Masterclasses in medicine

Recurrent uric acid stones

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Summary

A 46-year-old female had a history of recurrent uric acid stone formation, but the reason why uric acid precipitated in her urine was not obvious, because the rate of urate excretion was not high, urine volume was not low, and the pH in her 24-h urine was not low enough. In his discussion of the case, Professor McCance provided new insights into the pathophysiology of uric acid stone formation. He illustrated that measuring the pH in a 24-h urine might obscure the fact that the urine pH was low enough to cause uric acid to precipitate during most of the day. Because he found a low rate of excretion of NH₄⁺ relative to that of sulphate anions, as well as a high rate of citrate excretion, he speculated that the low urine pH would be due to a more alkaline pH in proximal convoluted tubule cells. He went on to suspect that there was a problem in our understanding of the function of renal medullary NH₃ shunt pathway, and he suggested that its major function might be to ensure a urine pH close to 6.0 throughout the day, to minimize the likelihood of forming uric acid kidney stones.

Introduction

In this case discussion, once again the central figure is Professor McCance, an imaginary consultant, who practiced medicine around 70 years ago. As usual, the overall objective is to demonstrate how the application of principles of integrative physiology at the bedside can be extremely helpful to reveal the pathophysiology of disease, to make better clinical diagnoses, and to plan optimal therapy. Using this approach, new concepts with respect to the pathophysiology of uric acid stone formation and the physiology of ammonium (NH₄⁺) excretion would be revealed.

The consultation

A 46-year-old female had a history of recurrent uric acid stones. There was no history of hyperuricaemia. The only positive findings on physical examination were a high blood pressure (150/100 mmHg) and moderate obesity. The composition of her urine is summarized in Table 1, which shows that the rate of excretion of total urates was not elevated and her 24-h urine pH was not low enough to cause uric acid to precipitate. Her glomerular filtration rate (GFR) and renal concentrating ability were normal. Because there was no obvious reason to explain why uric acid stones continued to
form, Professor McCance was asked to help. In his usual methodical way, their Professor used simple physiological concepts to analyse the data in depth—this led to new insights into this medical problem, and into the physiology of NH₄⁺ excretion as well.

Synopsis of uric acid production

The medical registrar began with a brief review of uric acid metabolism, which he had considered when trying to resolve their dilemma. Urates are the major end product of purine metabolism in humans because the gene that encodes for uricase—the enzyme that degrades uric acid—was inactivated very early in the Myocene period (Appendix). On a typical Western diet, humans excrete ~10 mg total urates per kg of body weight per day. Uric acid—and not the urate anion—is the focus of our attention, because its concentration can rise sufficiently to exceed its solubility product constant (Ksp) in the urine. The PK of uric acid is 5.35 at 37°C, while its Ksp is ~100 mg/l; supersaturation of the urine with uric acid occurs up to a concentration of ~200 mg/l. As shown in equation 1, there are two ways to elevate the concentration of uric acid in the urine: raise the total urate excretion rate or raise the urine H⁺ concentration.

\[ \text{H}^+ + \text{Urate}^- \rightleftharpoons \text{Uric acid} \] (1)

The patient did not have a high rate of urate excretion (600 mg/day). There did not seem to be a problem of a low urine volume, because she had been advised to drink much more water after passing her first kidney stone—her usual 24-h urine volume was now >2 l/day (equation 2). Hence the rate of excretion of uric acid could only be high if the urine had a very high H⁺ concentration (low urine pH), but her 24-h urine pH was 5.6, not low enough to cause uric acid to precipitate (Table 2).

\[ \text{[Uric acid]}_{\text{urine}} = \frac{\text{Uric acid content}}{\text{Urine volume}} \] (2)

The team was aware that the presence of a ‘nidus’ could cause a precipitate to form even if the urine was only mildly supersaturated with uric acid. This, however, did not seem to be the case in their patient because her urinary ionized calcium (Ca²⁺) multiplied by either the urine oxalate or divalent phosphate (HPO₄²⁻) concentration did not yield an ion product that was well above their respective Ksp values.

Having concluded the presentation, the registrar turned to Professor McCance to explain why their patient had recurrent uric acid stones. His first thoughts were that the total urinary urates might have been underestimated, or that there was a problem with the measurement of the urine pH. He turned to the group and asked, ‘How could the rate of excretion of total urates be underestimated?’

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Summary of data from the 24-h urine collection</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
</tr>
<tr>
<td>pH</td>
<td>6.0 ± 0.2</td>
</tr>
<tr>
<td>Volume (l/day)</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>Total urate (mg/day)</td>
<td>565 ± 25</td>
</tr>
</tbody>
</table>

Data represent means ± SEM for the controls (13 males and 4 females).

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Influence of the urine pH and volume on the concentration of uric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total urate (mg/day)</td>
<td>Urine pH</td>
</tr>
<tr>
<td>600</td>
<td>5.3</td>
</tr>
<tr>
<td>600</td>
<td>5.3</td>
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<tr>
<td>600</td>
<td>6.0</td>
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<tr>
<td>600</td>
<td>6.0</td>
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</tbody>
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This table is provided to illustrate the effects of the urine volume and the urine pH on the uric acid concentration when the total urate excretion rate is 600 mg/day.

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Question 1. How could the rate of excretion of total urates be underestimated?

Without waiting for an answer, Professor McCance pointed out that the excretion of urates could be underestimated if uric acid precipitated in a refrigerated urine sample prior to analysis. This is well known to clinical biochemists, so they add alkali to the urine collection vessel to dissolve uric acid crystals prior to assay. He went on to ask, ‘In what way might a 24-h urine collection lead to the false impression that the urine pH was not low?’

Question 2. In what way might a 24-h urine collection lead to the false impression that the urine pH was not low?

Physiology principle 1. Some compounds or ions in the urine have greater excretion rates at certain times of the day and lower ones at other times. This is called a diurnal or a circadian excretion pattern.² Professor McCance had observed large and reproducible variations in the urine pH in normal subjects. The lowest urine pH is usually found overnight, while higher values were observed close to noon. Hence mixing urine samples with a low pH and others with a high pH could mask times in the 24-h period when the urine pH was low enough to cause uric acid to precipitate. To illustrate this point, he suggested that they all participate in an experiment where their pattern of urine pH during the 24-hour period could be compared to that of their patient. Urine would be stored in separate vials after voiding at 2–3 h intervals during the day, while one overnight sample would be collected so as not to disturb sleep. They would add a preservative to the storage vials to avoid alkalization of the urine secondary to bacterial urease actions. For this experiment to be clinically relevant, only subjects who were healthy and not taking medications should participate. They should continue with their usual diet, water intake, and activities. The housestaff were excited by this idea, and also by the fact that their Professor would be a subject in the experiment as well. ‘Now we even have a control for age’, said one of the team with a smile. All agreed to meet as soon as the samples were analysed.

Results of the mini-experiment

Professor McCance was pleased that the intern had plotted the data, and was eager to examine the results. She began with an analysis of the diurnal changes in the urine flow rate.

(i) Diurnal variation in the urine flow rate. In the control subjects, the nadir in urine flow rate was in the overnight collection period (Figure 1). Therefore, there should be a higher total urate concentration in the urine for this portion of the 24-h period (unless the rate of excretion of urates underwent a marked diurnal variation), she said. This could be especially important for uric acid precipitation if a low urine pH were to occur in the overnight period. The housestaff were surprised by how low the urine flow rate became overnight. They asked Professor McCance, ‘What factors cause such a low urine flow rate in the overnight period?’

Question 3. What factors cause such a low urine flow rate in the overnight period?

Physiology principle 2. When vasopressin acts, the distal nephron is permeable to water. Therefore the urine flow rate is directly proportional to the

![Figure 1. Diurnal pattern for the urine flow rate and osmolality. Data represent means ± SEM for the controls (13 males and 4 females). Urine flow rate is depicted by the solid symbols connected by the solid line and \( U_{\text{osm}} \) by the open symbols connected by the dashed line. The nadir in the urine flow rate is in the overnight period, but there was no appreciable diurnal variation in the \( U_{\text{osm}} \).](image-url)
osmole excretion rate and inversely proportional to the $U_{osm}$ (equation 3).

$$\text{Urine flow rate} = \frac{\text{Osmole excretion rate}}{U_{osm}}$$

Return to the data: Because there was little variation in the urine osmolality ($U_{osm}$) throughout the 24-h period (Figure 1), the main reason for the low overnight urine flow rate was a low rate of excretion of osmoles. Professor McCance turned his attention to an analysis of the pattern of excretion of the individual urine osmoles. He knew that approximately half of them would be urea and the other half would be electrolytes. Therefore he asked about their respective excretion rates. As shown in Figure 2, there was little variation in the excretion or urea throughout the 24-hour cycle, but a lower electrolyte excretion rate overnight. At this point, one of the registrars asked; ‘Why was our salt excretion rate so low overnight if most of our salt intake usually occurs in the evening?’

**Question 4. Why was salt excretion rate so low overnight?**

**Physiology principle 3.** The signal to excrete Na$^+$ is related more directly to pressure than to central blood volume.

Return to the study: Our central blood volume is likely to be highest in the overnight period, because this follows the meal with our largest intake of NaCl, and also because we are no longer in an upright posture. Thus it is reasonable to suggest that the rate of excretion of Na$^+$ should be highest in the overnight period. Nevertheless, the opposite was observed (Figure 2). Therefore, it appears that the signal for the renal excretion of Na$^+$ is not simply due to an increased central blood volume; perhaps what is sensed is not a rise in volume, but a rise in central venous pressure. Professor McCance pointed out that even if these vessels contain a larger volume, there could be a fall in pressure if the venous tone were to decline. A possible explanation for the decrease in ‘pressure’ is that adrenergic stimulation, which increases venous tone, is lower during sleep. This lower excretion of Na$^+$ in the overnight period will permit undisturbed sleep, because it will slow the filling of the urinary bladder.

The housestaff, while impressed with the physiology, were curious about the clinical advice given to the patient—‘drink more water during the day’. They suggested that if the ingested water were excreted during daytime hours, the urine flow rate might remain low in the overnight period when the urine pH was lowest (Figure 3). Hence the concentration of uric acid would remain in a dangerous range overnight. Moreover, the 24-h urine volume would provide a false sense of security, if it reflected very large daytime flow rates while the overnight volume remained low. In contrast, more valuable information would be gained if multiple 2–3-hourly urine collections were obtained over the 24-h period. Armed with these insights, they were eager to examine their data on the urine pH.

(ii) *Diurnal variation in the urine pH.* These data also contained several surprises (Figure 3). First, the urine pH was close to 6.0 throughout the 24-h period in the control subjects. Second, the patient had a urine pH that was low enough to cause uric acid to precipitate during much of the 24-h period. Professor McCance pointed out that this information should be useful in the design of therapy, because alkali treatment would be most effective if it raised the urine pH at times when her
urine pH was low. Almost immediately, the housestaff asked, ‘Why did the patient have these low urine pH values for a significant portion of the 24-hour period?’ To deal with these issues, Professor McCance began a brief didactic discussion that focused on the urine pH.

Issues concerning a low urine pH

Professor McCance began by asking, ‘What might be the basis for the periods with a low urine pH in this patient?’

**Question 5.** What might be the basis for the periods with a low urine pH in this patient?

**Physiology principle 4.** The pH of a solution is dependent on two factors, the rate of addition of free $H^+$ and the availability of acceptors that can bind $H^+$ at the pH of that solution.

*Return to the bedside:* Applying this principle, one can deduce that there are two groups of causes for a low urine pH (Figure 4). First, there may be a higher rate of $H^+$ secretion in the distal nephron. Second, there may be diminished availability of acceptors for $H^+$ in the lumen of the collecting duct. This, in essence, means decreased entry of NH$_3$ into the medullary collecting duct (MCD) because, at a urine pH $\sim 6$, there is virtually no HCO$_3^-$ in the urine to titrate secreted $H^+$.

One of the housestaff quickly pointed out that measuring the rate of NH$_4^+$ excretion would separate these two possible aetiologies (equation 4). NH$_4^+$ excretion should be high if distal $H^+$ secretion is elevated, but low if NH$_3$ availability is low. Therefore they turned to the results of their experiment, and found that the patient had excreted 39 mmol of NH$_4^+$ in her 24-h urine. The intern concluded that this was a normal value, because it was very similar to the group’s mean NH$_4^+$ excretion rate. Professor McCance raised an eyebrow, and asked, ‘How do you define ‘normal’ when considering the composition of urine?’

**Question 6.** How do you define ‘normal’ when considering the composition of urine?

**Physiology principle 5.** In steady state, subjects should excrete metabolic wastes and ingested ions that are in excess of body needs (minus loss via non-renal routes) in their urine. Therefore a physiological rather than a statistical analysis is needed to define appropriate excretion rates.

*Illustrative example:* Professor McCance illustrated this physiological principle by examining the excretion of water. The urine flow rate should be assessed relative to the expected response in the presence of the stimulus of a surplus or a deficit of water in the body, rather than relative to what is the ‘usual’ rate of water excretion in a population with an unknown stimulus. Therefore, the expected urine flow rate will be as high as possible and the $U_{osm}$ as low as possible if the plasma sodium (Na$^+$) concentration ($P_{Na}$) is sufficiently low due to the ingestion of a large volume of water. On the other hand, the expected urine flow rate will be as low as possible and the $U_{osm}$ as high as
possible in response to a deficit of water (the signal
is a high PNa). Using this same logic, one cannot
determine if the rate of excretion of NH₄⁺ is normal
in a patient without assessing it relative to the
physiological stimulus for NH₄⁺ excretion. Therefore
the question is, ‘What is the physiological signal
for the rate of excretion of NH₄⁺?’

**Question 7. What is the physiological
signal for the rate of excretion of NH₄⁺?**

**Physiology principle 5 restated:** The rate of excre-
tion of NH₄⁺ should be compared to the stimulus
for NH₄⁺ excretion—NH₄⁺ excretion should be high
enough to prevent the development of metabolic
acidosis or if chronic metabolic acidosis is present,
its rate should be as high as can be achieved.

The non-volatile dietary H⁺ load is H₂SO₄ derived
from the metabolism of sulphur-containing amino
acids.³ Initially, these H⁺ are titrated by HCO₃⁻, and
this leaves the body with a deficit of HCO₃⁻. Neverthe-
less, SO₄²⁻ anions cannot bind a significant amount of H⁺ at the lowest possible
urine pH. Hence the kidneys must generate new HCO₃⁻ to restore HCO₃⁻ balance; this occurs
when SO₄²⁻ anions are excreted in the urine
with NH₄⁺. Therefore, in the absence of metabolic
acidosis, the number of mEq of NH₄⁺ should
be approximately equal to the number of mEq of
SO₄²⁻ anions in the urine.

Because this patient excreted 66 mEq of SO₄²⁻
anions but only 39 mEq of NH₄⁺ per day, she
had a low rate of excretion of NH₄⁺. Moreover,
this lower rate of NH₄⁺ excretion occurred when
her urine pH was decidedly low. Hence Professor
McCance concluded that her persistently low urine
pH reflected a low availability of NH₃ in her
medullary interstitial compartment. The question
they now needed to examine was, ‘What is the
cause for the low NH₃ concentration in the medul-
lar interstitial compartment?’ The nephrology
consultant took the lead at this point, because
Professor McCance was not aware of the new data
that were required to answer this question.

**Question 8. What is the cause of
the low NH₃ concentration in the
medullary interstitial compartment?**

Two steps are required for the generation of a high
medullary interstitial concentration of NH₃, said the
nephrology consultant. First, cells of the PCT must
produce NH₄⁺ (plus HCO₃⁻) from the metabolism of
 glutamine (Figure 5). Second, NH₄⁺ is recycled in
the loop of Henle (LOH) and transferred into the lumen
of the MCD.

(i) Production of NH₄⁺ by the kidney. Glutamine
must be selected as the main fuel for the prox-
imal convoluted tubule (PCT) by having a low
pH in these cells. There is an upper limit on this
production of NH₄⁺ set by the availability of ADP,
a required substrate for oxidation of glutamine.⁴

![Figure 5. High NH₃ concentration in the medullary interstitial compartment. The U-shaped structure is the loop of Henle (LOH). The first step in the process that raises the concentration of NH₃ in the medullary interstitial compartment is NH₄⁺ production in the PCT cells (site 1). The second step is the reabsorption of NH₄⁺ via NKCC in the mTAL (site 2). The third step is the entry of NH₄⁺ into the descending thin limb of the LOH (site 3).](image-url)
ADP is formed when the kidneys perform their work—reabsorb filtered Na⁺. Hence a low GFR leads to a diminished maximal rate of NH₄⁺ production in the PCT. Other fuels may compete with glutamine for oxidation in the PCT (e.g. free fatty acids provided, for example, during total parenteral nutrition) and therefore cause a lower rate of production of NH₄⁺.

(ii) NH₄⁺ recycling in the LOH. NH₄⁺ ions are reabsorbed in the thick ascending limb of the loop of Henle (LOH), replacing K⁺ on the Na-K-2 Cl- cotransporter (NKCC). This provides the ‘single effect’ for the recycling of NH₄⁺ in the LOH and the generation of a high concentration of NH₃ in the medullary interstitial compartment.

In summary, a low availability of NH₃ in the medullary interstitial compartment could be due to low production of NH₄⁺ in the PCT and/or a transfer defect due to a medullary interstitial disease, concluded the nephrology consultant.

Return to the data: Because the patient is able to concentrate her urine maximally, it is unlikely that her defect is in the LOH, said the nephrology consultant. Therefore I suspect a defect in the production of NH₃ in the PCT. A common cause for a low rate of production of NH₄⁺ is an alkaline PCT cell due to hyperkalaemia, but her plasma potassium (K⁺) concentration (P_k) was not elevated. The other common cause of a low rate of NH₄⁺ production is a low GFR, but her GFR was not low. Therefore I cannot identify a cause for his low rate of production of NH₄⁺, said the nephrology consultant.

Hence Professor McCance was now the focus of attention. He was asked to provide a possible explanation for the low rate of production of NH₄⁺. While the step-by-step analysis of the case appeared to be logical, he wondered if the patient could have an alkaline PCT cell in the absence of hyperkalaemia or an alkaline pH of blood. Professor McCance asked, ‘Is there a non-invasive way to gauge the pH of PCT cells in vivo?’

**Question 9. How can the pH of PCT cells be assessed in vivo?**

The nephrology consultant pointed out that the rate of excretion of citrate could provide a ‘window’ on the PCT cell pH. Metabolic acidosis and hyperkalaemia are conditions associated with a low pH in cells of the PCT, and there is a low rate of excretion of citrate in these settings. A notable exception is in patients with isolated proximal renal tubular acidosis (pRTA). Some of these patients have a high rate of excretion of citrate despite the systemic metabolic acidosis. Accordingly, it has been suggested that the underlying pathophysiology of this disorder is an alkaline PCT cell pH. Therefore, if this patient had an alkaline PCT cell pH, the consultant said he would expect to find a high rate of citrate excretion.

*Return to the experimental data: The rate of excretion of citrate in the patient was higher than in the control population (Table 1). Professor McCance was intrigued by this observation, and said that an alkaline PCT cell might provide an explanation for the low availability of NH₃ and thereby, the low urine pH. Obviously impressed by their Professor's thinking, the house-staff asked, ‘Why might her PCT cells have a more alkaline pH?’*

**Question 10. Why might her PCT cells have a more alkaline pH?**

Although it is possible to have a low rate of production of NH₄⁺ and a high rate of excretion of citrate due to eating an alkaline diet, this was not likely in our patient, because the urine pH would be high if this were the case. Therefore, to account for a somewhat more alkaline PCT cell pH, she might have a reduced rate of export of HCO₃⁻ or an increased rate of export of H⁺ from PCT cells, stated Professor McCance (Figure 6). He went on to point out that the putative lesion, however, should only involve PCT cells. ‘*Can anyone help me as to a likely candidate for this lesion?*’ he asked. The nephrology consultant said that a defect in the Na(HCO₃)₂⁻ cotransporter (NBC) in the basolateral membrane of PCT cells was a possibility. A lesion that increases the K_m of this transporter (the concentration of HCO₃⁻ needed for exit of HCO₃⁻ from PCT cells into the body)

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**Figure 6. Alkaline proximal cell pH.** An alkaline PCT cell pH could be due to a lesion that compromises the exit of HCO₃⁻ from cells via the Na(HCO₃)₂⁻ co-transporter. An alkaline PCT cell would lead to a diminished rate of production of NH₄⁺ (and hence less availability of NH₃ in the medullary interstitial compartment) and a high rate of excretion of citrate.
or decreases its maximum velocity ($V_{\text{max}}$) could lead to a steady state with a more alkaline intracellular pH. In fact, mutations in the gene encoding for NBC had been recently described in patients with isolated proximal RTA.9 The medical registrar pointed out that an alkaline PCT cell pH should lead to a diminished rate of $\text{HCO}_3^-$ reabsorption by PCT. If this occurred, the patient should have metabolic acidosis with a normal anion gap and a high urine pH, he argued.

**Question 11. Why might our patient not have bicarbonaturia?**

While admiring his younger colleague’s analysis, Professor McCance suggested that the degree of rise in the PCT cell pH could be small enough to cause only a small decrease in the rate of $\text{HCO}_3^-$ reabsorption in this nephron segment. If downstream nephron segments could reabsorb this small extra $\text{HCO}_3^-$ load that escaped reabsorption in the PCT, there would be no bicarbonaturia. Of course, the cause for an alkaline cell pH would have to be present in the PCT, but not in the distal nephron. The urine pH could be low if there was a low availability of $\text{NH}_3$ in the medullary interstitial compartment. Professor McCance was pleased to learn that patients with isolated proximal RTA typically have a low urine pH.10

While the processes were possible, Professor McCance needed more time to consolidate his ideas about $\text{NH}_4^+$ excretion and control of the urine pH. He needed to ask the nephrology consultant for more detailed information, and therefore he drew this portion of the consultation to a close, suggesting that they return to continue exploring this fascinating problem tomorrow.

**After the adjournment**

Professor McCance met the team in their seminar room the following morning. The nephrology consultant summarized the traditional view of the excretion of $\text{NH}_4^+$ as follows. The primary factor to augment the excretion of $\text{NH}_4^+$ is a very high $H^+$ concentration in the lumen of the MCD. This permits $\text{NH}_3$ to diffuse down its concentration difference from the interstitial compartment into the lumen of the MCD. Professor McCance chose to provide the team with reasons why he now had doubts about this traditional interpretation of the importance of a low urine pH to enhance the excretion of $\text{NH}_4^+$ during chronic metabolic acidosis—his doubts focused on the diffusion of $\text{NH}_3$ and the recently published data on this process. To explain this new understanding, Professor McCance summarized his analysis of the data and the responses to the questions he had asked the nephrology consultant.

**Question 12. Is diffusion of $\text{NH}_3$ in the medullary interstitial compartment a physiologically important pathway?**

**Physiology principle 6. Diffusion is a slow process with three major elements—a high concentration of the substance that diffuses, a very short distance for diffusion, and the absence of a barrier for diffusion.**

(a) Concentration of $\text{NH}_3$. Although $\text{NH}_3$ is transported across the basolateral membrane out of cells of the mTAL,11 with the prevailing pH of the medullary interstitium, the concentration of $\text{NH}_3$ will be low, only 1/100 that of $\text{NH}_4^+$.

(b) Distance for diffusion: Because the mTAL is in very close contact with the MCD, perhaps this is not a major issue.

(c) Barrier for diffusion: Both the basolateral and luminal membranes of cells of the MCD have lipid as a major constituent, Professor McCance had doubts that $\text{NH}_3$ would diffuse quickly across lipid barriers.

In summary, these reservations raised the possibility that $\text{NH}_4^+$ could be the species that is important for diffusion. For this to occur, there must be a way to transport $\text{NH}_4^+$ across cell membranes or a special way to convert $\text{NH}_4^+$ to $\text{NH}_3$ in cell membranes. I shall come back to this in a few minutes, he said. Professor McCance was intrigued by another question, ‘What is the quantitative importance of this medullary shunt pathway to the excretion of $\text{NH}_4^+$?’ His reasoning was that if the excretion of $\text{NH}_4^+$ was not its major function, perhaps this shunt pathway served a different purpose.

**Question 13. How much $\text{NH}_4^+$ is added in the MCD during chronic metabolic acidosis?**

Because invasive procedures are needed to obtain fluid from the end of the cortical collecting duct, the data to examine are from experiments performed in rats with chronic metabolic acidosis. Sajo et al.12 found that ~75% of $\text{NH}_4^+$ excretion in these rats was already present in the luminal fluid obtained from the end of the cortical collecting duct. Therefore, the medullary shunt of $\text{NH}_4^+$ could...
only account for approximately 25% of the NH$_4^+$ excreted during chronic metabolic acidosis.

Our Professor emphasized the results of a second experiment that he was informed about by the nephrology consultant.$^{13}$ Its objective was to assess the importance of this medullary shunt pathway for the excretion of NH$_4^+$. The premise was that the rate of excretion of NH$_4^+$ should decline when this recycling process in the LOH is inhibited, if its primary function were to increase the rate of excretion of NH$_4^+$. Nevertheless, the rate of excretion of NH$_4^+$ rose after a loop diuretic was administered.$^{13}$ This suggests that the medullary reabsorption of NH$_4^+$ and its shunt across the medullary interstitial compartment may serve a different function than simply increasing the excretion of NH$_4^+$. To deduce this function, he had asked, ‘What happened to the urine pH when the transport of NH$_4^+$ was inhibited?’ A striking finding was a fall in the urine pH said the nephrology consultant. ‘Aha’, said Professor McCance, ‘perhaps we now have an idea of the function of this shunt pathway, control of the urine pH’. This insight led to his final question for the nephrology consultant.

**Question 14. Is there a transporter for NH$_4^+$ across the basolateral and luminal membrane of the MCD cells?**

The nephrology consultant had read a recent review on this subject.$^{14}$ There were two different, but highly related transporters in cells of the MCD that carried out this function. They were both Rh-glycoproteins that might serve as cation exchangers where NH$_4^+$ and H$^+$ moved in opposite directions; one was in the luminal membrane and the other in the basolateral membrane of MCD cells. Professor McCance quickly pointed out that the net effect of this electroneutral cation exchange is the net unidirectional movement of NH$_3$. The nephrology consultant was amazed! He pointed out that these Rh glycoproteins actually are NH$_3$ channels, but with one additional property, they have a hydrophobic mouth, which strips a H$^+$ ion off of NH$_4^+$.$^{15}$ This is akin to lowering the pK of NH$_4^+$ by 3 log units in this local region.

Professor McCance speculated that the major function of the medullary NH$_3$ shunt pathway might not be to achieve high rates of excretion of NH$_4^+$, but possibly to prevent a large fall in the urine pH. This can be accomplished by having diffusion of NH$_3$ into the lumen of the MCD to remove H$^+$ secreted by the MCD.$^{15}$ In this process, distal H$^+$ secretion led to the formation of NH$_4^+$ into the lumen of the MCD. Hence this process would function as an adjuster of the urine pH if the NH$_3$ channel opening were modulated appropriately.

Professor McCance drew Figure 8 on the blackboard. Let us begin with the reabsorption of NH$_4^+$ from the loop of Henle, which adds NH$_3$ to the medullary interstitial compartment (the H$^+$ to convert it to NH$_4^+$ are added at site 3 in Figure 8). Recycling of NH$_4^+$ in the loop of Henle raises the concentration of NH$_4^+$ in the medullary interstitium$^6$ (site 1, Figure 8). NH$_4^+$ can diffuse rapidly enough through the renal medullary interstitial compartment because its concentration is high. NH$_4^+$ in the form of NH$_3$ diffuses across both lipid-containing...

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Figure 7. Low urine pH in patients with uric acid stones and an alkaline PCT cell pH. Alkaline PCT cells may cause a modest defect in HCO$_3^-$ reabsorption (step 1). Somewhat more HCO$_3^-$ is delivered to and reabsorbed in the distal nephron, because it does not exceed the rate of H$^+$ secretion in this portion of the nephron (step 2). The persistently low urine pH is due to a normal rate of secretion of H$^+$ in the MCD, together with the diminished availability of NH$_2^+$ and NH$_4^+$ in the medullary interstitium (step 3).
cell membranes of the MCD via these two different NH₃ channels, one on the basolateral and another on the luminal membrane of these cells (site 3). The NH₃ entry into the lumen of the MCD could adjust the urine pH upward (towards 6.0) by removing luminal H⁺ despite continuing H⁺ secretion by the H⁺-ATPase. The net result is a final urine pH that is approximately 6.0 with a somewhat higher rate of NH₄⁺ excretion. Professor McCance was intrigued by how the system is ‘smart enough’ to achieve a high rate of renal new HCO₃⁻ generation without requiring a large fall in the urine pH, with its associated danger of increasing the risk of forming uric acid kidney stones.

He had one more question for the nephrology consultant. He said that he could now understand why uric acid crystals would form in the urine, but it was unclear how they could be retained and grow within the lumen of the MCD.

**Question 15. What mechanism could permit uric acid deposits to grow over weeks or months of time, yet continue to be retained in the lumen of the MCD?**

The nephrology consultant had a smile on her face. Until recently, she too had been perplexed by this paradox. Fortunately, a recent publication by Evan and colleagues provided a possible answer to ProfessorMcCance’s excellent question.¹⁶ These investigators had found that the site where calcium oxalate stones began was very surprising—in the basolateral membrane of the thin ascending limb of the loop of Henle. The initial lesion was a deposit of apatite (Ca₃(PO₄)₂), a very difficult precipitate to form, because one needs an area with appreciable alkalinization to convert divalent phosphate to its trivalent form (PO₄³⁻). Once this nidus forms, solutes whose concentration exceeds their Ksp will be added at this site. Over time, the deposit enlarges.

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**Table 3  Summary of physiology principles**

<table>
<thead>
<tr>
<th>Physiology principle</th>
<th>Comment</th>
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<tbody>
<tr>
<td>1</td>
<td>Substances precipitate when their concentrations exceed their Ksp</td>
</tr>
<tr>
<td>2</td>
<td>Some metabolic wastes and ions have a diurnal excretion pattern</td>
</tr>
<tr>
<td>3</td>
<td>When vasopressin acts, the distal nephron becomes permeable to water</td>
</tr>
<tr>
<td>4</td>
<td>Urine flow rate is directly proportional to the osmole excretion rate and inversely to the Uosm</td>
</tr>
<tr>
<td>5</td>
<td>The urine pH depends on the rate of H⁺ secretion, the availability of H⁺ acceptors, and their pK</td>
</tr>
<tr>
<td>6</td>
<td>The expected rate of excretion of NH₄⁺ depends on the stimulus of an acid load</td>
</tr>
<tr>
<td>7</td>
<td>Diffusion is a slow process; it requires large concentration differences, short distances, and the absence of barriers</td>
</tr>
<tr>
<td>8</td>
<td>The medullary shunt of NH₄⁺ may not be a major contributor to NH₄⁺ excretion</td>
</tr>
</tbody>
</table>

Uric acid can precipitate if uric acid content rises or if urine volume falls. Use multiple small collections rather than 24-h urines for analyses.
to form what is called ‘Randall’s plaque’. Continuing growth and erosion lead to its exposure in the lumen of the papilla or the papillary-collecting duct. Once exposed, urine that is supersaturated with ionized calcium and oxalate will force crystals to deposit on its surface and the precipitate grows intermittently, but progressively.17 While the above is true for calcium oxalate stone formation in patients with hypercalciuria, there are no similar data published concerning the growth of uric acid stones.

Concluding remarks

Using an approach that emphasizes an understanding of simple physiological concepts, several new insights into the pathophysiology of uric acid stone formation and the physiology of NH₄⁺ excretion were revealed (Table 3). In the clinical evaluation of these patients, more valuable information about the pathophysiology of kidney stone formation would be available if multiple 2–3-hourly collections were obtained over the 24-h period rather than from a single 24-h urine collection. Regarding the physiology of NH₄⁺ excretion, it appears that the medullary reabsorption of NH₄⁺ and its shunt across the medullary interstitium serves a primary function of controlling the final urine pH rather than contributing significantly to achieve high rates of NH₄⁺ excretion. Therefore a persistently low urine pH could be due to three lesions (Figure 8): first, there could be a primary increase in the rate of H⁺ secretion in the distal nephron; second, there could be a diminished open probability of either of the NH₃ channels in the MCD; third, there could be a lower concentration of the substrate for these NH₃ channels (medullary interstitial NH₄⁺), most likely due to a lower rate of production of NH₄⁺ in the PCT.

In the patient discussed in this manuscript, a low rate of NH₄⁺ excretion together with a high rate of excretion of citrate suggested that her defect was a more alkaline pH in PCT cells.

Appendix: Possible advantages for deletion of the uricase gene in Paleolithic times

Most mammals possess the oxidative enzyme uricase in peroxisomes of hepatocytes, which degrades urate into the water-soluble product, allantoin, that is excreted by the kidneys. In contrast, in humans, the uricase gene is not expressed as a result of mutational silencing, and urate is the end-product of purine metabolism that is excreted by the human kidney.

During evolution, trade-offs were required to accommodate many and seemingly conflicting demands. These trade-offs should provide biological advantages for survival.18 These advantages, however, may not be obvious in our modern day industrialized society, and perhaps may be even considered as a disadvantage.

An intriguing hypothesis has been recently proposed by Johnson et al.19 concerning the low availability of NaCl in primitive diets. In their hypothesis, deletion of the uricase gene led to better conservation of NaCl and thereby defense of blood pressure. They showed that when experimental animals were given a drug to cause an acute increase in serum urate, there was both improved renal conservatism of NaCl and an increase in blood pressure, because of the action of urates to activate the renin-angiotensin system in response to a low salt diet. Higher plasma urate also induces renal microvascular and interstitial disease, which leads to salt sensitivity and a chronic increase in blood pressure. While this may have provided survival advantage during early development in modern society, the switch to a high salt diet in conjunction with this mutation may play an important role in the current epidemic of hypertension and cardiovascular disease. In this regard, plasma urates are an independent risk factor for both hypertension and atherosclerotic heart disease.20

References


