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Review

Dogmas and controversies in the handling of nitrogenous wastes: Excretion of nitrogenous wastes in human subjects

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Summary

Two major nitrogenous waste products, urea and ammonium (NH_4^+), are produced in humans when proteins are oxidized, and in this manuscript their excretions are examined from two perspectives. First, the specific physiology of each nitrogenous waste is reviewed and the current dogmas summarized. Second, their excretions are considered in the context of integrative physiology, i.e. the need to ensure that the urine composition is appropriate to minimize the risk of kidney stone formation. After the latter analysis, weak links in our understanding of the overall physiology become apparent and a conundrum is defined. The conundrum for the excretion of urea focuses on the fact that urea is not an effective osmole in the medullary-collecting duct when vasopressin acts. As a result, it appears that urinary urea cannot prevent a large decline in the urine flow rate and thereby minimize the risk of forming kidney stones in electrolyte-poor urine. The conundrum for the excretion of NH_4^+ is: high rates of NH_4^+ excretion require a low urine pH, yet a pH ~6.0 must be maintained in order to reduce the risk of precipitating uric acid in the urine. Possible ways of resolving these conundrums require novel physiological interpretations.

Key words: acid–base, ammonium, urea, urine osmolality, water, human.

Introduction

Protein intake is essential for survival. Its oxidation yields two major nitrogen-containing waste products, urea and ammonium (NH_4^+), which must be removed by renal excretion. While noting the specific physiology that influences their individual excretion rates, integrative aspects should also be considered, i.e. the need to ensure that these excretions do not alter the composition of the urine and thereby cause urinary solutes to precipitate within the urinary tract. Urea is not an effective urine osmole; accordingly, in this paper urea excretion is analyzed in the context of controlling the minimum urine flow rate. In addition to considering the physiology of acid balance (Kamel et al., 2002), NH_4^+ excretion is analyzed in the context of the need to maintain a urine pH of ~6.0 to minimize the risk of kidney stone formation (Coe and Parks, 2000).

I. Excretion of urea and the urine volume

Traditional physiology to define the dogma

Two aspects are explored in this section: first, we comment on the urine flow rate needed to minimize the risk of forming kidney stones; second, we consider how the minimum urine flow rate may be higher when urea is excreted.

What is a safe urine flow rate?

To illustrate this point, the rise in the ion product for calcium × oxalate, the constituents of the commonest form of kidney stones in humans (Coe and Parks, 2000), are calculated at a 24 h average urine flow rate (1.2 ml min^{-1}), at the usual mean overnight urine flow rate of 0.6 ml min^{-1} (Halperin et al., 2002), and at a lower urine flow rate of 0.3 ml min^{-1}. For the purpose of this calculation, we shall assume that the excretion rates for calcium and oxalate remain constant (Table 1). The calcium × oxalate ion product is 4- and 16-fold higher at the 0.6 and 0.3 ml min^{-1} flow rates than at the 1.2 ml min^{-1} rate. The major danger of precipitation occurs when the urine flow rate decreases from 0.6 to 0.3 ml min^{-1}.

Control of the urine flow rate

Vasopressin is released when the sodium (Na^+) concentration in plasma (P_{Na}) exceeds its low-normal value of 138 mmol l^{-1} (Robertson, 2000). This hormone causes water channels (aquaporin 2; AQP-2) to be inserted into the luminal membrane of the late distal convoluted tubule (DCT) and the collecting ducts (Nielsen et al., 2002). In this setting, the urine volume should be directly proportional to the number of

Table 1. *Effect of the urine flow rate on the ion product of calcium × oxalate*

Flow rate (ml min^{-1})	Calcium (mmol l^{-1})	Oxalate (mmol l^{-1})	Calcium × oxalate (mmol l^{-1})
1.2	X	Y	XY
0.6	2X	2Y	4 XY
0.3	4X	4Y	16 XY

For simplicity, the concentrations of calcium and oxalate are assigned values of X and Y mmol l^{-1}, respectively, at an average 24 h urine flow rate of 1.2 ml min^{-1}. Their ion products become 4 and 16 if the excretion rates for these ions is constant, but the urine flow rate is the usual overnight value of 0.6 ml min^{-1} or with an excessive degree of oliguria (0.3 ml min^{-1}).

effective urine osmoles and inversely proportional to their concentration in the urine (Equation 1):

$$\text{Urine volume} = \frac{\text{number of 'effective' solutes excreted}}{[\text{effective solutes}]_{\text{urine}}} . \quad (1)$$

Of great interest, vasopressin also causes the insertion of urea transporters into the luminal membrane of the inner medullary collecting duct (MCD) (Sands, 1999). In rats consuming their usual diet, the MCD is sufficiently permeable to urea that its concentrations are similar in the urine and in the renal papilla (Gowrishankar et al., 1998) (Table 2). Hence urea is not an effective osmole in the urine and it does not control the urine flow rate in this setting.

Conundrum

When the urine is electrolyte-poor, its flow rate could decline sufficiently to cause a precipitate to form because of the low rate of excretion of effective osmoles (Equation 1, Table 1). An example of this conundrum is a human in a hot environment who sweats and ingests little salt or water. A deficit of Na^+, Cl^- and water will develop and the urine will contain very few of the usual effective osmoles (Na^+ and Cl^-).

Possible ways to resolve this conundrum

(i) Increase the excretion of new effective osmoles

Ketonuria of prolonged fasting seems to waste useful energy. Moreover, to achieve acid–base balance, these ketoacid anions must be excreted with NH_4^+, adding to the apparent disadvantage in their excretion because the source of the nitrogen would be from lean body mass (Halperin et al., 1989). Kamel et al. (1998) postulated that there was an advantage in excreting NH_4^+ plus ketoacid anions instead of urea during prolonged fasting, when viewed as a physiological adaptation to allow for the excretion of urine with a flow rate in a safer range (~0.5 ml min^{-1}). In more detail, when 2 mmoles of NH_4^+ are excreted along with 2 mmoles of ketoacid anions, this results in acid–base balance; however, the urine will contain four effective milliosmoles containing the same two nitrogens that would be present in one millimole of the ineffective urine osmole, urea. While useful in the prolonged fasted state, this mechanism does not provide a means to ensure a safe minimum urine flow rate in fed mammals.

(ii) Make urea become an effective osmole in electrolyte-poor urine when vasopressin acts

The only non-electrolyte osmole that is excreted in a sufficient quantity in a fed mammal is urea, but urea does not seem to be an effective urine osmole when vasopressin acts in rats consuming the usual amount of electrolytes (Table 2).

To gain insights into how to resolve this conundrum, the concentration of urea was measured in the urine and in the interstitial compartment of the papilla in rats consuming a low-electrolyte diet while they received vasopressin. The concentration of urea was significantly higher in the urine than in the papillary interstitial compartment (Table 2). This implies that urea is not freely permeable in the inner MCD when the urine is electrolyte-poor. Accordingly, we speculated that under this circumstance, urea could become an effective osmole in the inner MCD despite the continuing presence of vasopressin (Fig. 1). In fact, the change in urea permeability

Table 2. *Osmole concentrations in the papilla*

	No salt		NaCl	
{Osmole]	Papilla	Urine	Papilla	Urine
Na^+ (mmol l^{-1})	372±20	80±12*	379±34	426±33
K^+ (mmol l^{-1})	60±3	38±10	76±7	210±12*
NH_4^+ (mmol l^{-1})	145±23	303±37*	107±35	246±24*
Osmolality (mOsm kg^{-1} H_2O)	2332±104	2219±190	1927±94	2343±153
Urea (mmol l^{-1})	1176±44	1565±101*	803±78	750±91

Six rats in each group were pair-fed the low electrolyte diet for 4 days. This diet has its alkali source removed and the rats have high rates of excretion of NH_4^+ (Cheema-Dhadli et al., 2002). One subgroup was given 5.8±1.3 mmol of NaCl in their drinking water; desamino,D-arginine vasopressin (dDAVP; 2 mg/rat) was administered 4 h prior to the collection of the urine and excision of the renal papilla. Papillary samples were obtained from anaesthetized rats as previously described (Gowrishankar et al., 1998).

*P<0.05 for comparison between the urine and the papilla.

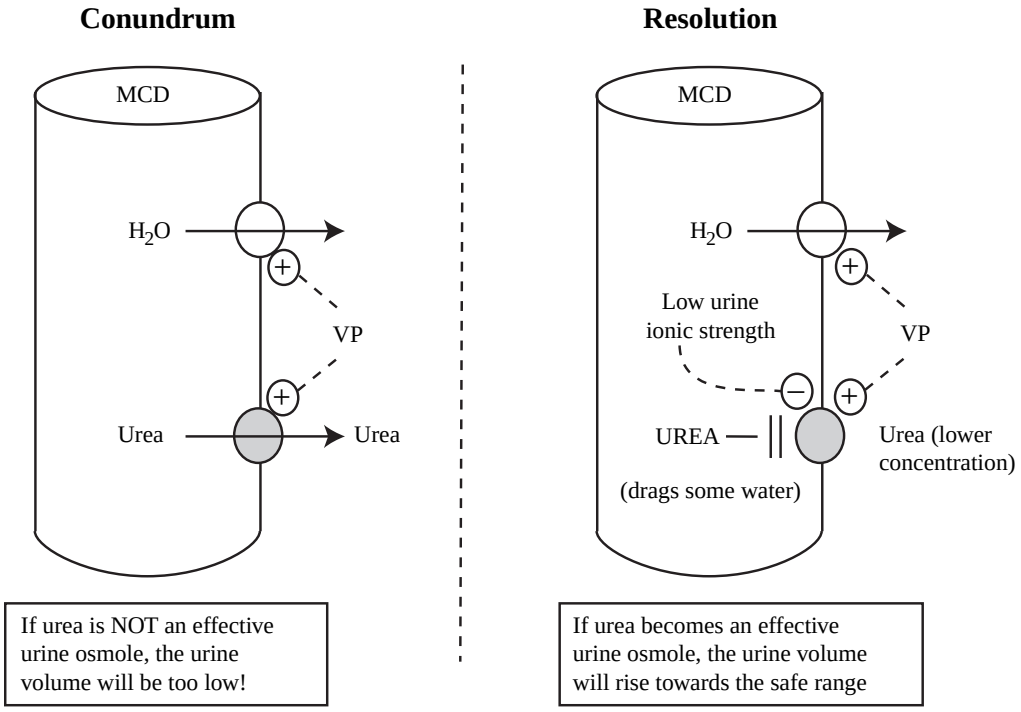

Fig. 1. Urea excretion and the avoidance of oliguria in a subject with a normal protein intake and a deficit of Na^+, Cl^- and water. The barrel-shaped structure represents the inner MCD; AQP-2 is shown as a clear oval and the urea transporter is shown as a shaded oval in its luminal membrane. The issues in the conundrum are shown to the left of the vertical broken line and features for its resolution are shown to the right of this line. To resolve the conundrum, the hypothesis is that urea can become an effective urine osmole in urine with a low ionic strength. This view is supported by the fact that the concentration of urea is higher in the luminal urine than the interstitial compartment (papilla; Table 2). VP, vasopressin; MCD, medullary collecting duct.

need only be modest to ensure the needed small rise in the urine flow rate.

In summary, to allow for water conservation, urea is not an effective osmole when vasopressin acts in fed mammals (Gamble et al., 1934). Nevertheless, when the urine is electrolyte-poor, there is a risk of precipitate formation in the urinary tract if urea does not become an effective osmole to ensure a safe minimum urine flow rate. The mechanism that could permit this physiological alteration in urea reabsorption is a subject of ongoing studies.

II. Excretion of NH_4^+ and control of the urine pH

The excretion of NH_4^+ is examined from two similar perspectives. The first focuses on its primary function, allowing the body to achieve acid–base balance when the kidney must eliminate a H^+ load. The second perspective is the requirement to maintain a urine pH of ~6.0 to minimize the risk of uric acid precipitation (Fig. 2).

Uric acid stone formation, importance of the urine pH

Uric acid is a sparingly soluble constituent of the urine; its concentration rises when the urine pH is too low (Equation 2):

$$H^+ + \text{Urate anions} \leftrightarrow \text{Uric acid}, \quad (2)$$

and its solubility product constant K_{sp} is close to 90 mg l^{-1} (Asplin, 1996). Because its pK is 5.3, half of the total urates will be in the form of uric acid at a urine pH of 5.3. Hence, to favour the urate anion form, the urine pH must rise (its $[H^+]$ must fall). At a urine pH of 6.0, approximately one-sixth of the total urates are in the insoluble uric acid form (100 mg of the total daily excretion of 600 mg; Table 3). Hence, a daily urine volume of 0.6 l at pH 6.0 should prevent uric acid stone formation because the urine can become supersaturated with uric acid up to a concentration of 180 mg dl^{-1} (Asplin, 1996).

Table 3. *Effect of urine pH and volume on its concentration of uric acid*

Total urate (mg day^{-1})	Urine pH	Urine volume (l day^{-1})	[Uric acid] (mg l^{-1})
600	5.3	1	300
600	5.3	3	100
600	6.0	1	100
600	6.0	0.6	167

For details, see text.

The values illustrate the interaction of urine volume and urine pH for a given 24 h total urate excretion rate of 600 mg day^{-1}.

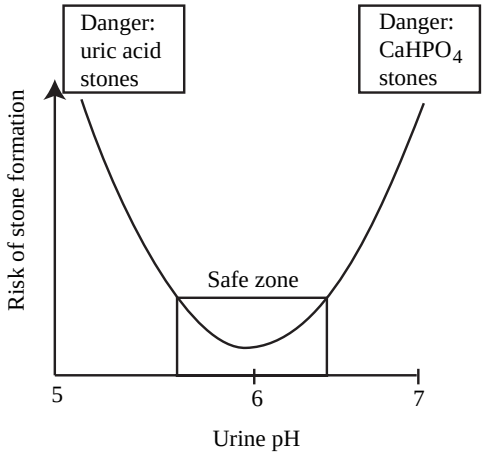

Fig. 2. Urine pH and the risk of kidney stone formation. A urine pH that is close to 6.0 minimizes the risk of forming certain kidney stones. The urine pH that is most dangerous with respect to the formation of uric acid stones, is a value significantly lower than 6.0. In contrast, a urine pH significantly greater than 6.0 must also be avoided to minimize the risk of calcium phosphate ($CaHPO_4$) stone formation.

Traditional view of acid balance; define the dogma

H^+ are produced when new anions are formed during the metabolism of neutral compounds **(**Equation 3**)**:

$$\text{Neutral compound (e.g. glucose)} \rightarrow H^+ + \text{organic anions (e.g. L-lactate}^-) \; . \quad (3)$$

The major non-volatile acid that requires renal disposal as its NH_4^+ salt in the fed state is sulphuric acid (H_2SO_4) (Halperin and Jungas, 1983). H_2SO_4 is produced from the complete oxidation of sulphur-containing amino acids (Relman et al., 1961). The immediate fate of these H^+ is to react with bicarbonate ions (HCO_3^-), resulting in the production of CO_2 that is exhaled *via* the lungs (Equation 4):

$$H^+ + HCO_3^- \rightarrow CO_2 \uparrow \text{(lungs)} + H_2O \; . \quad (4)$$

As a result, the body is left with a deficit of HCO_3^-. Acid balance is achieved when sulphate anions (SO_4^{2-}) are excreted in the urine with an equivalent amount of NH_4^+ because NH_4^+ is formed along with HCO_3^- in the kidney **(**Fig. 3**)**.

The excretion of NH_4^+ can be thought of as having two components. First, NH_4^+ is synthesized in the proximal convoluted tubule (PCT) along with HCO_3^-; its precursor is the electroneutral amino acid, glutamine (Halperin et al., 1989). The selection of this glutamine pathway of metabolism in PCT cells depends on having a low intracellular fluid (ICF) pH. Generation of this new HCO_3^- is not complete until NH_4^+ is made into an end-product of metabolism; NH_4^+ must be excreted in the urine otherwise it would be returned to the liver and converted to urea + H^+.

Second, for high rates of secretion of NH_4^+, ammonia (NH_3) must diffuse across the renal medullary interstitial compartment to the MCD. In this process, NH_4^+ are reabsorbed in the thick ascending limb of the loop of Henle, replacing K^+ on the Na^+, K^+, 2-Cl^- cotransporter (NKCC). This generates the 'single effect' for recycling of NH_4^+ in the loop of Henle

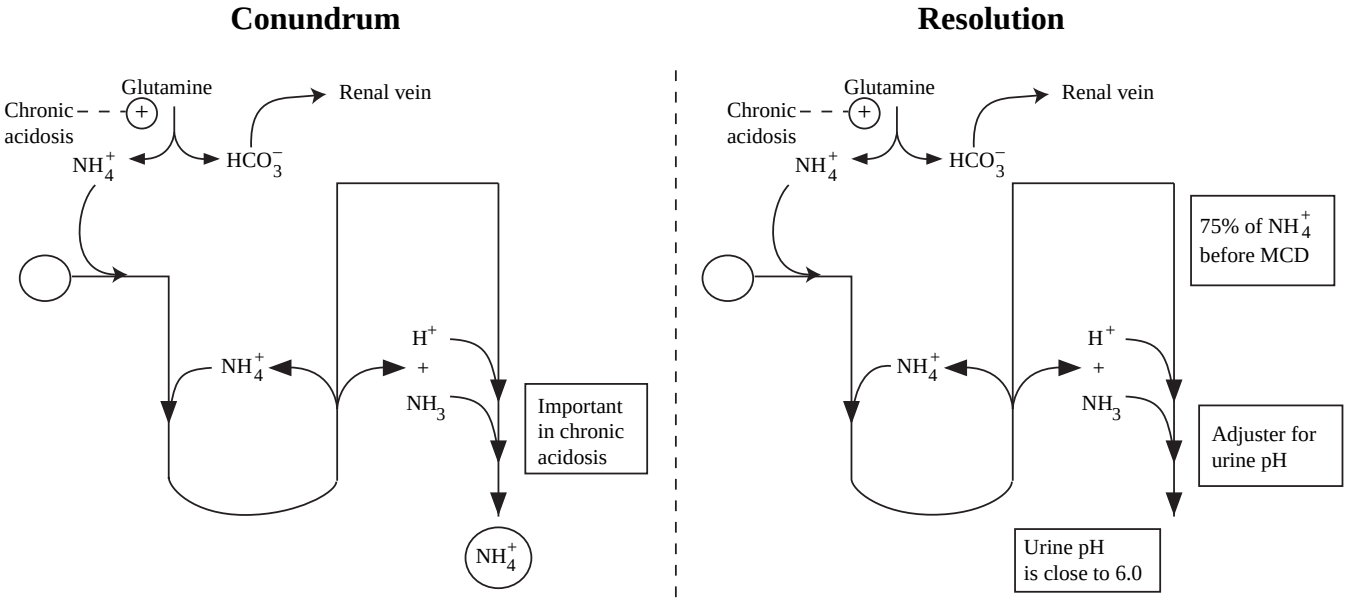

Fig. 3. Physiology of NH_4^+ excretion and acid–base balance. There are two major steps as shown on the left. First, NH_4^+ + HCO_3^- are produced when glutamine is metabolized in the proximal convoluted tubule (PCT). Second, NH_4^+ is transferred *via* the medullary interstitial compartment to the lumen of the medullary collecting duct (MCD) because of a high medullary concentration of NH_3 and a low luminal NH_3 concentration, the result of distal H^+ secretion. Sajo et al. (1981), however, demonstrated that most of the NH_4^+ destined for urinary excretion was added before entering the MCD in rats with chronic metabolic acidosis. This conundrum could be resolved if the major function of events in the inner medulla could be to adjust the urine pH to ~6.0 without compromising the excretion of NH_4^+ (see Fig. 4).

and the build-up of the high concentration of NH_3 in the medullary interstitial compartment to facilitate its diffusion into the lumen of the MCD **(**Fig. 3**)**. To complete the transfer of NH_3 to the final urine as NH_4^+, the pH of the luminal fluid in this nephron segment should be less than 6.0 (Knepper et al., 1989); this low urine pH is caused by H^+ secretion in the MCD, primarily by the H^+-ATPase.

Conundrum

A low urine pH is needed to achieve high rates of excretion of NH_4^+. Hence the price to pay to defend acid–base balance would be to accept a risk of precipitation of uric acid in the terminal nephron. This led us to reconsider the traditional view of the physiology of the excretion of NH_4^+ and the role of its medullary shunt pathway.

Resolution; the physiology of NH_4^+ excretion revisited

(i) Is a low urine pH required for high rates of NH_4^+ excretion?

As judged from the data in subjects fed NH_4Cl on a chronic basis (Madison and Seldin, 1958; Simpson, 1971) and during the ketoacidosis of prolonged fasting (Kamel et al., 1998), a low urine pH is not needed to augment the excretion of NH_4^+ because maximum excretion rates occur when the urine pH is ~6.0.

(ii) Is a medullary shunt required for high rates of NH_4^+ excretion?

We address this question under three headings: theoretical considerations, an experiment using loop diuretics in rats to inhibit the reabsorption of NH_4^+ in the loop of Henle, and data from rats with chronic metabolic acidosis that permit a quantitative analysis of the contribution of the medullary interstitial shunt pathway to NH_4^+ excretion.

Theoretical concerns

Diffusion is a slow process, which requires a high concentration of the substance that will diffuse and the absence of a barrier for its diffusion. Although NH_3 is the species of NH_4^+/NH_3 transported across the basolateral membrane out of cells of the medullary thick ascending limb of the loop of Henle (Kikeri et al., 1989), owing to the interstitial fluid pH and the pK for NH_4^+, the interstitial concentration of NH_3 is 1/100 that of NH_4^+. Of greater importance, there are barriers for the diffusion of NH_3 in the renal medullary interstitial compartment, i.e. the lipid component of cell membranes of the MCD.

Experiment using loop diuretics

Another way to gain insights into the importance of a pathway is to inhibit it. Because NH_4^+ is reabsorbed in the loop of Henle, a loop diuretic should markedly reduce medullary NH_4^+ shunting. Following action of the loop diuretic, ethacrynic acid, rather than reducing the rate of excretion of NH_4^+ as one would expect if this NH_3 shunt pathway were critical for NH_4^+ excretion, the rate of NH_4^+ excretion rose significantly (Vasuvattakul et al., 1993). Because the urine pH fell in the experiments employing ethacrynic acid, we used the loop diuretic, furosemide, which has a minor carbonic anhydrase effect that prevents the fall in the urine pH. Furosemide led to a marked rise in the rate of excretion of NH_4^+, but in these experiments, this rise could not be attributed to a fall in the urine pH (Table 4). Therefore this medullary shunt pathway lowers rather than raises the rate of NH_4^+ excretion. Hence another hypothesis is needed to identify a possible function of this shunt pathway.

Table 4. *Effect of furosemide on the rate of NH_4^+ excretion in rats fed a low-electrolyte diet*

	Furosemide (mg kg^{-1})	
Urine	0	4.0
NH_4^+ (nmol min^{-1})	0.8±0.07	3.1±0.50*
Flow rate (µl min^{-1})	3.3±0.2	50±6*
Na^+ (µmol min^{-1})	0.3±0.05	6.3±0.9*
Osmolality (mOsm kg^{-1} H_2O)	2449±102	753±22*
pH	5.7±0.30	6.2±0.03

Six rats consumed a low-electrolyte diet for 4 days. On day 3, dDAVP was given prior to collecting overnight urine. At 10:00 h, all rats were given furosemide and dDAVP by the intraperitoneal route and urine was collected over the next 2 h.

Results are reported as means ± S.E.M.

*P<0.01 for the effect of furosemide.

Quantitative analysis of the medullary shunt process

Sajo et al. (1981) measured NH_4^+ excretion in rats with chronic metabolic acidosis using a microcatheterization technique to sample fluid from the junction of the cortical and medullary collecting ducts. They found that approximately 75% of the NH_4^+ excreted was added prior to entry into the MCD (Fig. 3). Therefore a high rate of shunting of NH_4^+ from the loop of Henle to the MCD is not required to achieve a high rate of excretion of NH_4^+ in the rat.

Reinterpretation of the physiology of NH_4^+ excretion; an attempt at resolution

The major function of the medullary NH_3 shunt pathway is not 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 a robust luminal NH_4^+/H^+ ion exchanger to remove H^+ secreted by the MCD during chronic metabolic acidosis (Verlander et al., 2003; Weiner and Verlander, 2003). In this hypothesis, distal H^+ secretion will provide the driving force for the transport of NH_4^+ into the lumen of the MCD and hence this process functions as an adjuster of the urine pH.

The overall process is described in more detail in Fig. 4. It begins 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 4 in Fig. 4). Recycling of NH_4^+ in the loop of Henle raises the

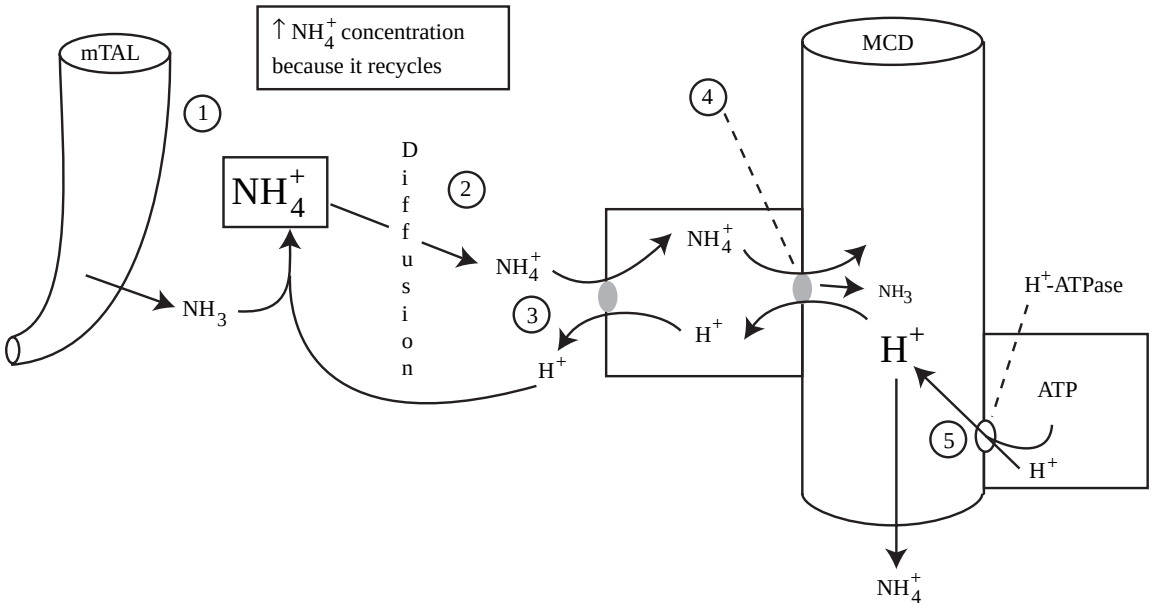

Fig. 4. Transfer of NH_4^+ from the loop of Henle (LOH) to the medullary collecting duct (MCD). The medullary thick ascending limb (mTAL) of the LOH is shown on the far left and the MCD is shown on the right side of the figure. Reabsorption of NH_4^+ from the mTAL adds NH_3 to the interstitial compartment (the H^+ to convert it to NH_4^+ arrives at site 4). Recycling of NH_4^+ in the LOH raise the concentration of NH_4^+ in the medullary interstitium (site 1). NH_4^+ diffuses through the renal medullary interstitial compartment because its concentration is high and that of NH_3 is too low for rapid rates of diffusion (site 2). NH_4^+ crosses the cell membranes of the MCD using two different NH_4^+/H^+ exchangers, one on each of these cells (sites 3 and 4). The combination of NH_4^+ entry into and H^+ exit from the lumen of the MCD (site 4) adjusts the urine pH upward (towards 6.0) despite continuing H^+ secretion by the H^+-ATPase (site 5). The net result is a final urine pH that is close to 6.0 and a somewhat higher rate of NH_4^+ excretion.

concentration of NH_4^+ in the medullary interstitium (Knepper et al., 1989) (site 1, Fig. 4). NH_4^+ can diffuse rapidly enough through the renal medullary interstitial compartment because its concentration is high. NH_4^+ crosses both lipid-containing cell membranes of the MCD *via* two different Rh-glycoproteins that function as NH_4^+/H^+ exchangers, one on the basolateral and another on the luminal membrane of these cells (sites 3 and 4 in Fig. 4) (Verlander et al., 2003; Weiner and Verlander, 2003). The combination of H^+ exit from, and NH_4^+ 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 ~6.0 and a somewhat higher rate of NH_4^+ excretion.

In summary, we suggest that NH_4^+ plays a direct role in both the traditional physiology (generates new HCO_3^-) and in the integrative physiology (adjusts the urine pH to ~6.0). For the latter, NH_4^+ diffuses across the medullary interstitial compartment and that there is a H^+-linked counter-transport system with NH_4^+ across both polar membranes of MCD cells. Moreover, the driving force for this process is the secretion of H^+ by the MCD, because the concentration of NH_4^+ in the urine is higher than in the papillary interstitial compartment (Table 2). Thus, the activity of the NH_4^+/H^+ exchanger in the luminal membrane might act as an 'adjuster' for the final urine pH to minimize the risk of uric acid stone formation (Fig. 4).

Concluding remarks

Our goal was to consider the renal excretion of the major nitrogenous waste products, urea and NH_4^+, from the perspectives of their specific physiology and of integrative physiology, i.e. the need to ensure that the composition of the urine would minimize the risk of forming kidney stones. For urea, a system was needed to make it become an effective urine osmole in electrolyte-poor urine and thereby avoid a dangerously low urine flow rate. A special consideration for the excretion of NH_4^+ was to ensure that the urine pH was close to 6.0 without sacrificing acid–base balance. During chronic metabolic acidosis, the high rate of excretion of NH_4^+ needs to be achieved while maintaining a urine pH of 6.0. The medullary shunt process for NH_3, which requires a low luminal pH, does not appear to be important for NH_4^+ excretion. Rather, this shunt pathway utilizes the diffusion of NH_4^+ plus two NH_4^+/H^+ exchangers in the MCD, and it can prevent a large fall in the urine pH due to distal H^+ secretion.

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