[1]Renal Control of Acid/Base Balance

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Acid/Base refers to anything having to do with the concentrations of H^+ ions in aqueous solutions. In medical physiology, we are concerned with the processes that keep the body's H^+ ion concentration stable during health (a H^+ concentration that generates a pH of approximately 7.40), the pathology that alters H^+ ion concentration during disease (acid/base disorders), and the diagnosis and treatment of these disorders. Although the normal intracellular pH is very similar to extracellular fluid pH (although slightly more acidic intracellularly due to the large number of acidic proteins), we monitor extracellular pH in medicine, because that is the compartment where we can easily sample H^+ ion concentration (ie, plasma is extracellular fluid).

Blood pH

The concentration of H^+ ions in plasma is very low compared to other ions. For example, you know that normal plasma Na^+ ion concentration is about 140 milliequivalents per liter of plasma (expressed as mEq/L of plasma). This is some 3 million times greater than the normal plasma H^+ ion concentration, which at average pH (7.40) is about **40 nEq/L** (nanoequivalents/L plasma). Because H^+ is a univalent ion, this can also be expressed as *nanomoles/liter* (nMol/L). The term "**pH**", which is most often used to describe the H^+ ion concentration in biological solutions, is simply the negative log of the H^+ ion concentration:

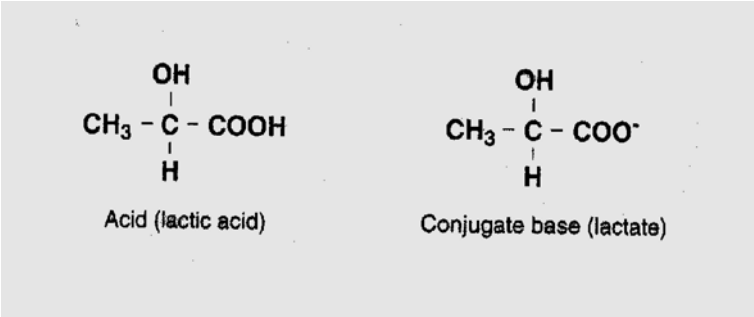
$$pH = - \log [H^+]$$

Furthermore, understand that in a biological solution where the pH is measured as 7.0, this means that the H^+ ion concentration is approximately 10^{-7} moles/liter (mol/L). In other words, 0.0000007 moles of H^+ ions per liter of fluid.

As you are aware, the pH scale ranges from 1 to 14, and a solution with a pH lower than 7.0 (i.e. *more* than 10^{-7} mol/L of H^+ ions) considered acidic (the lower the pH number, the more acidic, because you have more mols of free H^+ ions in solution), and a solution with a pH higher than 7.0 is considered alkaline, because you have less free H^+ ions in solution. A pH of 7.0 is considered a *neutral* solution, neither acidic nor alkaline, in a solution that is 25° C. However, a pH of 7.0 is really not neutral at body temperature (37° C). In fact, in pure water at 37° C, a neutral pH is about 6.8. Therefore, a pH of 7.0 at body temperature is really slightly alkaline. Thus, healthy blood is also somewhat alkaline, with a pH of about **7.40**. Acceptable ranges of pH are between about **7.38 and 7.42**. Anything above 7.42 is considered an **alkalemia**, and a plasma pH below 7.38 is generally considered an **acidemia**. The control of blood pH is critically important, because rapid swings of just .10 or .20 pH units in either direction can lead to impaired cardiopulmonary performance and neurological impairment.

Acid and Base

What is an "acid", and what is a "base"? The first definition was proposed by Bronsted and Lowry, who defined an acid as a substance that can donate (ie, "give up") a proton, and a base as a substance that can accept (ie, "pick up") a proton. When an acid loses it's proton, it becomes the *conjugate base* of that acid. By definition, a conjugate base is exactly the same as the parent acid, except that it is missing a proton. An example of an acid and it's conjugate base is lactic acid and lactate:



When an acid and base are related through the loss or gain of a proton, they are referred to as a *conjugate acid-base pair*. Examples of such pairs are lactic acid and lactate as seen above, but also such compounds as ammonium ion (NH_4^+) and ammonia (NH_3). In a conjugate pair, the acid always has a more positive charge by +1 (because it has the extra proton). However, as the ammonium ion/ammonia conjugate acid-base pair demonstrates, there is no reason why the acid must be neutral in charge, or the conjugate base must be an anion. You will see later that this ammonium ion/ammonia conjugate acid-base pair are extremely important in buffering and eliminating excess H^+ ions into the urine.

Biological Buffers

There are **3 major buffering systems** in biological fluids; the bicarbonate buffer system, the protein buffer system , and the phosphate buffer system. Part of the protein buffering system includes the role of albumin, the major plasma protein. As you know, proteins are constructed of many different amino acids. The most important buffering sites on plasma proteins like albumin are on the amino acid *histidine*, which has a dissociable proton with a pK that makes it a good buffer. Plasma proteins like albumin account for about 20% of the non-bicarbonate buffering capacity.

Another important protein is *deoxygenated hemoglobin*, which buffers mostly within the red blood cells. Like albumin, it is the histidine residues in hemoglobin that allow it to be a good buffer. It accounts for about 80% of the non-bicarbonate buffering power. Phosphoric acid (H_3PO_4) is a triprotic acid, meaning it has 3 dissociable protons. As the protons dissociate, it becomes $H_2PO_4^{1-}$ (dihydrogen phosphate), and HPO_4^{2-} (monohydrogen phosphate), and finally PO_4^{3-} (phosphate). As you will see later, only the $H_2PO_4^{1-}$ <-------> HPO_4^{2-} make a good buffering pair, because this reaction has a pK of about 6.8 (ie, somewhat near physiological pH). The phosphate buffers are the least important of the extracellular non-bicarbonate buffers, mainly because their concentrations in the extracellular fluid is so low.

The Isohydric Principle

The isohydric principle simply makes a point of the fact that, even though there are 3 principle types of buffering systems in biological fluids, in an acid/base crisis, they all work together. This is because the H^+ ion is common to all of them. The take home message is that *any insult that changes one buffering system, will also change the other two in the same way*. Class discussion will involve the major plasma buffer, the bicarbonate buffering system, as that is the one we focus on in medicine.

The Henderson-Hasselbalch Equation

Not all acids are equally weak or strong; strong acids dissociate more readily than weak acids when in solution. As a rule, mineral acids (HCl, KCl, H_2SO_4, etc) are much stronger acids than organic acids (lactic acid, citric acid, butyric acid, etc.). The Henderson-Hasselbalch equation equation was constructed many years ago to measure pH, based on the ratios of conjugate acids to their conjugate bases:

$$pH = pK + \log [A^-]/[HA]$$

Therefore, to estimate the pH of a buffered solution, you simply do a chemical analysis to determine $[A^-]$ and [HA] of the acid-base pair that is acting as the buffer, then calculate the ratio. You then simply determine the log of the ratio, and add that number to the pK of the acid in the acid-base pair that is acting as the buffer. The pK is simply the pH of a solution that an acid is 50% dissociated; the lower the pK, the stronger the acid. The pK of all acid / base pairs is known and can be simply looked up in a textbook. The pK of the bicarbonate buffering systems happens to be 6.1.

As you know at this point, **the conjugate base of the bicarbonate buffering system is HCO_3^-, and the conjugate acid of this system is $C0_2$**. Only $C0_2$ dissolved in the plasma or ECF can participate in acid base balance, and we can calculate how much dissolved $C0_2$ is present in plasma or ECF based on it's **partial pressure** ($pC0_2$). The solubility of $C0_2$ is known to be:

0.03 mMol / L / mm Hg

This tells us that, for each 1 mm Hg of partial pressure exerted by $C0_2$ in contact with water, the equilibrium concentration of $[C0_2]$ will be 0.03 mMol/L. Therefore, if the $PC0_2$ in arterial blood is measured as 40 mm Hg (the average 'normal' value), the concentration of $C0_2$ dissolved in that arterial blood is about **1.2 mMol/L** (40 x 0.03 = 1.2). Therefore, when we say a patient with lung disease has a $PC0_2$ of 80, we are really saying that this patient's arterial $[C0_2]$ is twice normal, or 2.4 mMol/L. The average 'normal' concentration of HCO_3^- in the blood is about 24 mMol/L, and as you just saw above, the normal amount of dissolved $C0_2$ in blood is about 1.2 mMol/L. So when we have normal values for this bicarbonate buffering system acid / base pair present in blood, the pH will also be normal, as calculated by the **Henderson Hasselbach equation**:

$$pH = pK + \log [A^-] / [HA]$$

$$pH = 6.1 + \log [HCO_3^-] / \ 0.03 \times pC0_2$$

$$pH = 6.1 + \log (24 / 1.2)$$

$$pH = 6.1 + \log (20)$$

$$pH = 6.1 + 1.3$$

$$pH = 7.4$$

It is important to recognize that it is the RATIO of the conjugate base (HCO_3^-) to the conjugate acid ($C0_2$) present in a physiological solution (such as the ECF) that dictates what the pH will be. For example, in the bicarbonate buffering system, the normal ratio of HCO_3^- to dissolved $C0_2$ is around 20 to 1 (24 / 1.2 = 20). Therefore, even if the plasma levels of dissolved $C0_2$ *doubled* to 2.4 mMol/L, if a compensatory doubling of the HCO_3^- concentration to 48 mMol/L occurred as well, the pH would still be maintained at a normal 7.4 (if you don't believe it, plug the numbers into the Henderson Hasselbalch equation above, and you will see that the calculated pH is 7.4). We will talk more about this issue of 'compensation' in acid base balance in another lecture.

Review of Dietary Acid Production

There are two main sources of acids that acidify the body. As you saw above, the first is production of CO_2 from tissue metabolism. CO_2 can combine with water and form bicarbonate and a proton, as illustrated below:

$$CO_2 + H_20 \rightarrow H_2C0_3 \rightarrow H^+ + HCO_3^-$$

CO_2 is considered a weak acid. However, the second acidifying process produces strong acids and is referred to as 'endogenous acid production' (EAP). A number of mechanisms are involved in EAP, but physiologically we can say that EAP comprises all mechanisms of acid production in the body EXCEPT CO_2 production.

The acids produced during EAP include *organic* acids such as lactic acid and beta hydroxybutyrate, and *inorganic* acids such as sulfuric and hydrochloric acid. Although the organic acids are not as 'strong' as the inorganic acids, their pK's are still substantially lower than the pH of body fluids, so they are dissociated in the body water almost completely. So mole for mole, organic acids release about as many protons as inorganic acids into the body fluids.

One useful way to think of EAP's is to coneptualize them as coming from one of two sources: a metabolic source, and a gastrointestinal source. The acids production from metabolic pathways is sometimes called '*metabolic acid production'* (MAP), whereas the acids manufactured in the GI tract are referred to as gastrointestinal acid production (GAP). Therefore, we can summarize by saying:

$$EAP = GAP + MAP$$

Metabolic reactions do one of three things in our body: 1. Liberate protons; 2. Consume protons; 3. Neither liberate nor consume protons. There are 5 major types of metabolic reactions that occur in our bodies:

1. Organic cations to neutral molecules (protons liberated)
2. Organic anions to neutral molecules (protons consumed)
3. Sulfur-containing amino acids to sulfuric acid (protons liberated)
4. Neutral molecules to organic acids (protons liberated)
5. Neutral molecules to neutral molecules (no effect on protons)

Production of acids in the GI tract (GAP) is something you will learn more about when digestive physiology is covered. However, a summary of this process is that in the upper part of the GI tract (essentially the stomach), cells lining the stomach secrete protons into the stomach fluid, and secrete bicarbonate out the basolateral side of the cell, alkalinizing the ECF. Below the stomach, cells lining the GI tract secrete bicarbonate into the lumen of the intestine/colon, and protons into the ECF, acidifying the ECF. On balance, the GI tract secretes more protons than bicarbonate into the ECF, so overall the GI tract can be thought of as an acidifying organ.

REGARDLESS OF WHERE THEY COME FROM, EXCESS PROTONS PRODUCED IN THE ECF MUST BE BUFFERED, TO PREVENT LARGE CHANGES IN THE PLASMA PH FROM OCURRING!! WHEN BUFFERED BY THE BICARBONATE BUFFERING SYSTEM, BICARBONATE IS 'USED UP' AND EVENTUALLY THIS ECF BICARBONATE MUST BE REPLACED.

As you will see below, HCO_3^- is regenerated in the kidneys to replace lost HCO_3^-, and the regeneration of HCO_3^- is linked to two mechanisms: **the excretion of titratable acids (TA), and the excretion of NH_4^+** both of which ultimately lead to loss of excess H^+ ions into the urine.

Renal Acid / Base Handling

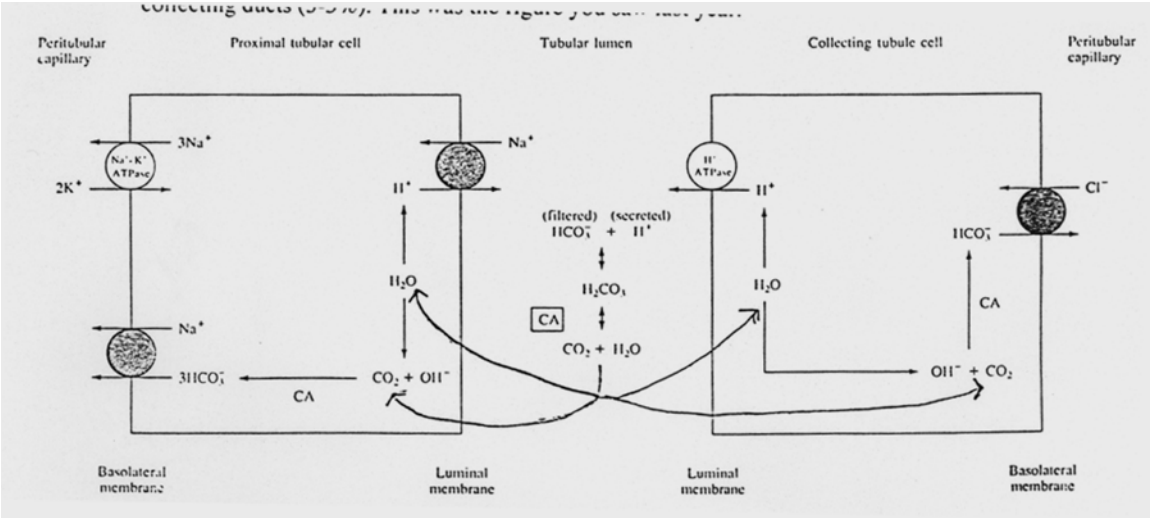
The kidneys have at least three important jobs with regards to acid/base maintenance. They must *recapture* the daily filtered load of HCO_3^- ions by reabsorbing them. They must *excrete* into the urine any excess free H^+ ions which are added to the body fluids daily, and they must also *replace* any HCO_3^- used up titrating these excess acids daily. Lets look at reclaiming filtered HCO_3^- first.

I. *Reclaiming Filtered HCO_3^-*

Normal plasma HCO_3^- concentration is about **24 mEq/L**. If 180 liters of plasma (average) are filtered into the nephrons daily, then approximately 4320 mEq's of HCO_3^- are filtered into the nephrons daily (180 x 24 = 4320). You are familiar with this equation:

(Eq. 2) $CO_2 + H_20 \rightarrow H_2C0_3 \rightarrow H^+ + HCO_3^-$

Because plasma HCO_3^- is constantly buffering free H^+ ions, the direct *loss* of any plasma HCO_3^- ions is equivalent to the *addition* of H^+ ions to the plasma, which will produce some degree of acidemia (depending on the amount of HCO_3^- lost). Normally, we lose little or no filtered HCO_3^- into the urine because it is prevented by very efficient reabsorption of filtered HCO_3^-, (about 99% of the filtered bicarbonate is usually reclaimed from the glomerular filtrate):



In the above figure, the mechanism for reabsorbtion of filtered HCO_3^- in the proximal tubule is shown on the left, and the mechanism in the collecting tubule is shown on the right. The majority of this HCO_3^- reabsorption occurs in the proximal tubule (around 85%), but also in the loops of Henle (10-20%), distal tubules and collecting ducts (3-5%). The mechanism will be discussed in class. As you can see, however, in both cases it involves use of secreted H^+ ions. In

both cases, the secreted H^+ ion combines with a filtered HCO_3^- ion, and the HCO_3^- ion is then disassembled into CO_2 and H_2O molecules. These molecules then diffuse into the cells, and are reassembled into a HCO_3^- ion, which diffuses out the basolateral side, is picked up by peritubular blood and returned to the ECF. The H^+ ion is simply recycled. In the proximal tubule, H^+ ions are secreted from tubular cells into the lumen of the nephron, by coupling with the reabsorbtion of Na^+ ion, via a Na^+ / H^+ antiporter present on the apical membrane here. In the collecting tubules, secretion of H^+ ion is accomplished by a H^+ ATPase on the apical membrane. This is an extremely important mechanism, since excessive loss of filtered HCO_3^- into the urine would rapidly result in a metabolic academia.

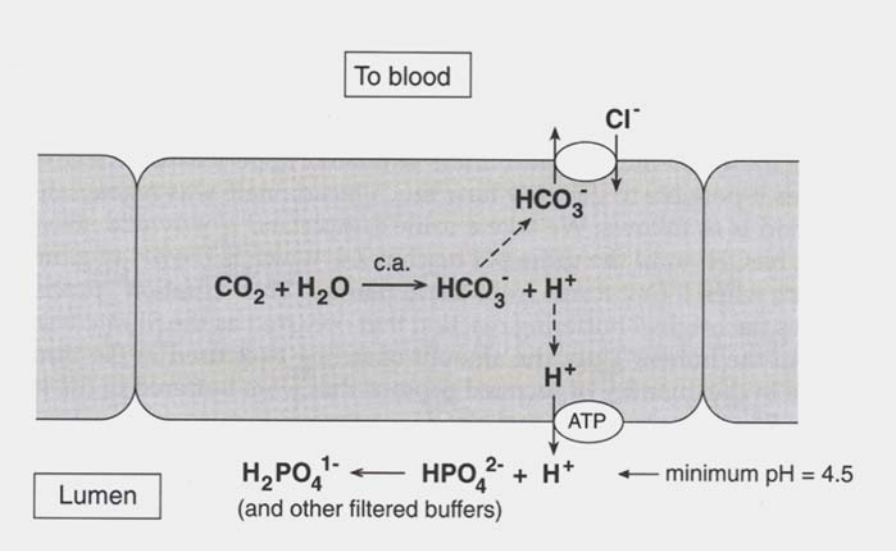
II. *Renal Generation of New HCO_3^-*

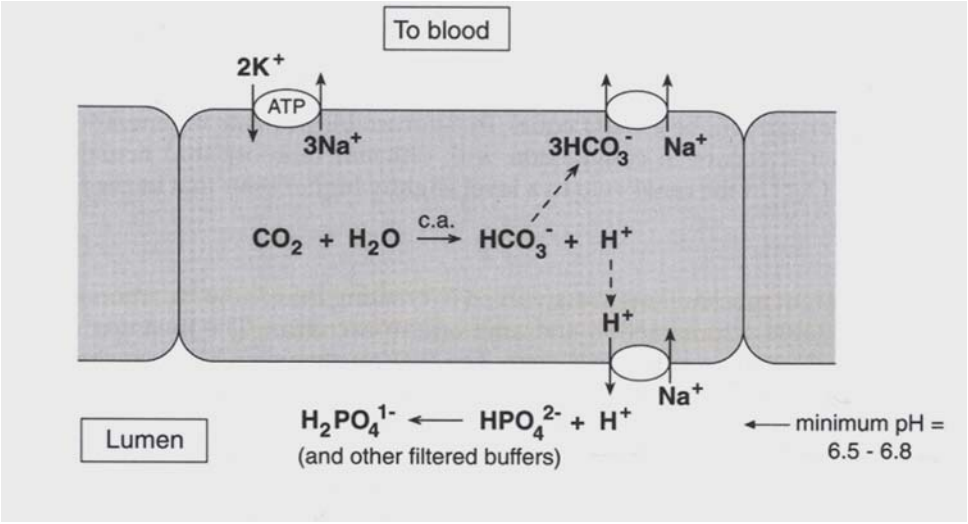
It is not only important for the kidneys to recapture filtered bicarbonate to keep it from being lost in the urine (as you saw above), but they also play an important role in replacing the plasma HCO_3^- used up titrating fixed acids we are exposed to daily (see below), thus keeping us in acid base balance. For example, when a *metabolic acidemia* occurs, which is the most common acid/base disorder, bicarbonate in the ECF is *used up* to buffer the excess H^+ ion present. In fact, one of the hallmarks of the presence of a metabolic acidosis is a low plasma HCO_3^- and an elevated plasma H^+ ion concentration (plasma pCO_2 goes down as well, due to a significant increase in respirations). Again, the metabolic acidosis drives equation 2 above to the left.

The bicarbonate ions that buffer these excess free H^+ ions during a metabolic acidosis are now **gone**. Again, looking at equation 2 above, you can see that when the HCO_3^- combines with the free H^+ ion, H_2C0_3 is formed, which in turn quickly dissociates to CO_2 and H_20. The CO_2 is blown off, and the H_20 pool now contains the extra H^+ ion, which must go to the kidney to be excreted. Therefore, this HCO_3^- that was lost MUST BE REPLACED to bring the plasma HCO_3^- concentration back to around 24 mEq/L. By the same token, the excess H^+ ion MUST BE EXCRETED by the kidney to return the plasma H^+ ion concentration to around 40 nMol/L. When you think about, buffering is simply a way of buying time – it does not get rid of excess protons or bases, it just keeps them from changing our pH too dramatically. The excess protons /

bases must ultimately be eliminated from our bodies to return to acid base balance, and this is the job of the kidneys.

III. *Linking HCO_3^- Regeneration with Excretion of Titratable Acid*





These two figures above are simply different versions of the same figure you saw previously in this handout for reabsorbtion of filtered bicarbonate. In titratable acid secretion,

protons secreted into and utilized in the lumen of the nephron are derived from the same source as they are for bicarbonate reabsorbtion – a Na^+ / H^+ antiporter on the apical membrane in the proximal tubule, and H^+ ATPase in the collecting ducts (the top figure on the previous page shows the mechanism in the distal tubules and collecting ducts, while the bottom figure is the mechanism in the proximal tubules). The only difference here is that instead of the secreted H^+ ion being buffered by a filtered bicarbonate ion, it is buffered by a filtered non-bicarbonate buffer, predominately **Na_2HPO_4** (sodium monohydrogen phosphate), most of which comes from our diets. After the secreted H^+ ion combines with a filtered monohydrogen phosphate to form dihydrogen phosphate, the dihydrogen phosphate will now carry this secreted H^+ ion on into the urine, *thus removing it from the body* (as opposed to the situation with simply reabsorbing filtered $H_2CO_3^-$, where the H^+ ion was simply recycled back into the tubular cells).

Notice in the figures above that it is essential that the secreted H^+ ion be lost in the urine, in order to generate 'new' bicarbonate ion. If the proton were not lost, no 'new' HCO_3^- would be gained by this process. The single Na^+ ion liberated from the monohydrogen phosphate will combine with the newly formed HCO_3^- ion, and the $NaHCO_3^-$ will then be returned to the systemic circulation through the basolateral membrane. Thus, the net result of this process is that **for each H^+ ion secreted and excreted into the urine, a new $NaHCO_3^-$ ion is produced to replace the $NaHCO_3^-$ ion that was used up buffering that H^+ ion in the plasma in the first place.**

IMPORTANT CONCEPT

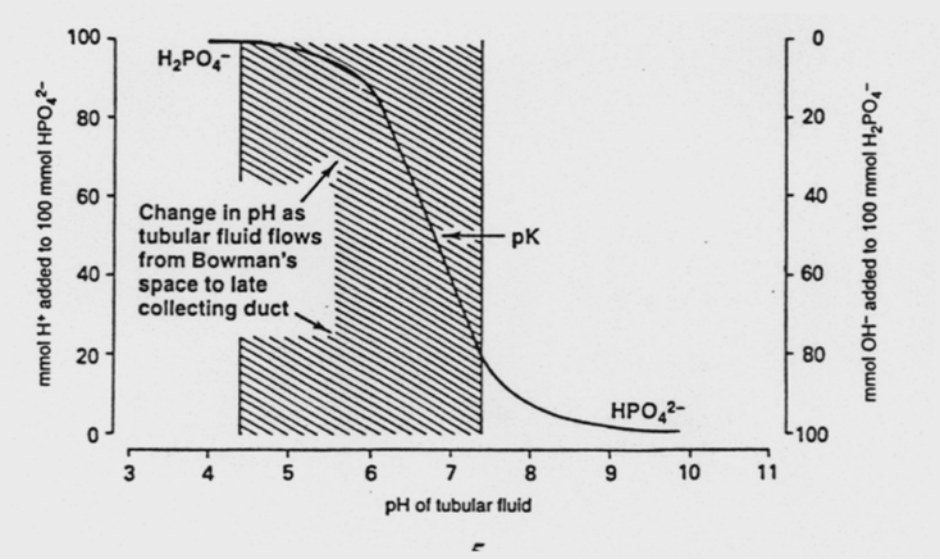
Consider for a moment why urinary buffering of the secreted H^+ ion is so important, in terms of actually getting it out of the body via the urine. Lets assume an individual has to excrete 100 mEq of H^+ ion a day to stay in acid / base balance (which as noted above, is an amount you can easily acquire just by your diet). The minimum pH that can be achieved by the urine is about 4.5. Although urine with a pH of 4.5 has a H^+ concentration about 1000 times greater than healthy plasma (about 3 log units, if you assume 'normal' pH is 7.5 instead of 7.4), the H^+ ion concentration of this urine with a pH of 4.5 is still only about 40 uMol/L. Thus, to get 100 mEq's of H^+ ion into the urine each day **you would have to produce about 2500 liters of this urine** !!

(2500 x 40 uMol = 100,000 uMol, or 100 mMol…..mMol and mEq are the same thing when you are referring to an ion that has only a single charge, such as H^+ ion).

Defining Titratable Acidity

The amount of strong base (such as NaOH) that it takes to titrate a patient's urine that is acidic back to pH 7.40 is approximately equal to the amount of titratable acid that was in the urine. *Dihydrogen phosphate is the major titratable acid in urine*. Other filtered buffers that can act as titratable acids include creatinine, citrate, acetate, and beta hydroxybutyrate, but because they are present in the urine in low concentrations, and have a low pK, their contribution to to the titratible acidity is small. As was noted previously, healthy individual can easily generate some **50 to 100 mEq's** of H^+ ions daily, just from catabolism of proteins in the diet. Obviously, a person with a metabolic acidosis is generating even more than this.

However, titratable acidity normally can account for the excretion of only about **10 to 40 mEq of H^+ ion per day**. This is because the amount of filtered sodium monohydrogen phosphate available is limited, and as it passes farther and farther down the nephron, **almost all of it will be in the form of sodium dihydrogen phosphate when the urine pH reaches about 5.2**. This is shown graphically below:



This figure demonstrates that, as the filtrate passes from Bowman's space to the collecting tubules, the pH can drop all the way to about 4.5. This is an important concept,

because **urinary pH cannot drop below approximately 4.5, as noted above**. This is probably because H^+ ion can't be secreted into the lumen against a H^+ ion concentration gradient exceeding 1:1000 (the pH of the cells surrounding the lumen is around 7.4; if the pH of the lumen is 4.4 or 4.5, this about a 1000 fold greater concentration of H^+ ions present in the lumen).

If titratable acids were the only compounds available to buffer secreted H^+ ions (so they could then be *excreted*) we would have major problems excreting a daily acid load into our urine, because of this saturation of titratable acids at a urine pH of about 5.2. This would result in the *almost immediate cessation* of urinary H^+ ion secretion (and therefore, excretion as well). Now you have a problem. Especially if you have a metabolic acidosis, because you have many more mEq's of H^+ ion to excrete than the 40 or so mEq's of H^+ ions you can get rid of using titratable acids alone, and the kidneys are the only way out. This is where you will see the importance of ammonia as a urinary buffer.

Ammonia Buffering

Many years ago, it was observed that in those patients experiencing acidosis, there was not only a rise in urinary TA's, but also in urinary ammonium ion **(NH_4^+)**. This of course made clinicians and researchers suspect that perhaps ammonia played a role in renal H^+ ion buffering.

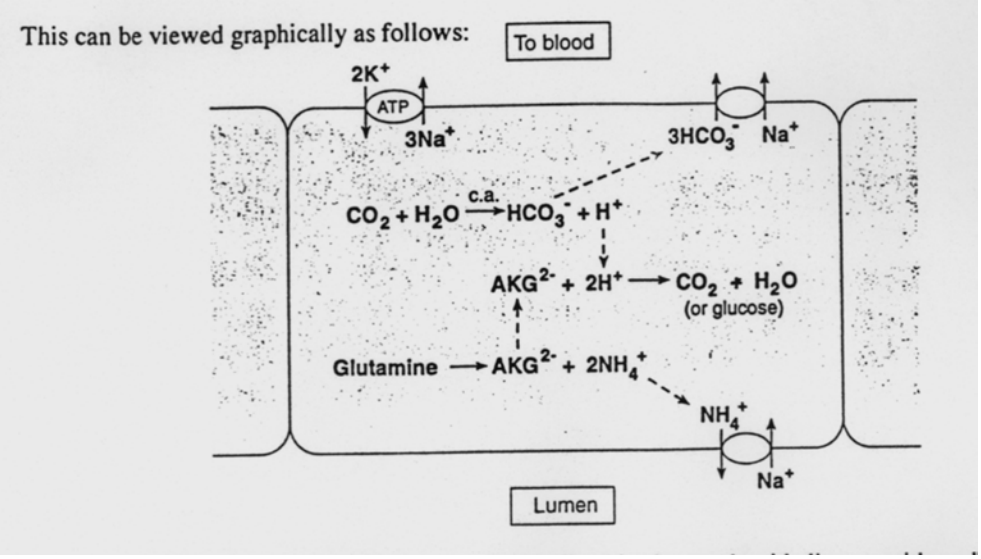
In fact, we now know that ammonia is a very important urinary buffer, because the amount available is not directly dependant on diet or filtration, like the case with titratable acids such as monohydrogen phosphate. Ammonium ion can actually be produced in the cells lining the nephron, particularly in the proximal tubule, mostly (but not exclusively) from the deamination of **glutamine**. The amount of ammonium ion produced can be altered to meet physiological needs (ie, unlike titratable acids, the amount available is *not fixed*; it changes depending on acid/base status).

The synthesis of ammonium ion in the proximal tubule occurs as follows:

$$\text{Glutamine} \longrightarrow 2NH_4^+ + \alpha\text{-ketoglutarate}$$

The subsequent metabolism of α -ketoglutarate (not shown) results in the **formation of two new HCO_3^- ions**, which are then returned to the general circulation. Therefore, once again you will see that, *with ammonia buffering, like buffering with titratable acids*, **the secretion and subsequent excretion into the urine of each NH_4^+ ion is linked to the generation of a new HCO_3^- ion, which will then be returned to the circulation to replace the HCO_3^- lost buffering excess plasma H^+ ions**. This makes sense, since each H^+ ion lost into the urine is by defination a H^+ that appeared in the body for some reason, in excess of the 40 nMol/L that we want in order to maintain a pH of 7.4. And since every 'excess' H^+ ion that appears in the body by defination caused the loss of one HCO_3^- ion when it was buffered, this "one H^+ ion out, one new into the ECF" makes perfect sense stoichiometrically.

The figure below shows this process, including how the newly generated HCO_3^- ions are transported into the peritubular blood via a HCO_3^-/Na^+ symporter on the basolateral membrane, and returned to the plasma, and how the NH_4^+ molecules are extruded into the lumen by substituting for H^+ ion on the apical membrane Na^+/H^+ antiporter.



Once produced, α -ketoglutarate is metabolized in the renal epithelium to either glucose or CO_2 and water. Both pathways consume two protons as noted above. The ammonium ion (NH_4^+) that was transported into the luminal fluid by substituting for H^+ on the Na^+/H^+ antiporter is passed out into the urine. **The excretion of NH_4^+ plays no direct role in removing**

protons: NH_4^+ is merely a side product, or marker, of the formation of new HCO_3^-. Nonetheless, NH_4^+ must be excreted. If it were not, it would eventually diffuse into the renal blood vessels, exit the kidney via the renal vein, and be carried to the liver. There it would be converted to urea, a reaction that generates protons. These protons would then be buffered by the body's HCO_3^- ions, thus canceling the gain of HCO_3^- made by the kidneys through metabolism of α-ketoglutarate.