aim of understanding the renal contribution to acid–base balance amounts to deducing its effects on the independent variables for a specified body compartment. It has been reported that the concepts of SID and weak acids may be blurred. For example, pH may influence the behaviour of species as either strong ions (components of SID) or weak acids [27], and this applies, for instance, to phosphates and proteins. Furthermore, neither Na^+ nor Ca^{2+} is invariably and totally dissociated, as implied by the common SID construct [28].

One important but thus far undeveloped aspect of the Stewart approach to whole body acid balance problems is that the independent variables for the extracellular compartment normally in focus may be only partly relevant to

the much larger intracellular compartment. Excretion of large amounts of potassium, for example, may be minimally relevant to SID in the extracellular compartment but may, depending on the circumstances, be crucial to intracellular SID [29].

It is evident that there will be differences in the approach to accounting for acid–base balance in the classical compared with the physicochemical approach. In the classical setting we must perform difficult titrations [4] and measurements of NH_4^+, P_{CO_2} and pH to compute a [HCO_3^-] after correction of pK for ionic strength. Every part of this is complicated, and the overall results with regard to our understanding of whole body balance are not universally accepted [4]. In the physicochemical approach, renal involvement in acid–base balance is manifested in its influence on independent variables – nothing more and nothing less. For a first approximation, this is the urine excretion of SID components, principally Na^+ and Cl^- when extracellular homeostasis alone is considered. It will be a practical matter to determine the extent to which the Stewart approach will be complicated by problems in computing both SID and weak acids in urine.

In the physicochemical approach, the urinary excretion of NH_4^+ or organic anions will be important for acid–base balance only to the extent that it influences SID in a body compartment. Excretion of organic anions is from this perspective a way to excrete Na^+ without Cl^- and thereby decrease SID in the body. This will result in increasing plasma H^+, no matter what the nature of the organic anion is. This hypothesis can be tested experimentally. On a similar footing, NH_4^+ excretion could be understood as means to excrete Cl^- without Na^+ in order to increase SID in the body. However, apart from their influence on SID, the excretion of these substances may convey important information about underlying pathophysiological processes. Hence, Kellum [30] has proposed that, when analyzing the mechanism of hyperchloraemic acidosis, an initial distinction could be made between states in which the kidney reacted normally (i.e. increasing the excretion of Cl^- relative to Na^+ and K^+ by augmenting NH_4^+ excretion and so causing urine SID to be more negative) and situations where, in spite of acidosis, the kidney continues to decrease whole body SID by excreting more Na^+ and K^+ than Cl^-. This will typically be the case in distal RTA (dRTA) without increased NH_4^+ excretion during acidosis.

Overview on renal tubular acidoses

Several types of RTA may be discerned [31]: proximal (type 2), distal (type 1), mixed (type 3), and a heterogeneous group of disorders characterized by hyperkalaemia and acidosis (type 4). RTA is a hyperchloraemic rather than an anion-gap-type metabolic acidosis. Typically, renal function (glomerular filtration rate) is unimpaired and the acidosis is not simply caused by absence of renal clearance. RTA must be separated from other forms of hyperchloraemic acidosis, some of which (e.g. the hyperchloraemic acidosis that occurs following saline infusion) are very important in the intensive care setting [32,33].

Proximal renal tubular acidosis (type 2)

Proximal RTA is classically characterized by impaired proximal reclamation of bicarbonate. This may be isolated or combined with other proximal tubular defects, and it may be congenital or acquired.

Proximal bicarbonate reabsorption is still incompletely understood [34]. Most of the bicarbonate [35] leaves the tubule lumen as CO_2 following sodium dependent H^+ secretion via Na^+/H^+ exchanger isoforms or (to a minor extent) vacuolar H^+-ATPase, apical anion exchange via formate enhanced Slc26a6, or other mechanisms [36], but some bicarbonate transport may also be paracellular [37]. The transport requires both membrane bound carbonic anhydrase (CA) type 4 and intracellular CA-2.

Among hereditary forms of RTA type 2 [38] is a very rare autosomal dominant disorder, the mechanism of which is unknown, but isoform 3 of the Na^+/H^+ exchanger (solute carrier [SLC]9A3) is a candidate. More common is an autosomal recessive form with ocular abnormalities, related to mutations in kidney Na^+/HCO_3^- cotransporter (kNBC)1 (SLC4A4) gene, which encodes the basolateral, electrogenic $Na^+/3(HCO_3^-)$ cotransporter. kNBC1 activity leads to a depolarization of the membrane and to extracellular accumulation of HCO_3^-. A recently identified potassium channel, named TASK2, recycles K^+ and repolarizes the potential, and mice that are deficient in this channel had metabolic acidosis associated with insufficient proximal bicarbonate reabsorption [39]. Recent studies of the regulation of kNBC1 and integrated transport in the proximal tubule have shown that, in addition to a substrate interaction, there is also a true macromolecular interaction between CA-2 and kNBC1 [40].

Sporadic forms, which are not yet characterized, also occur. However, most cases of proximal RTA are secondary and a host of associations have been described. Blockade of CA-4 by acetazolamide leads predictably to proximal RTA. Important are other genetic diseases that cause a generalized proximal tubular syndrome (Fanconi's; e.g. cystinosis, fructose intolerance, etc.) and drugs and toxins (e.g. ifosfamide [41], lead, mercury and cadmium), but light chain disease occurs among the elderly with proximal RTA. A number of medications have been related to proximal RTA [42].

Characteristic of proximal RTA is the presence of bicarbonaturia, with a fractional bicarbonate excretion of more than 15% when bicarbonate is given. Eventually, acid–base balance and urine acidification is achieved as plasma bicarbonate drops low enough for reabsorption to keep pace. Treatment may be difficult because administered base is often excreted before the desired normalization is achieved.

Explaining acidosis in proximal RTA from the conventional point of view is straightforward because the defining loss of urinary bicarbonate will inevitably deplete the body and result in hyperchloraemic acidosis. From the point of view of the physicochemical approach, the reciprocal retention of Cl^- and resulting decline in SID will also explain the findings.

In the conventional notion of acid–base regulation, proximal bicarbonate reabsorption is thought to be regulated by pH. However, based on studies of bicarbonate transport in the perfused rabbit proximal tubules, Boron and coworkers [43] concluded that the observed regulation would require both a CO_2 sensor and a HCO_3^- sensor. A pH sensor would not be enough. Stoichiometrically, a HCO_3^- sensor transmits the same information as a hypothetical SID sensor, and the results thus indicate that the proximal tubule senses the two important independent variables in the Stewart model. These quite new results could indicate that the physicochemical approach is highly relevant to our understanding of the mechanisms that underlie regulation of acid–base physiology.

Distal renal tubular acidosis (type 1)

dRTA is characterized by impaired ability to acidify the urine in the distal tubules and it is often accompanied by hypokalaemia, low urinary NH_4^+ and hypocitraturia. In contrast to proximal RTA, nephrocalcinosis and nephrolithiasis frequently occur. Clinically, dRTA occurs as a primary (persistent or transient) or secondary disorder. Secondary dRTA occurs in a great number of circumstances related to autoimmune diseases, drugs and toxins, and genetic or structural disruptions of renal tubules. Treatment of dRTA is simple and involves substituting about 1 mEq/kg of alkali per day.

The molecular details of some forms of primary dRTA are being pursued in great detail. α-Intercalated cells secrete H^+ by means of a vacuolar-type H-ATPase [44] (and possibly also a H^+/K^+-type ATPase), and bicarbonate is exchanged for Cl^- by means of anion exchanger (AE1) at the basolateral side. An autosomal dominant form of mutation in 17q21-22 of SLC4A1 leads to dysfunction of AE1 possibly related to mistargeting of the protein [45]. Also, AE1 mutations causing autosomal recessive dRTA and haemolytic anaemia have been described [46]. Otherwise, recessive forms of dRTA are related to mutations in the proton pump in α-intercalated cells. Some are accompanied by sensorineural deafness. The gene involved (ATP6V1B1) is located on chromosome 2, and encodes the B1-subunit of H^+-ATPase expressed apically on α-intercalated cells and also in the cochlea. dRTA with less impaired hearing is related to mutation in ATP6V0A4 on chromosome 7, which encodes a4, an accessory subunit of H^+-ATPase. As far as presently known, the H^+ pumps are electrogenic and, at least under some circumstances, they also involve shunting of the potential by Cl^-, although reverse transport of K^+ may also occur [44,47]. The Cl^- shunt pathway has not been elucidated yet nor aligned with any of the many known Cl^- channels [44]. Likewise, functional Cl^- channels (ClC5) are necessary to acidify transport vesicles in Dent's disease, pointing to the link between H^+ and Cl^- transport [48].

Jentsch and coworkers [49] recently presented a detailed examination of a mouse model that was knocked out for a K^+/Cl^- cotransporter, KCC4, which is located in the basolateral membrane in α-intercalated cells in the collecting duct. These animals had metabolic acidosis with alkaline urine, but electrolyte excretion in urine was unchanged compared with controls. The investigators measured a high intracellular $[Cl^-]$ and inferred a high intracellular pH also, driven by the basal HCO_3^-/Cl^- exchanger AE1. Although intracellular pH was not actually measured, and the defective cotransporter would be expected also to result in increased intracellular $[K^+]$, the results seem difficult to reconcile with a dominant effect of intracellular SID to set intracellular pH and with the notion that urine SID will have to change to explain acidosis in RTA. Details are awaited for this model; the authors also failed to document that conventional accounting for acid–base balance would explain the findings (decreased NAE would also change electrolyte excretion).

Recently, examination of the dRTA that is sometimes seen in cyclosporine A treatment has led to deeper insights into the tubular handling of protons and bicarbonate, but also – and importantly – that of Cl^-. In a study [50] of perfused rabbit collecting ducts, cyclosporine A inhibited acidosis induced downregulation of unidirectional HCO_3^- secretory flux in β-intercalated cells and prevented downregulation of the linked Cl^- resorption. Detailed examination of the apical and basolateral exchanges indicates that, rather than responding to, for example, intracellular pH, intracellular $[Cl^-]$ could be the regulated entity [51]. If true, this interpretation is compatible with a Stewart-based perspective.

A number of drugs and chemicals (e.g. amphotericin B [52], foscarnet and methicillin) have been found occasionally to cause dRTA [42], although details of the underlying mechanisms are not available.

Type 3 renal tubular acidosis (carbonic anhydrase dysfunction)

Type 3 RTA is caused by recessive mutation in the CA-2 gene on 8q22, which encodes carbonic anhydrase type 2 [53]. It is a mixed type RTA that exhibits both impaired proximal HCO_3^- reabsorption and impaired distal acidification, and more disturbingly osteopetrosis, cerebral calcification and mental retardation. The mechanisms that underlie the clinical picture in type 3 RTA, apart from much slower conversion of carbonic acid to and from bicarbonate, apparently also involve direct interaction between CA and the Na^+/HCO_3^- cotransporter kNBC1 [54] or Cl^-/HCO_3^- exchanger SLC26A6 [55]. From the physicochemical interpretation, acidosis is expected under these circumstances because of impaired transport of SID components.

Type 4 (hyperkalaemic) renal tubular acidosis

RTA type 4 or hyperkalaemic RTA is a heterogeneous group of disorders that is characterized by low urine NH_4^+, which is probably caused by the hyperkalaemia or by aldosterone deficiency or defective signalling. Causes include various types of adrenal failure or pseudohypoaldosteronism (PHA)1 due to defects in the mineralocorticoid receptor or the epithelial Na^+ channel, all characterized by salt loss and hypotension. A similar picture may be seen in obstructive uropathy or drug-induced interstitial nephritis. Furthermore, a number of drugs may impair signalling in the renin–aldosterone system and cause hyperkalaemia and metabolic acidosis (e.g. potassium sparing diuretics, trimethoprim, cyclo-oxygenase inhibitors, angiotensin converting enzyme inhibitors).

Lately, much interest has been given to a group of rare autosomal dominant diseases characterized by hyperkalaemia and acidosis and age-related hypertension [56]. In spite of hypervolaemia, aldosterone is not low and the disorders have been collectively termed pseudohypoaldosteronism type 2 (PHA2) [57]. Two of the mutations have been mechanistically characterized in some detail. Mutations in 17q21 in the *WNK4* gene may change the function of the protein, whereas a mutation in the intron to the *WNK1* gene at 12p increases transcription of the protein. Briefly, *WNK4* normally inhibits the thiazide-sensitive cotransporter (TSC) in the distal convolute tubule (DCT), and inhibits the renal outer medullar K^+ channel (ROMK) in the collecting duct (CD), but enhances paracellular Cl^- transport in both DCT and CD. Mutations in the *WNK4* gene that cause PHA2 are found to release the normal inhibition of TSC, but at the same time PHA2 enhances the inhibition of ROMK and enhances the paracellular Cl^- flux (but not Na^+ flux) through claudins. Hence, the hyperkalaemia is explained both by inhibition of ROMK and by decreased delivery of Na^+ to CD because of enhanced absorption in the DCT, and the good effect of thiazides on the hypertension is readily explained. The normal explanation for metabolic acidosis is based on the decreased delivery of Na^+ to CD and thereby inhibition of generation of lumen negative potential to enhance H^+ secretion in combination with the decreased delivery of NH_4^+ secondary to the hyperkalaemia [58].

The effect of the molecular abnormalities on Cl^- transport is barely considered in the explanation of the findings using the conventional model of acid–base. From the physicochemical approach it is evident that acidosis is well explained by the dominant and primary enhancement of Cl^- absorption in this disorder. Even if only the TSC effect were invoked, an isotonic expansion of body volume with Na^+ and Cl^- would be expected to yield acidosis. In any case, SID in plasma will decrease and pH will too. Very recently it was described that *WNK1* activates the epithelial Na^+ channel [59], and this was felt to explain the finding that not all patients with PHA2 are equally sensitive to thiazides. This would be expected to relieve the voltage imposed inhibition of H-ATPase in CD and likewise lessen the degree of hyperkalaemia. Electrolyte and NAE balance studies across different mutations may help to clarify how acid–base balance is actually constructed in these rare diseases.

Diagnosis and differential diagnosis

Traditionally, dRTA is recognized by the inability to decrease urine pH below 5.5 in spite of metabolic acidosis. These patients are also characterized by an inability to augment NH_4^+ excretion [60]. A high urine P_{CO_2} after bicarbonate loading has traditionally been the criterion for declaring distal H^+ secretion to be normal [61], and it was also recently found to identify patients with confirmed dRTA due to a proton pump problem [25].

Proximal RTA is characterized by high fractional excretion of bicarbonate (>15%) during loading, and an ability to achieve a urine pH below 5.5 during acidosis. Approaches are well described by Soriano [31] and Smulders and coworkers [62].

When assessing urine to gauge whether the physicochemical approach or the classical theory is best able to explain the acidosis in RTA, it is possible that both will do so successfully. From the physicochemical approach, the lack of urine NH_4^+ in distal RTA will force excretion of urine with a relatively high SID and this will explain the acidosis. An old study did in fact indicate that, in type 1 RTA, Na^+ loss and to a lesser degree Cl^- handling was abnormal in spite of long-term correction of acidosis [63].

The classical theory also explains the acidosis by a lack of amplification of NH_4^+ excretion. Likewise, for proximal RTA bicarbonate loss and high SID excretion will be equivalent. It was recently suggested that even though it may be difficult mechanistically to separate the implications of the theories, by using the physicochemical approach the focus is forced toward movements of Na^+ and Cl^-, and this may lead to a new understanding [2]. Indeed, analysis of *WNK* mutations confirms this expectation.

Conclusion

From the clinical viewpoint, the advantage of employing the physicochemical approach is that the renal contribution to acid–base homeostasis, even in complicated settings, can be ascertained in principle by simple chemical analysis of the urine. It is possible to explain RTA in general as a hyperchloraemic form of metabolic acidosis that can be described as a low SID acidosis, which has focused attention primarily on the net handling of SID constituents, namely Na^+, K^+, and Cl^-. This handling of SID constituents has not had a central position in our understanding of the various disease states, and in some cases only seems to be a consequence of anions necessarily being filled in by Cl^- as HCO_3^- goes down and reversely. However, in the future efforts will focus on which transport mechanism is active (e.g. is Cl^- moving with H^+ or K^+ or against it to shunt the potential generated by

the vacuolar H-ATPase [44]) and on which moiety is actually regulated by the tubular processes. A number of studies have recently focused on apical anion handling in the collecting duct via a newly characterized transporter, namely pendrin [64]. This exchanger seems well poised to react to Cl^- balance [65] and could therefore also be sensitive to the independent variable in acid–base regulation (i.e. SID) [66].

One defining point in the physicochemical approach that has an impact on the interpretation of acid–base phenomena is the concept of $[H^+]$ as a dependent variable, which tends to imply that clinical or physiological phenomena might more fundamentally depend on the baseline independent variables (e.g. SID, weak acids and P_{CO_2}). The necessity when analyzing renal phenomena to differentiate metabolic and respiratory acidosis may be an indicator that pH as such is not actually the sensed quantity.

In fact, how derangements in acid–base balance are sensed by the kidneys remains elusive, although there it is a general belief that such detection happens there. Quite recently, a protein, Pyk2, that was sensitive to pH and that regulated isoform 3 of the Na^+/H^+ exchanger in the proximal tubules was described [67]. Furthermore, in experiments identifying this alleged pH sensor, SID was directly varied but P_{CO_2} did not change. Hence, it is not evident that pH was really sensed, and in an accompanying editorial Gluck [68] expressed reservations regarding this notion. As explained above in relation to proximal RTA, recent studies conducted by Boron and coworkers [43] indicate that that bicarbonate and P_{CO_2} are the regulated entities, rather than pH, which is in accordance with the physicochemical approach to acid–base physiology insofar as bicarbonate and SID are equivalent.

Finally, if whole body acid–base balance is to be untangled, then the intracellular domains, which are likely to vary, must also be understood. In exercise physiology [69] advances have been made using the Stewart approach in elucidating plasma acid–base balance as it is perturbed by transfer of putative independent influences, but modelling cells or whole organs themselves from this point of view has not been done. This will entail such difficulties as determining water structure in cells and small confines [70] and modelling the pH effects of the structural proteins and nucleic acids as they fold and integrate. Modelling potassium balance in order to draw inferences regarding intracellular SID will likewise be necessary and interesting.

A recent study of patients in acute renal failure [71], employing state of the art methods, found that almost 80% of total body water appeared to be extracellular. This indicates that a great deal of experimental work must be done before analytical solutions [72] to the whole body multicompartment system can be derived and applied in clinical practice. We suggest that the physicochemical approach will prove useful in formulating hypotheses for future work aimed at developing a coherent, unpretentious and practical understanding of mechanisms involved in renal acid–base regulation.

Competing interests

The author(s) declare that they have no competing interests.

References

1. Corey HE: **Stewart and beyond: New models of acid-base balance.** *Kidney Int* 2003, **64:**777-787.
2. Laing CM, Toye AM, Caposso G, Unwin RJ: **Renal tubular acidosis: developments in our understanding of the molecular basis.** *Int J Biochem Cell Biol* 2005, **37:**1151-1161.
3. Lemann J Jr, Bushinsky DA, Hamm LL: **Bone buffering of acid and base in humans.** *Am J Physiol* 2003, **285:**F811-F832.
4. Cohen RM, Feldman GM, Fernandez PC: **The balance of acid, base and charge in health and disease.** *Kidney Int* 1997, **52:** 287-293.
5. Cheema-Dhadli S, Lin S-H, Halperin ML: **Mechanisms used to dispose of progressively increasing alkali loads in rats.** *Am J Physiol* 2002, **282:**F1049-F1055.
6. Brown JC, Packer RK, Knepper MA: **Role of organic anions in renal response to dietary acid and base loads.** *Am J Physiol* 1989, **257:**F170-F176.
7. Kamel KS, Briceno LF, Sanchez MI, Brenes L, Yorgin P, Kooh SW, Balfe JW, Halperin ML: **A new classification for renal defects in net acid excretion.** *Am J Kidney Dis* 1997, **29:**136-146.
8. Burbea Z-H, Gullans SR, Ben-Yaakov S: **ΔAlkalinity: a simple method to measure cellular net acid-base fluxes.** *Am J Physiol* 1987, **253:**C525-C534.
9. Osther PJ, Engel K, Kildeberg P: **Renal response to acute acid loading.** *Scand J Urol Nephrol* 2004, **38:**62-68.
10. Atkinson DE, Bourke E: **Metabolic aspects of the regulation of systemic pH.** *Am J Physiol* 1987, **252:**F947-F956.
11. Nagami GT: **Renal ammonia production and excretion.** In *The Kidney, Physiology and Pathophysiology*, 3rd ed. Edited by Seldin DW, Giebisch G. Philadelphia: Lippincott Williams & Wilkins; 2000:1995-2013.
12. Hosch M, Muser J, Hulter HN, Krapf R: **Ureagenesis: evidence for a lack of hepatic regulation of acid-base equilibrium in humans.** *Am J Physiol* 2004, **286:**F94-F99.
13. Gennari FJ, Maddox DA: **Renal regulation of acid-base homeostasis: integrated response.** In *The Kidney, Physiology and Pathophysiology*, 3rd ed. Edited by Seldin DW, Giebisch G. Philadelphia: Lippincott Williams & Wilkins; 2000:2015-2053.
14. Kurtz I, Dass PD, Cramer S: **The importance of renal ammonia metabolism to whole body acid-base balance: a reanalysis of the pathophysiology of renal tubular acidosis.** *Miner Electrolyte Metab* 1990, **16:**331-340.
15. Weiner ID: **The Rh gene family and renal ammonium transport.** *Curr Opin Nephrol Hypertens* 2004, **13:**533-540.
16. Frank AE, Weiner ID: **Effects of ammonia on acid-base transport by the B-type intercalated cell.** *J Am Soc Nephrol* 2001, **12:**1607-1614.
17. Frank AE, Wingo CS, Andrews PM, Ageloff S, Knepper MA, Weiner ID: **Mechanisms through which ammonia regulates cortical collecting duct net proton secretion.** *Am J Physiol* 2002, **282:**F1120-F1128.
18. Stewart PA: **Modern quantitative acid-base chemistry.** *Can J Physiol Pharmacol* 1983, **61:**1444-1461.
19. Stewart PA: *How to understand acid–base. A Quantitative acid–base primer for biology and medicine*. London: Edward Arnold; 1981.
20. Stewart PA: **Independent and dependent variables of acid–base control.** *Respir Physiol* 1978, **33:**9-26.
21. Corey HE: **Bench-to-bedside review: fundamental principles of acid-base physiology.** *Crit Care* 2004, **8:**184-192.
22. Lindinger MI, Heigenhauser GJF, McKelvie RS, Jones NL: **Blood ion regulation during repeated maximal exercise and recovery in humans.** *Am J Physiol* 1992, **262:**R126-R136.
23. Ring T, Andersen PT, Knudsen F, Nielsen FB: **Salicylate-induced hyperventilation [letter].** *Lancet* 1985, **i:**1450.
24. Geers C, Gros G: **Carbon dioxide transport and carbonic anhydrase in blood and muscle.** *Phys Rev* 2000, **80:**681-715.

25. Kim S, Lee JW, Park J, Na KY, Joo KW, Ahn C, Kim S, Lee JS, Kim GH, Kim J, *et al.*: **The urine-blood PCO2 gradient as a diagnostic index of H+-ATPase defect in distal renal tubular acidosis.** *Kidney Int* 2004, **66:**761-767.
26. Purkerson JM, Schwartz GJ: **Expression of membrane-associated carbonic anhydrase isoforms IV, IX, XII, and XIV in the rabbit: induction of CA IV and IX during maturation.** *Am J Physiol* 2005, **288:**R1256-R1263.
27. Wooten EW: **Analytic calculation of physiological acid-base parameters in plasma.** *J Appl Physiol* 1999, **86:**326-334.
28. Staempfli HR, Constable PD: **Experimental determination of net protein charge and Atot and Ka of nonvolatile buffers in human plasma.** *Am J Physiol* 2003, **95:**620-630.
29. Gowrishankar M, Chen CB, Mallie JP, Halperin ML: **What is the impact of potassium excretion on the intracellular fluid volume: importance of urine anions.** *Kidney Int* 1996, **50:**1490-1495.
30. Kellum JA: **Determinants of blood pH in health and disease.** *Crit Care* 2000, **4:**6-14.
31. Soriano JR: **Renal tubular acidosis: the clinical entity.** *J Am Soc Nephrol* 2002, **13:**2160-2170.
32. Kellum JA: **Metabolic acidosis in the critically ill: lessons from physical chemistry.** *Kidney Int* 1998, **Suppl 66:**s-81-s-86.
33. Constable PD: **Hyperchloremic acidosis: the classic example of strong ion acidosis.** *Anesth Surg* 2003, **96:**919-922.
34. Weinstein AM: **Mathematical models of renal fluid and electrolyte transport: acknowledging our uncertainty.** *Am J Physiol* 2003, **284:**F871-F884.
35. Petrovic S, Barone S, Weinstein AM, Soleimani M: **Activation of the apical Na+/H+ exchanger NHE3 by formate: basis of enhanced fluid and electrolyte reabsorption by formate in the kidney.** *Am J Physiol* 2004, **287:**F336-F346.
36. Wang Z, Wang T, Petrovic S, Tuo B, Riederer B, Lorenz JN, Seidler U, Aronson PS, Soleimani M: **Renal and intestinal transport defects in Slc26a6-null mice.** *Am J Physiol* 2005, **288:**C957-C965.
37. Guo P, Weinstein AM, Weinbaum S: **A dual-pathway ultrastructural model for the tight junction of rat proximal tubule epithelium.** *Am J Physiol* 2003, **285:**F241-F257.
38. Igarashi T, Sekine T, Inatomi J, Seki G: **Unravelling the molecular pathogenesis of isolated proximal renal tubular acidosis.** *J Am Soc Nephrol* 2002, **13:**2171-2177.
39. Warth R, Barrière H, Meneton P, Bloch M, Thomas J, Tauc M, Heitzmann D, Romeo E, Verrey F, Mengual R, *et al.*: **Proximal renal tubular acidosis in TASK2 K+ channel-deficient mice reveals a mechanism for stabilizing bicarbonate transport.** *Proc Natl Acad Sci USA* 2004, **101:**8215-8220.
40. Pushkin A, Abuladze N, Gross E, Newman D, Tatishchev S, Lee I, Fedotoff O, Bondar G, Azimov R, Nguyen M, *et al.*: **Molecular mechanism of kNBC1-carbonic anhydrase II interaction in proximal tubule cells.** *J Physiol* 2004, **559:**55-65.
41. Skinner R: **Chronic ifosfamide nephrotoxicity in children.** *Med Pediatr Oncol* 2003, **41:**190-197.
42. Hemstreet BA: **Antimicrobial-associated renal tubular acidosis.** *Ann Pharmacother* 2004, **38:**1031-1038.
43. Zhou Y, Zhao J, Bouyer P, Boron WF: **Evidence from renal proximal tubules that HCO3- and solute transport are acutely regulated not by pH but by basolateral HCO3- and CO2.** *Proc Natl Acad Sci USA* 2005, **102:**3875-3880.
44. Wagner CA, Finberg KE, Breton S, Marshansky V, Brown D, Geibel JP: **Renal vacuolar H+-ATPase.** *Physiol Rev* 2004, **84:**1263-1314.
45. Devonald MAJ, Karet FE: **Renal epithelial traffic jams and one-way streets.** *J Am Soc Nephrol* 2004, **15:**1370-1381.
46. Karet FE: **Inherited diatal renal tubular acidosis.** *J Am Soc Nephrol* 2002, **13:**2178-2184.
47. Paroutis P, Touret N, Grinstein S: **The pH of the secretory pathway: measurement, determinants, and regulation.** *Physiology* 2004, **19:**207-215.
48. Jentsch TJ: **Chloride transport in the kidney: lessons from human disease and knockout mice.** *J Am Soc Nephrol* 2005, **16:**1549-1561.
49. Boettger T, Hübner CA, Maier H, Rust MB, Beck FX, Jentsch TJ: **Deafness and renal tubular acidosis in mice lacking the K-Cl cotransporter Kcc4.** *Nature* 2002, **416:**874-878.
50. Watanabe S, Tsuruoko S, Vijayakumar S, Fischer G, Zhang Y, Fujimura A, Al-Awqati Q, Schwartz GJ: **Cyclosporin A produces distal renal tubular acidosis by blocking peptidyl prolyl *cis-trans* isomerase activity of cyclophilin.** *Am J Physiol* 2005, **288:**F40-F47.
51. Schwartz GJ, Tsuruoka S, Vijayakumar S, Petrovic S, Mian A, Al-Awqati Q: **Acid incubation reverses the polarity of intercalated cell transporters, and effect mediated by hensin.** *J Clin Invest* 2002, **109:**89-99.
52. Goldman RD, Koren G: **Amphotericin B nephrotoxicity in children.** *J Pediatr Hematol Oncol* 2004, **26:**421-426.
53. Shah GN, Bonapace G, Hu PY, Strisciuglio P, Sly WS: **Carbonic anhydrase II deficiency syndrome (osteopetrosis with renal tubular acidosis and brain calcification): novel mutations in CA2 indentified by direct sequencing expand the opportunity for genotype-phenotype correlation.** *Hum Mutat* 2004, **24:**272.
54. Pushkin A, Abuladze N, Gross E, Newman D, Tatishchev S, Lee I, Fedotoff O, Bondar G, Azimov R, Ngyuen M, *et al.*: **Molecular mechanisms of kNBC1-carbonic anhydrase II interaction in proximal tubule cells.** *J Physiol* 2004, **559:**55-65.
55. Alvarez BV, Vilas GL, Casey JR: **Matabolon disruption: a mechanism that regulates bicarbonate transport.** *EMBO J* 2005, **24:**2499-2511.
56. Gamba G: **Role of WNK kinases in regulating tubular salt and potassium transport and in the development of hypertension.** *Am J Physiol* 2005, **288:**F245-F252.
57. Kahle KT, Wilson FH, Lifton RP: **Regulation of diverse ion transport pathways by WNK4 kinase: a novel molecular switch.** *Trends Endocrinol Metab* 2005, **20:**1-6.
58. DuBose TD, Good DW: **Chronic hyperkalemia impairs ammonium transport and accumulation in the inner medulla of the rat.** *J Clin Invest* 1992, **90:**1443-1449.
59. Xu BE, Stippec S, Chu PY, Lazrak A, Li XJ, Lee BH, English JM, Ortega B, Huang CL, Cobb MH: **WNK1 activates SGK1 to regulate the epithelial sodium channel.** *Proc Natl Acad Sci USA* 2005, **102:**10315-10320.
60. Halperin ML, Richarson RMA, Bear RA, Magner PO, Kamel K, Ethier J: **Urine ammonium: the key to the diagnosis of diatal renal tubular acidosis.** *Nephron* 1988, **50:**1-4.
61. DuBose TD, Caflisch CR: **Validation of the difference in urine and blood carbon dioxide tension during bicarbonate loading as an index of distal nephron acidification in experimental models of distal renal tubular acidosis.** *J Clin Invest* 1985, **75:**1116-1123.
62. Smulders YM, Frissen PHJ, Slaats EH, Silberbusch J: **Renal tubular acidosis. Pathophysiology and diagnosis.** *Arch Intern Med* 1996, **156:**1629-1636.
63. Sebastian A, McSherry E, Morris RC Jr: **Impaired renal conservation of sodium and chloride during sustained correction of systemic acidosis in patients with type 1, classic renal tubular acidosis.** *J Clin Invest* 1976, **58:**454-469.
64. Frische S, Kwon T-H, Frøkiær J, Madsen KM, Nielsen S: **Regulated expression of pendrin in rat kidney in response to chronic NH4Cl or NaHCO3 loading.** *Am J Physiol* 2003, **284:**F584-F593.
65. Wall SM, Kim YH, Stanley L, Glapion DM, Everett LA, Green ED, Verlander JW: **NaCl restriction upregulates renal Slc26a4 through subcellular redistribution. Role in Cl- conservation.** *Hypertension* 2004, **44:**982-987.
66. Quentin F, Chambrey R, Trinh-Trang-Tan MM, Fysekidis M, Cambillau M, Paillard M, Aronson PS, Eladari D: **The Cl-/HCO3- exchanger pendrin in the rat kidney is regulated in response to chronic alterations in chloride balance.** *Am J Physiol* 2004, **287:**F1179-F1188.
67. Li S, Sato S, Yang X, Preisig PA, Alpern RJ: **Pyk2 activation is integral to acid stimulation of sodium/hydrogen exchanger 3.** *J Clin Invest* 2004, **114:**1782-1789.
68. Gluck SL: **Acid sensing in renal epithelial cells.** *J Clin Invest* 2004, **114:**1696-1699.
69. Putman, CT, Jones NL, Heigenhauser GJF: **Effects of short-term training on plasma acid-base balance during incremental exercise in man.** *J Physiol* 2003, **550:**585-603.
70. Truskett TM: **The subtleties of water in small spaces.** *Proc Natl Acad Sci USA* 2003, **100:**10139-10140.
71. Ikizler TA, Sezer MT, Flakoll PJ, Hariacher S, Kanagasundaram NS, Gritter N, Knights S, Shyr Y, Paganini E, Hakim RM, *et al.*: **Urea space and total body water measurements by stable isotopes in patients with acute renal failure.** *Kidney Int* 2004, **65:**725-732.
72. Wooten EW: **Calculation of physiological acid-base parameters in multicompartmental systems with application to human blood.** *J Appl Physiol* 2003, **95:**2333-2344.