4.4: Renal Regulation of Acid-Base Balance

2.4.1: Role of the Kidneys

The organs involved in regulation of external acid-base balance are the **lungs** and the **kidneys**.

The lungs are important for excretion of carbon dioxide (the respiratory acid) and there is a huge amount of this to be excreted: at least 12,000 to 13,000 mmols/day.

In contrast the kidneys are responsible for excretion of the fixed acids and this is also a critical role even though the amounts involved (70-100 mmols/day) are much smaller. The main reason for this renal importance is because there is no other way to excrete these acids and it should be appreciated that the amounts involved are still very large when compared to the plasma [H\(^+\)] of only 40 nanomoles/litre.

There is a second extremely important role that the kidneys play in acid-base balance, namely the reabsorption of the filtered bicarbonate. Bicarbonate is the predominant extracellular buffer against the fixed acids and it important that its plasma concentration should be defended against renal loss.

In acid-base balance, the kidney is responsible for 2 major activities:

- Reabsorption of filtered bicarbonate: 4,000 to 5,000 mmol/day
- Excretion of the fixed acids (acid anion and associated H\(^+\)): about 1 mmol/kg/day.

Both these processes involve secretion of H\(^+\) into the lumen by the renal tubule cells but only the second leads to excretion of H\(^+\) from the body.
The renal mechanisms involved in acid-base balance can be difficult to understand so as a simplification we will consider the processes occurring in the kidney as involving 2 aspects:

- Proximal tubular mechanism
- Distal tubular mechanism

### 2.4.2: Proximal Tubular Mechanism

The contributions of the proximal tubules to acid-base balance are:

- firstly, reabsorption of bicarbonate which is filtered at the glomerulus
- secondly, the production of ammonium

The next 2 sections explain these roles in more detail.

### 2.4.3: Bicarbonate Reabsorption

Daily filtered bicarbonate equals the product of the daily glomerular filtration rate (180 l/day) and the plasma bicarbonate concentration (24 mmol/l). This is 180 x 24 = 4320 mmols/day (or usually quoted as between 4000 to 5000 mmols/day).

About 85 to 90% of the filtered bicarbonate is reabsorbed in the proximal tubule and the rest is reabsorbed by the intercalated cells of the distal tubule and collecting ducts.

The reactions that occur are outlined in the diagram. Effectively, $H^+$ and $HCO_3^-$ are formed from $CO_2$ and $H_2O$ in a reaction catalysed by carbonic anhydrase. The actual reaction involved is probably formation of $H^+$ and $OH^-$ from water, then reaction of $OH^-$ with $CO_2$ (catalysed by carbonic anhydrase) to produce $HCO_3^-$. Either way, the end result is the same.

The $H^+$ leaves the proximal tubule cell and enters the PCT lumen by 2 mechanisms:

- Via a $Na^+-H^+$ antiporter (major route)
- Via $H^+-ATPase$ (proton pump)

Filtered $HCO_3^-$ cannot cross the apical membrane of the PCT cell. Instead it combines with the secreted $H^+$ (under the influence of brush border carbonic anhydrase) to produce $CO_2$ and $H_2O$. The $CO_2$ is lipid soluble and easily crosses into the cytoplasm of the PCT cell. In the cell, it combines with $OH^-$ to produce bicarbonate. The $HCO_3^-$ crosses the basolateral membrane via a $Na^+-HCO_3^-$ symporter. This symporter is electrogenic as it transfers three $HCO_3^-$ for every one $Na^+$. In comparison, the $Na^+-H^+$ antiporter in the apical membrane is not electrogenic because an equal amount of charge is transferred in both directions.

The basolateral membrane also has an active $Na^+-K^+$ ATPase (sodium pump) which transports 3 $Na^+$ out per 2 $K^+$ in.
This pump is electrogenic in a direction opposite to that of the $\text{Na}^+-\text{HCO}_3^-$ symporter. Also the sodium pump keeps intracellular $\text{Na}^+$ low which sets up the $\text{Na}^+$ concentration gradient required for the $\text{H}^+-\text{Na}^+$ antiport at the apical membrane. The $\text{H}^+-\text{Na}^+$ antiport is an example of secondary active transport.

The net effect is the reabsorption of one molecule of $\text{HCO}_3^-$ and one molecule of $\text{Na}^+$ from the tubular lumen into the blood stream for each molecule of $\text{H}^+$ secreted. This mechanism does not lead to the net excretion of any $\text{H}^+$ from the body as the $\text{H}^+$ is consumed in the reaction with the filtered bicarbonate in the tubular lumen.

Note

The differences in functional properties of the apical membrane from that of the basolateral membranes should be noted. This difference is maintained by the tight junctions which link adjacent proximal tubule cells.

These tight junctions have two extremely important functions:

*Gate function*: They limit access of luminal solutes to the intercellular space. This resistance can be altered and this paracellular pathway can be more open under some circumstances (ie the gate can be opened a little).

*Fence function*: The junctions maintain different distributions of some of the integral membrane proteins. For example they act as a fence to keep the $\text{Na}^+-\text{H}^+$ antiporter limited to the apical membrane, and keep the $\text{Na}^+-\text{K}^+$ ATPase limited to the basolateral membrane. The different distribution of such proteins is absolutely essential for cell function.

The 4 major factors which control bicarbonate reabsorption are:

- Luminal $\text{HCO}_3^-$ concentration
- Luminal flow rate
- Arterial $\text{pCO}_2$
- Angiotensin II (via decrease in cyclic AMP)

An increase in any of these four factors causes an increase in bicarbonate reabsorption. Parathyroid hormone also has an effect: an increase in hormone level increases cAMP and decreases bicarbonate reabsorption.
The mechanism for $\text{H}^+$ secretion in the proximal tubule is described as a high capacity, low gradient system:

The high capacity refers to the large amount (4000 to 5000 mmols) of $\text{H}^+$ that is secreted per day. (The actual amount of $\text{H}^+$ secretion is 85% of the filtered load of $\text{HCO}_3^-$).

The low gradient refers to the low pH gradient as tubular pH can be decreased from 7.4 down to 6.7-7.0 only.

Though no net excretion of $\text{H}^+$ from the body occurs, this proximal mechanism is extremely important in acid-base balance. Loss of bicarbonate is equivalent to an acidifying effect and the potential amounts of bicarbonate lost if this mechanism fails are very large.

2.4.4: Ammonium Production

Ammonium ($\text{NH}_4^+$) is produced predominantly within the proximal tubular cells. The major source is from glutamine which enters the cell from the peritubular capillaries (80%) and the filtrate (20%). Ammonium is produced from glutamine by the action of the enzyme glutaminase. Further ammonium is produced when the glutamate is metabolised to produce alpha-ketoglutarate. This molecule contains 2 negatively-charged carboxylate groups so further metabolism of it in the cell results in the production of $2 \text{HCO}_3^-$ anions. This occurs if it is oxidised to $\text{CO}_2$ or if it is metabolised to glucose.

The pKa for ammonium is so high (about 9.2) that both at extracellular and at intracellular pH, it is present entirely in the acid form $\text{NH}_4^+$. The previous idea that lipid soluble $\text{NH}_3$ is produced in the tubular cell, diffuses into the tubular fluid where it is converted to water soluble $\text{NH}_4^+$ which is now trapped in the tubule fluid is incorrect.

The subsequent situation with ammonium is complex. Most of the ammonium is involved in cycling within the medulla. About 75% of the proximally produced ammonium is removed from the tubular fluid in the medulla so that the amount of ammonium entering the distal tubule is small. The thick ascending limb of the loop of Henle is the important segment for
removing ammonium. Some of the interstitial ammonium returns to the late proximal tubule and enters the medulla again (ie recycling occurs).

An overview of the situation so far is that:

- The ammonium level in the DCT fluid is low because of removal in the loop of Henle
- Ammonium levels in the medullary interstitium are high (and are kept high by the recycling process via the thick ascending limb and the late PCT)
- Tubule fluid entering the medullary collecting duct will have a low pH if there is an acid load to be excreted (and the phosphate buffer has been titrated down).

If H\(^+\) secretion continues into the medullary collecting duct this would reduce the pH of the luminal fluid further. A low pH greatly augments transfer of ammonium from the medullary interstitium into the luminal fluid as it passes through the medulla. The lower the urine pH, the higher the ammonium excretion and this ammonium excretion is augmented further if an acidosis is present. This augmentation with acidosis is ‘regulatory’ as the increased ammonium excretion by the kidney tends to increase extracellular pH towards normal.

If the ammonium returns to the bloodstream it is metabolised in the liver to urea (Krebs-Henseleit cycle) with net production of one hydrogen ion per ammonium molecule.

Note

Section 2.4.7 discusses the role of urinary ammonium excretion.

2.4.5: Distal Tubular Mechanism

This is a low capacity, high gradient system which accounts for the excretion of the daily fixed acid load of 70 mmols/day. The maximal capacity of this system is as much as 700 mmols/day but this is still low compared to the capacity of the proximal tubular mechanism to secrete H\(^+\). It can however decrease the pH down to a limiting pH of about 4.5: this represents a thousand-fold (ie 3 pH units) gradient for H\(^+\) across the distal tubular cell. The maximal capacity of 700 mmols/day takes about 5 days to reach.

The processes involved are:

- Formation of titratable acidity (TA)
- Addition of ammonium (NH\(_4^+\)) to luminal fluid
- Reabsorption of Remaining Bicarbonate

1. Titratable Acidity

H\(^+\) is produced from CO\(_2\) and H\(_2\)O (as in the proximal tubular cells) and actively transported into the distal tubular lumen via a H\(^+\)-ATPase pump. Titratable acidity represents the H\(^+\) which is buffered mostly by phosphate which is present in significant concentration. Creatinine (pKa approx 5.0) may also contribute to TA. At the minimum urinary pH, it will
account for some of the titratable acidity. If ketoacids are present, they also contribute to titratable acidity. In severe diabetic ketoacidosis, beta-hydroxybutyrate (pKa 4.8) is the major component of TA.

The TA can be measured in the urine from the amount of sodium hydroxide needed to titrate the urine pH back to 7.4 hence the term titratable acidity.

2. Addition of Ammonium

As discussed previously, ammonium is predominantly produced by proximal tubular cells. This is advantageous as the proximal cells have access to a high blood flow in the peritubular capillaries and to all of the filtrate and these are the two sources of the glutamine from which the ammonium is produced.

The medullary cycling maintains high medullary interstitial concentrations of ammonium and low concentrations of ammonium in the distal tubule fluid. The lower the urine pH, the more the amount of ammonium that is transferred from the medullary interstitium into the fluid in the lumen of the medullary collecting duct as it passes through the medulla to the renal pelvis. [Note: The medullary collecting duct is different from the distal convoluted tubule.]

The net effect of this is that the majority of the ammonium in the final urine was transferred from the medulla across the distal part of the tubule even though it was produced in the proximal tubule. [Simplistically but erroneously it is sometimes said that the ammonium in the urine is produced in the distal tubule cells.]

Ammonium is not measured as part of the titratable acidity because the high pK of ammonium means no H⁺ is removed from NH₄⁺ during titration to a pH of 7.4. Ammonium excretion in severe acidosis can reach 300 mmol/day in humans.

Ammonium excretion is extremely important in increasing acid excretion in systemic acidosis. The titratable acidity is mostly due to phosphate buffering and the amount of phosphate present is limited by the amount filtered (and thus the plasma concentration of phosphate). This cannot increase significantly in the presence of acidosis (though of course some additional phosphate could be released from bone) unless other anions with a suitable pKa are present. Ketoanions can contribute to a significant increase in titratable acidity but only in ketoacidosis when large amounts are present.

In comparison, the amount of ammonium excretion can and does increase markedly in acidosis. The ammonium excretion increases as urine pH falls and also this effect is markedly augmented in acidosis. Formation of ammonium prevents further fall in pH as the pKa of the reaction is so high.

In review

- Titratable acidity is an important part of excretion of fixed acids under normal circumstances but the amount of phosphate available cannot increase very much.
- Also as urine pH falls, the phosphate will be all in the dihydrogen form and buffering by phosphate will be at its maximum.
- A further fall in urine pH cannot increase titratable acidity (unless there are other anions such as keto-anions present in significant quantities)
The above points mean that titratable acidity cannot increase very much (so cannot be important in acid-base regulation when the ability to increase or decrease renal H\(^+\) excretion is required)

In acidosis, ammonium excretion fills the regulatory role because its excretion can increase very markedly as urine pH falls.

A low urine pH itself cannot directly account for excretion of a significant amount of acid: for example, at the limiting urine pH of about 4.4, [H\(^+\)] is a negligible 0.04 mmol/l. This is several orders of magnitude lower than H\(^+\) accounted for by titratable acidity and ammonium excretion. (ie 0.04 mmol/l is insignificant in a net renal acid excretion of 70 mmols or more per day)

3. Reabsorption of Remaining Bicarbonate

On a typical Western diet all of the filtered load of bicarbonate is reabsorbed. The sites and percentages of filtered bicarbonate involved are:

- Proximal tubule 85%
- Thick ascending limb of Loop of Henle 10-15%
- Distal tubule 0-5%

The decrease in volume of the filtrate as further water is removed in the Loop of Henle causes an increase in [HCO\(_3\)-] in the remaining fluid. The process of HCO\(_3\)- reabsorption in the thick ascending limb of the Loop of Henle is very similar to that in the proximal tubule (ie apical Na\(^+\)-H\(^+\) antiport and basolateral Na\(^+\)-HCO\(_3\)- symport and Na\(^+\)-K\(^+\) ATPase).

Bicarbonate reabsorption here is stimulated by the presence of luminal frusemide. The cells in this part of the tubule contain carbonic anhydrase.

Any small amount of bicarbonate which enters the distal tubule can also be reabsorbed. The distal tubule has only a limited capacity to reabsorb bicarbonate so if the filtered load is high and a large amount is delivered distally then there will be net bicarbonate excretion.

The process of bicarbonate reabsorption in the distal tubule is somewhat different from in the proximal tubule:

- H\(^+\) secretion by the intercalated cells in DCT involves a H\(^+\)-ATPase (rather than a Na\(^+\)-H\(^+\) antiport)
- HCO\(_3\)- transfer across the basolateral membrane involves a HCO\(_3\)--Cl\(^-\) exchanger (rather than a Na\(^+\)-HCO\(_3\)- symport)

The net effect of the excretion of one H\(^+\) is the return of one HCO\(_3\)- and one Na\(^+\) to the blood stream. The HCO\(_3\)-effectively replaces the acid anion which is excreted in the urine.

The net acid excretion in the urine is equal to the sum of the TA and [NH\(_4\)^+] minus [HCO\(_3\)-] (if present in the urine). The [H\(^+\)] accounts for only a very small amount of the H\(^+\) excretion and is not usually considered in the equation (as
In metabolic alkalosis, the increased bicarbonate level will result in increased filtration of bicarbonate provided the GFR has not decreased. The kidney is normally extremely efficient at excreting excess bicarbonate but this capacity can be impaired in certain circumstances. (See Section 7.2 and 7.3)

Outline of Reactions in Distal Tubule Lumen & Cells

2.4.6: Regulation of Renal H⁺ Excretion

The discussion above has described the mechanisms involved in renal acid excretion and mentioned some factors which regulate acid excretion.

The major factors which regulate renal bicarbonate reabsorption and acid excretion are:

1. Extracellular volume

Volume depletion is associated with Na⁺ retention and this also enhances HCO₃⁻ reabsorption. Conversely, ECF volume expansion results in renal Na⁺ excretion and secondary decrease in HCO₃⁻ reabsorption.

2. Arterial pCO₂

An increase in arterial pCO₂ results in increased renal H⁺ secretion and increased bicarbonate reabsorption. The converse also applies. Hypercapnia results in an intracellular acidosis and this results in enhanced H⁺ secretion. The cellular processes involved have not been clearly delineated. This renal bicarbonate retention is the renal compensation for a chronic respiratory acidosis.
3. Potassium & Chloride Deficiency

Potassium has a role in bicarbonate reabsorption. Low intracellular $K^+$ levels result in increased $HCO_3^-$ reabsorption in the kidney. Chloride deficiency is extremely important in the maintenance of a metabolic alkalosis because it prevents excretion of the excess $HCO_3^-$ (i.e., now the bicarbonate instead of chloride is reabsorbed with $Na^+$ to maintain electroneutrality). (See discussion in Section 7.3)

4. Aldosterone & cortisol (hydrocortisone)

Aldosterone at normal levels has no role in renal regulation of acid-base balance. Aldosterone depletion or excess does have indirect effects. High aldosterone levels result in increased $Na^+$ reabsorption and increased urinary excretion of $H^+$ and $K^+$ resulting in a metabolic alkalosis. Conversely, it might be thought that hypoaldosteronism would be associated with a metabolic acidosis but this is very uncommon but may occur if there is coexistent significant interstitial renal disease.

5. Phosphate Excretion

Phosphate is the major component of titratable acidity. The amount of phosphate present in the distal tubule does not vary greatly. Consequently, changes in phosphate excretion do not have a significant regulatory role in response to an acid load.

6. Reduction in GFR

It has recently been established that a reduction in GFR is a very important mechanism responsible for the maintenance of a metabolic alkalosis. The filtered load of bicarbonate is reduced proportionately with a reduction in GFR.

7. Ammonium

The kidney responds to an acid load by increasing tubular production and urinary excretion of $NH_4^+$. The mechanism involves an acidosis-stimulated enhancement of glutamine utilisation by the kidney resulting in increased production of $NH_4^+$ and $HCO_3^-$ by the tubule cells. This is very important in increasing renal acid excretion during a chronic metabolic acidosis. There is a lag period: the increase in ammonium excretion takes several days to reach its maximum following an acute acid load. Ammonium excretion can increase up to about 300 mmol/day in a chronic metabolic acidosis so this is important in renal acid-base regulation in this situation. Ammonium excretion increases with decreases in urine pH and this relationship is markedly enhanced with acidosis.

2.4.7: What is the Role of Urinary Ammonium Excretion?

There are different views on the true role of $NH_4^+$ excretion in urine. How can the renal excretion of ammonium which has a $pK$ of 9.2 represent $H^+$ excretion from the body?
One school says the production of ammonium from glutamine in the tubule cells results in production of alpha-ketoglutarate which is then metabolised in the tubule cell to new bicarbonate which is returned to the blood. The net effect is the return of one bicarbonate for each ammonium excreted in the urine. By this analysis, the excretion of ammonium is equivalent to the excretion of acid from the body as one plasma $H^+$ would be neutralised by one renal bicarbonate ion for each ammonium excreted. Thus an increase in ammonium excretion as occurs in metabolic acidosis is an appropriate response to excrete more acid.

The other school says this is not correct. The argument is that metabolism of alpha-ketoglutarate in the proximal tubule cells to produce this new $\text{HCO}_3^-$ merely represents regeneration of the $\text{HCO}_3^-$ that was neutralised by the $H^+$ produced when alpha-ketoglutarate was metabolised to glutamate in the liver originally so there can be no direct effect on net $H^+$ excretion. The key to understanding is said to lie in considering the role of the liver. Consider the following:

Every day protein turnover results in amino acid degradation which results in production of $\text{HCO}_3^-$ and $\text{NH}_3^+$. For a typical 100g/day protein diet, this is a net production of 1,000mmol/day of $\text{HCO}_3^-$ and 1,000mmol/day of $\text{NH}_4^+$. (These are produced in equal amounts by neutral amino acids as each contains one carboxylic acid group and one amino group.) The high pK of the ammonium means it cannot dissociate to produce one $H^+$ to neutralise the $\text{HCO}_3^-$ so consequently amino acid metabolism is powerfully alkalinising to the body. The body now has two major problems:

- How to get rid of 1,000mmol/day of alkali?
- How to get rid of 1,000mmol/day of the highly toxic ammonium?

The solution is to react the two together and get rid of both at once. This process is hepatic urea synthesis (Krebs-Henseleit cycle). The cycle consumes significant energy but solves both problems. Indeed, the cycle in effect acts as an ATP-dependent pump that transfers $H^+$ from the very weak acid $\text{NH}_4^+$ to $\text{HCO}_3^-$. The overall reaction in urea synthesis is:

$$2 \text{NH}_4^+ + 2 \text{HCO}_3^- \rightarrow \text{urea} + \text{CO}_2 + 3\text{H}_2\text{O}$$

The body has two ways in which it can remove $\text{NH}_4^+$:

- Urea synthesis in the liver
- Excretion of $\text{NH}_4^+$ by the kidney

The key thing here is that the acid-base implications of these 2 mechanisms are different.

For each ammonium converted to urea in the liver one bicarbonate is consumed. For each ammonium excreted in the urine, there is one bicarbonate that is not neutralised by it (during urea synthesis) in the liver. So overall, urinary excretion of ammonium is equivalent to net bicarbonate production -but by the liver! Indeed in a metabolic acidosis, an increase in urinary ammonium excretion results in an exactly equivalent net amount of hepatic bicarbonate (produced from amino acid degradation) available to the body. So the true role of renal ammonium excretion is to serve as an alternative route for nitrogen elimination that has a different acid-base effect from urea production.

The role of glutamine is to act as the non-toxic transport molecule to carry $\text{NH}_4^+$ to the kidney. The bicarbonates...
consumed in the production of glutamine and then released again with renal metabolism of ketoglutarate are not important as there is no net gain of bicarbonate.

Overall: renal NH$_4^+$ excretion results indirectly in an equivalent amount of net hepatic HCO$_3^-$ production.

Other points are:

- Glutamate metabolism in the proximal tubule converts ADP to ATP and the low availability of ADP limits the maximal rate of NH$_4^+$ production in the proximal tubule cells. Further as most ATP is consumed in the reabsorption of Na$^+$, then it is ultimately the amount of Na$^+$ reabsorbed in the proximal tubule that sets the upper limit for NH$_4^+$ production.
- The anion that is excreted with the NH$_4^+$ is also important. Excretion of beta-hydroxybutyrate (instead of chloride) with NH$_4^+$ in ketoacidosis leads to a loss of bicarbonate as this anion represents a potential bicarbonate.

Finally: The role of urine pH in situations of increased acid secretion is worth noting. The urine pH can fall to a minimum value of 4.4 to 4.6 but as mentioned previously this itself represents only a negligible amount of free H$^+$.

As pH falls, the 3 factors involved in increased H$^+$ excretion are:

1. **Increased ammonium excretion** (increases steadily with decrease in urine pH and this effect is augmented in acidosis) [This is the **major and regulatory factor** because it can be increased significantly].

2. **Increased titratable acidity**:
   - Increased buffering by phosphate (but negligible further effect on H$^+$ excretion if pH < 5.5 as too far from pKa so minimal amounts of HPO$_4^{2-}$ remaining)
   - Increased buffering by other organic acids (if present) may be important at lower pH values as their pKa is lower (eg creatinine, ketoanions)

(As discussed also in section 2.5.4, increases in TA are limited and are not as important as increases in ammonium excretion)

3. **Bicarbonate reabsorption is complete** at low urinary pH so none is lost in the urine (Such loss would antagonise the effects of an increased TA or ammonium excretion on acid excretion.)

**Comment**

The above discussion focuses on the 'traditional approach' to acid-base balance and a shortcoming of that approach is that the explanations are wrong. The Stewart approach (see Chapter 10) provides the explanations and the insights into what is occurring. For example, the focus on excretion of H$^+$ and excretion of NH$_4^+$ by the kidney is misleading. 'Acid handling' by the kidney is mostly mediated through changes in Cl$^-$ balance. NH$_4^+$ is a weak anion that when excreted with Cl$^-$ allows the body to retain the strong ions Na$^+$ and K$^+$. The urinary excretion of Cl$^-$ without excretion of an equivalent amount of strong ion results in a change in the SID (or 'strong ion difference') and it is this change which causes the change in plasma pH. The explanatory focus should be on the
excretion of $\text{Cl}^-$ without strong ions and not on the excretion of $\text{NH}_4^+$. See Chapter 10 for an introduction to the Stewart approach.