Studies to identify the basis for an alkaline urine pH in patients with calcium hydrogen phosphate kidney stones

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Abstract

Background. Patients with CaHPO$_4$ kidney stones belong to a diagnostic category that has a high urine pH as its common feature. Our objective was to provide a new clinical approach to examine the basis for this high pH.

Methods. The study group consisted of 26 CaHPO$_4$ stone formers and 28 normal volunteers. Urine was collected q2h plus an overnight sample to identify patients with a urine pH > 6.5 for 12/24 h. Urine ammonium (U$_{NH_4}$), sulphate (U$_{SO_4}$) and citrate were measured and diet net alkali was calculated.

Results. Of the 26 patients, 13 had persistently alkaline urine. In 7/13, U$_{NH_4}$ (68 ± 13 mEq/day) and U$_{SO_4}$ (57 ± 7 mEq/day) were both high. In 6/13 patients, U$_{NH_4}$ was the usual 31 ± 3 mEq/day; in 4/6, U$_{NH_4}$/U$_{SO_4}$ was 0.9 ± 0.1; the cause of the alkaline urine pH seemed to be a dietary alkali load because the rise in urine pH was episodic and coincided with a high net diet alkali load and peak citrate excretion rates. The remaining two patients had a high U$_{NH_4}$/U$_{SO_4}$ (2.2 and 1.6). Citrate excretion was very low in the male, but not in the female patient.

Conclusions. There are heterogeneous causes for a persistently high urine pH. Two of the patients had a possible molecular basis: the lesion could be a low proximal convoluted tubule cell pH in the male and an increased entry of NH$_3$ into the late distal nephron in the female.

Keywords: alkali ingestion; ammonium; citrate; kidney stones; sulphate; urine pH

Introduction

Nephrolithiasis is a common disorder that is associated with significant morbidity and a substantial cost to the health care system. About 15% of kidney stone formers produce stones that are composed predominantly of calcium hydrogen phosphate (CaHPO$_4$) [1]. In a recent study, Evan et al. [2] demonstrated that patients with this type of kidney stone develop progressive parenchymal damage and nephron loss due to plugging of the terminal collecting duct with CaHPO$_4$ crystals. These findings emphasize the importance of understanding the underlying pathophysiology that leads to the precipitation of these crystals so that an effective, individualized therapy could be designed.

CaHPO$_4$ precipitates are formed when the ion product for ionized calcium and divalent phosphate (HPO$_{2}^-_4$) exceeds its solubility product constant (K$_{sp}$) and its ability to remain dissolved in a supersaturated solution. Only ~1/6 of the urine phosphate is in the form of HPO$_{2}^-_4$ when the urine pH is ~6.1 whereas half of the urine phosphate will be in its HPO$_{2}^-_4$ form at a urine pH of 6.8 (the pK for this buffer system in urine. Table 1) [3]; hence, a urine pH of 6.8 increases the potential risk for precipitation of CaHPO$_4$ by 3-fold. On the other hand, there is little extra risk when the urine pH rises from 7.1 to 7.5.

Patients with CaHPO$_4$ kidney stones belong to a diagnostic category that have a common feature—a high urine pH (1)—rather than representing a specific disease entity. It is important to establish the basis for this high urine pH, to understand why recurrent CaHPO$_4$ kidney stones are formed in an individual patient, because there are many possible explanations for this alkaline urine pH. For example, patients with distal renal tubular acidosis (RTA) caused by a low net distal H$^+$ secretion rate have a high urine pH. Because of acidemia, they also have a low rate of excretion of citrate and thereby, an increased risk of forming CaHPO$_4$ kidney stones [4]. Since the pathophysiology in this subgroup of patients is
well-characterized, these patients were excluded from the present investigation.

The focus of this investigation is on a different subgroup of patients. Although they too have a persistently high urine pH, they are easily distinguished from patients with distal RTA because metabolic acidosis is not present and they do not have a low rate of excretion of NH$_4^+$ [5]. The rate of excretion of NH$_4^+$ should be examined relative to the dietary non-volatile acid load that requires the excretion of NH$_4^+$ (U$_{NH4}$) for H$^+$ elimination. This is revealed for the most part by the rate of excretion of sulphate anions (SO$_4^{2-}$) in the urine (U$_{SO4}$). In control subjects, the U$_{NH4}$/U$_{SO4}$ is close to 1 in mEq terms [6].

Our results show that in a subgroup of patients, the rate of excretion of NH$_4^+$ was high, but the U$_{NH4}$/U$_{SO4}$ was close to 1—this indicated that the high medullary interstitial NH$_3$ in these patients was due to a high diet acid load (equation 1). In another subgroup, the rate of excretion of NH$_4^+$ was not high; these patients however, represented two distinct causes based on their U$_{NH4}$/U$_{SO4}$. In some, the U$_{NH4}$/U$_{SO4}$ was close to one; hence the alkaline urine pH seemed to be driven by a high episodic dietary alkali intake. Two patients had high U$_{NH4}$/U$_{SO4}$ (2.2 and 1.6)—their rate of excretion of citrate was markedly different, and this suggested two different possible lesions to explain their persistently alkaline urine pH.

$$H^+ + NH_3 \rightarrow NH_4^+$$  \hspace{1cm} (1)

Subjects and methods

The Research Ethics Board at St. Michael’s Hospital approved the study protocol; informed consent was obtained from all patients and control subjects.

Control subjects

There were 28 normal volunteers, 14 males and 14 females, mean age 32 ± 3 years, with no history of kidney stones.

Patients

Twenty-six patients with recurrent CaHPO$_4$ stones were studied; >50% of each of their stones consisted of CaHPO$_4$ and Ca$_3$(PO$_4$)$_2$ by X-ray diffraction crystallography.

Procedures

Data were obtained while the patients and the control subjects consumed their usual diet, and did not take medications for at least 1 week. To select patients with persistently high urine pH values, urine was voided voluntarily q2h while awake plus an overnight collection (to permit undisturbed sleep) over the 24-h period [6]. Time and volume of voiding were recorded. Thymol was added to the urine as the preservative. To characterize the pathophysiology of the alkaline urine pH, the rate of excretion of NH$_4^+$, SO$_4^{2-}$ and citrate was measured in 24-h urine collections and net dietary alkali was calculated as described below. Glomerular filtration rate (GFR) was calculated by endogenous creatinine clearance.

Net diet alkali. The potential alkali load of the diet was calculated as the sum of the excretion of urinary cations (Na$^+ + K^+ + Ca^{2+} + Mg^{2+}$) minus the sum of the excretion of urinary anions (Cl$^-$ + inorganic phosphate) in mEq terms [6,7]. The excretions of NH$_4^+$ and SO$_4^{2-}$ were not included in this analysis because they represent the daily H$_2$SO$_4$ load and its elimination.

Analytical techniques

Bicarbonate (HCO$_3^-$), pH, sodium (Na$^+$), potassium (K$^+$), chloride (Cl$^-$), phosphate, calcium (Ca$^{2+}$), magnesium (Mg$^{2+}$), NH$_4^+$, SO$_4^{2-}$, creatinine, urea, osmolality and citrate were measured in plasma and urine as previously described [6].

Results

The urine pH was close to 6.0 in the control subjects throughout the day (Figure 1). In addition, the rates of excretion of NH$_4^+$ (28 ± 2 mEq/day) and SO$_4^{2-}$ (31 ± 3 mEq/day) were very similar while citrate excretion was 9 ± 1 mEq/day (Table 2). The ratio of U$_{SO4}$ to nitrogen (U$_N$) in the urine was 2.2% in mmol terms largely reflecting the proportion of sulphur-containing amino acids in their ingested protein.

In 13/26 patients, the urine pH was not >6.5 for more than 12/24 h; hence these patients were excluded from further study (Figure 2). Although, this definition is arbitrary, our purpose was to focus on patients with persistently high urine pH values.

To characterize the pathophysiology of the alkaline urine pH, patients were separated into two groups based on the rate of excretion of NH$_4^+$ (Figure 2). Seven patients (7/13) had an NH$_4^+$ excretion rate that was greater than 50 mEq/day (68 ± 13 mEq/day); this high NH$_4^+$ excretion rate was accompanied by a rate of excretion of SO$_4^{2-}$ that was almost 2-fold higher than in the control subjects (57 ± 7 vs 31 ± 3 mEq/day).
In the remaining 6/13 patients, the NH$_4^+$ excretion rate was 31 ± 2 mEq/day. These patients, however, represented two distinct subgroups based on their UNH$_4$/USO$_4$ that was close to unity, and two patients had markedly higher UNH$_4$/USO$_4$ (values in the rectangles below).

In the remaining 6/13 patients, the NH$_4^+$ excretion rate was 31 ± 3 mEq/day. These patients, however, represented two distinct subgroups based on their UNH$_4$/USO$_4$. In 4/6 patients, the UNH$_4$/USO$_4$ was close to unity (0.9 ± 0.1). In the diurnal pattern of their urine pH, there was a period where the urine pH was distinctly higher than other values. In a representative patient, the time when there was a higher net alkali excretion rate (Figure 3A), there was a lower rate of excretion of NH$_4^+$ but not SO$_4^{2-}$ (Figure 3B), and a small rise in the rate of excretion of citrate (Figure 3C). This pattern suggests that the basis for the high urine pH might have been a high intake of alkali. The other two patients had very high UNH$_4$/USO$_4$ ratios (1.6 and 2.2). The first patient, a 40-year-old male, passed his first stone at age 10 and the second patient, a 17-year-old female, passed her first stone at age 7. Both patients had urine collections on two occasions that were separated by a period of more than 4 weeks, while consuming their usual diet and taking no medications. While the findings in each patient were similar in both collections, the data shown in Table 2 are from a single 24-h collection in each patient that was selected because the urine flow rate and the ratio of USO$_4$/UN were similar to control subjects. This later ratio was used to ensure that the rate of excretion of SO$_4^{2-}$ reflects the diet acid load from metabolism of sulphur-containing amino acids. The endogenous creatinine clearance was 120 l/day in the male patient and 123 l/day in the female patient.

The pattern of the urine pH throughout the 24-h cycle in the control subjects and these two patients is shown in Figure 1. The control group had urine pH values that were consistently close to 6.0. In contrast, the two patients had substantially higher urine pH values for the bulk of the 24-h period; the urine pH values were higher in the male patient.

The next factor evaluated was the rate of excretion of NH$_4^+$ and SO$_4^{2-}$ and its variation in the 24-h period. In the male patient, the rate of excretion of NH$_4^+$ was greater than the rate of excretion of SO$_4^{2-}$ throughout the day (Figure 4). It is noteworthy that this was achieved with a urine pH that was ~7.0. In the female patient, there was an 8-h period where the rates of excretion of NH$_4^+$ and SO$_4^{2-}$ were similar, and a 16-h period where the rate of excretion of NH$_4^+$ was considerably greater than that of SO$_4^{2-}$. Hence, it is possible that there are different mechanisms operating in the two patients.

The final factor evaluated was the rate of excretion of citrate and its variation in the 24-h period. In the male patient, the rate of excretion of citrate was strikingly low throughout the day (Figure 5). This markedly low rate of excretion of citrate was not due to a reduced dietary intake of alkali, as his calculated dietary net alkali load was high (Table 2). In contrast,
the rate of excretion of citrate was similar to that in control subjects in the female patient (Figure 5).

Discussion

The purpose of this study was to examine the pathophysiology of the alkaline urine pH in patients with CaPHO4 stones who do not have distal RTA. A second aim was to identify patients in whom dietary factors do not seem to play a central role in the pathophysiology of their alkaline urine pH. Accordingly, the latter patients may have specific transport defects as a basis for their disease.

The label ‘incomplete RTA’ originated in an era where the lynchpin in the diagnosis of distal RTA was a high urine pH in patients with hyperchloraemic metabolic acidosis [8,9,10]. Hence, it is not surprising that when patients present with recurrent CaHPO4 stones accompanied by a high urine pH in the absence of hyperchloraemic metabolic acidosis, the term used to describe this clinical scenario was ‘incomplete RTA’. These same findings, however, are present in three different settings. The first subgroup of patients does not have a renal lesion—their high urine pH is the expected response to the intake of a high ‘alkaline-ash’ diet [7]. The second subgroup of patients has the subtype of distal RTA that is due to a decreased distal secretion of H+ [6] or augmented distal secretion of HCO3- [11], yet they do not have hyperchloraemic metabolic acidosis because it is masked by the intake of a diet that yields a low net H+ load. The diagnosis in these subgroups may be confirmed by the finding of a low rate of excretion of NH4+ Patients with a low net distal H+ secretion will have a low rate of excretion of NH4+ in response to a chronic acid load (NH4Cl). Uniquely, those with the distal secretion of HCO3- subtype have been observed to have a high urine PCO2 in alkaline urine [11]. A third group of patients has what could be called ‘true incomplete RTA’, because they have a high urine pH and a high rate of NH4+ excretion (as compared with that of SO42-) [5]. The basis of their alkaline urine pH is an increased entry of NH3 into the lumen of the collecting duct; nevertheless, these patients represent a heterogeneous group with regard to the pathophysiology of this disorder. Since the rate of excretion of NH4+ in these patients is already high, a urine minus blood PCO2 test and an NH4Cl loading test are not needed to include a patient in this diagnostic category.

In summary, the term ‘incomplete RTA’ probably should be abandoned at this point in time (the Appendix). Alternatively, if one wished to use this term, it should be redefined—reserving it for one special subgroup of patients—those with persistently high urine pH values and a high rate of excretion of NH4+ as compared with that of SO42- [6,12].
two pathophysiological reasons for their alkaline urine pH. It is for these reasons that we described our workup of patients with CaHPO₄ stones using pathophysiological categories (e.g. high urine pH along with high rate of excretion of NH₄⁺) to avoid misunderstandings due to terminology (Figure 1).

The key to our analysis of the basis of alkaline urine pH is an understanding of how acid balance and base balance are achieved [13,14]. Before discussing the data, we provide a brief synopsis of pertinent issues.

**Acid-balance.** Although the major acids requiring renal disposal are H₂SO₄ and phosphoric acid, only the H⁺ from H₂SO₄ require NH₄⁺ excretion to eliminate these protons because of the low affinity of SO₂⁻/C₀₄ for H⁺. Acid balance is normally achieved when SO₂⁻ is excreted with an equivalent amount of NH₄⁺ in the urine (Table 2) [15–17]. To examine the stimulus for the production of NH₄⁺ in the proximal convoluted tubule (PCT), we measured the rate of citrate excretion as a ‘window’ on the PCT cell pH [18].

**Base balance.** Dietary alkali is derived from the metabolism of ingested fruits and vegetables, but it is not eliminated by the excretion of an appreciable quantity of HCO₃⁻ in the urine as the urine pH in our control subjects was consistently close to 6.0 (Figure 1). Therefore, dietary alkali is eliminated by excreting a family of organic anions, including citrate [14]. If there were a defect in the excretion of organic anions, excreting HCO₃⁻ may be the means to eliminate some of the dietary alkali, but this would raise the urine pH. Accordingly, we measured the rate of excretion of citrate to help evaluate the base balance [18]. We assessed net diet alkali using the calculation described in Subjects and methods.

On initial screening, 13/26 patients with a history of CaHPO₄ stones were excluded because their urine pH was >6.5 for considerably less than 12/24 h. In 7/13 patients, the basis of a persistently high urine pH was a high addition of NH₃ to the medullary collecting duct that was secondary to the renal response to a chronic dietary acid load (Figure 6). This subset was identified because they had high rates of excretion of NH₄⁺ and SO₂⁻. The urine pH values in these patients, however, are rather higher than those observed in normal control subjects who were given a chronic acid load by ingesting NH₄Cl. These subjects excreted large amounts of NH₃ at the time when the urine pH is ~6.0 [19–21]. Therefore, it is important to note that, while dietary factors seem to be important in the pathophysiology of their alkaline urine pH, it is also possible that a transport defect akin to the ones described below may also be present. To characterize the pathophysiology, these patients will need to be examined while consuming a standardized diet.

In 4/13 patients, a contributing factor for their high urine pH seemed to be a large alkali intake. This was identified by finding an episodic diet net alkali load with a coincident rise in the urine pH and citrate excretion rate, as well as a fall in the rate of NH₄⁺ excretion that was not due to a diminished rate of excretion of SO₂⁻ (Figure 3).

In 2/13 patients, the pathophysiology of the alkaline urine pH did not seem to be related to dietary factors.
In more detail, both had a rather high rate of excretion of NH$_4^+$ relative to their dietary acid load (rate of excretion of SO$_4^{2-}$). These two patients were studied in more detail to identify possible transport defects. Of note, both patients had the onset of their stone disease at young age.

**Possible basis for the high urine pH in the male patient**

The male patient had marked hypocitraturia in the absence of systemic acidosis or hypokalaemia (Figure 5); this suggested that he might have intracellular acidosis restricted to PCT cells [4]. Accordingly, one explanation for his high urine pH might be the need to dispose of dietary alkali by excreting HCO$_3^-$ instead of citrate because of enhanced proximal reabsorption of citrate [14]. The second reason for the high urine pH could be an increased medullary interstitial availability of NH$_3$ caused by the high PCT [H$^+$], which stimulated ammoniagenesis [22]. This will require, however, that gating of the NH$_3$ channel be modulated by the availability of NH$_3$ or a related compound or ion (Figure 6); this will be discussed in more detail when the data in the female patient are analysed.

**Possible basis for the high urine pH in the female patient**

The female patient, while similar in many respects, did not have a low rate of excretion of citrate (Figure 5). Therefore, it is unlikely that she had an acidified PCT cell pH. Newer insights into the physiology of the renal transport of NH$_4^+$ suggest a different possibility (Figure 6) [23]. There is an NH$_3$ channel whose mouth is very hydrophobic, which allows for $\sim$3 order of magnitude fall in the pK for NH$_4^+$ within the channel mouth [23]. This raises the concentration of NH$_3$ within the channel by $\sim$1000-fold, and provides the concentration difference needed for diffusion of NH$_3$ into the lumen of the medullary collecting duct. Accelerated entry of NH$_3$ into its lumen can raise both the $U_{\text{NH4}}$ and the urine pH. There are two types of Rh-glycoproteins that may form these NH$_3$ channels, one in the basolateral and the other in the luminal membrane of the medullary collecting duct cells in rat and mouse kidneys [24]. It is possible that a lesion that causes a higher open-probability and/or a greater number of NH$_3$ channels enhances the entry of NH$_3$ into the lumen of medullary collecting duct. This will drive the urine pH upward as well as increase the rate of excretion of NH$_4^+$.

**Conclusions**

Our data reveal that patients with CaHPO$_4$ kidney stones are heterogeneous with respect to the pathophysiology of their alkaline urine pH. Two possible novel lesions that may cause persistently alkaline urine pH values in a subset of patients with CaHPO$_4$ nephrolithiasis were described. Recognizing this difference in pathophysiology may lead to novel strategies for therapy.

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**References**


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**Appendix**

**Incomplete RTA**

This diagnostic category is based on finding a persistently high urine pH in patients who do not have ‘complete’ distal RTA of the subtype with low net distal H$^+$ secretion [5]—i.e. patients who do not have hyperchloremic metabolic acidosis. Notwithstanding, it includes more than one specific disease or pathophysiological entity. Ruling out other causes of a high urine pH should improve diagnostic specificity.

**Conditions to remove from the 'incomplete RTA' diagnostic category.**

(i) Patients with distal RTA due to low net distal secretion of H$^+$ who consume a low net H$^+$ load

Although these patients usually have a degree of acidaemia, a low blood pH may not be present if they ingest a low net H$^+$ load. In more detail, if their diet were to have more alkali (e.g. fruit and vegetables) and/or less precursors of H$_2$SO$_4$ (e.g. low protein intake), acidaemia may be absent. Accordingly, the key to the diagnosis is a low rate of excretion of NH$_4^+$ (i.e., U$_{NH_4}$< U$_{SO_4}$) (equations A1 and A2). One would expect that these patients should develop acidaemia if they consume a typical Western diet. The diagnosis could be confirmed by finding of a low rate of excretion of NH$_4^+$ in response to a chronic acid (NH$_4$Cl) load [25] and a low urine PCO$_2$ in alkaline urine [26].

**Normal subjects**

\[
\text{H}^+ + \text{NH}_3 \leftrightarrow \text{NH}_4^+ \quad (\text{NH}_4^+ = \text{SO}_4^{2-}) \quad \text{(A1)}
\]

**Low net distal H$^+$ secretion**

\[
\text{H}^+ + \text{NH}_4^+ \leftrightarrow \text{NH}_3 + \text{H}_2\text{SO}_4 \quad (\text{NH}_3 < \text{SO}_4^{2-}) \quad \text{(A2)}
\]

(ii) Large intake of alkali in a normal subject

A second category of patients who have a normal plasma pH and plasma HCO$_3^−$ concentration along with a high urine pH includes normal individuals with a large dietary intake of alkali precursors. Clues to examine include the rate of excretion of NH$_4^+$ as compared with SO$_4^{2−}$ excretion in mEq terms [6] (see equations A1 and A2) and the rate of excretion of net alkali [14] (e.g. organic anions including citrate).
In more detail, shortly after dietary alkali is ingested, the rate of excretion of NH\textsuperscript{+} as compared with SO\textsubscript{2}\textsuperscript{−}/CO\textsubscript{2}\textsuperscript{−} should fall while the rate of excretion of organic anions plus citrate should rise. To exclude these patients from the ‘true incomplete RTA’ category, they should be studied on a typical western diet to see if a high alkali ingestion may have been responsible for their high urine pH. Thus, this category resembles the patients with distal RTA due to low net H\textsuperscript{+} secretion and a low net H\textsuperscript{+} load described above, except that they will not develop acidaemia when they consume the typical Western diet.

Patients with true ‘incomplete RTA’. These patients also come to medical attention because they have CaHPO\textsubscript{4} kidney stone disease. Their initial diagnostic workup also reveals a high urine pH for much of the day. The key diagnostic step in this group of patients is finding a high rate of excretion of NH\textsubscript{4} as compared with that of SO\textsubscript{2}\textsuperscript{−} (equations A1 and A2). Contrary to normal subjects in whom the daily excretion of NH\textsubscript{4} and SO\textsubscript{2} are in a 1:1 proportion and in the patients with distal RTA and a low net dietary intake of H\textsuperscript{+} precursors who excrete less NH\textsubscript{4} than SO\textsubscript{2}, their daily excretion of NH\textsubscript{4} is considerably greater than their daily excretion of SO\textsubscript{2}. Since the rate of excretion of NH\textsubscript{4} in these patients is already high relative to their dietary non-volatile acid load, an NH\textsubscript{4}Cl loading test is not required to confirm this diagnosis.

Since these patients achieve high rates of excretion of NH\textsubscript{4} while their urine pH is also high, the basis of their disease is a subgroup of disorders that have in common an increased entry of NH\textsubscript{3} into the lumen of the collecting duct. We described two subtypes of ‘true incomplete RTA’ in the paper—those with an acidified proximal convoluted tubular cell pH and those with a primary increase in the medullary NH\textsubscript{3} shunt [27].