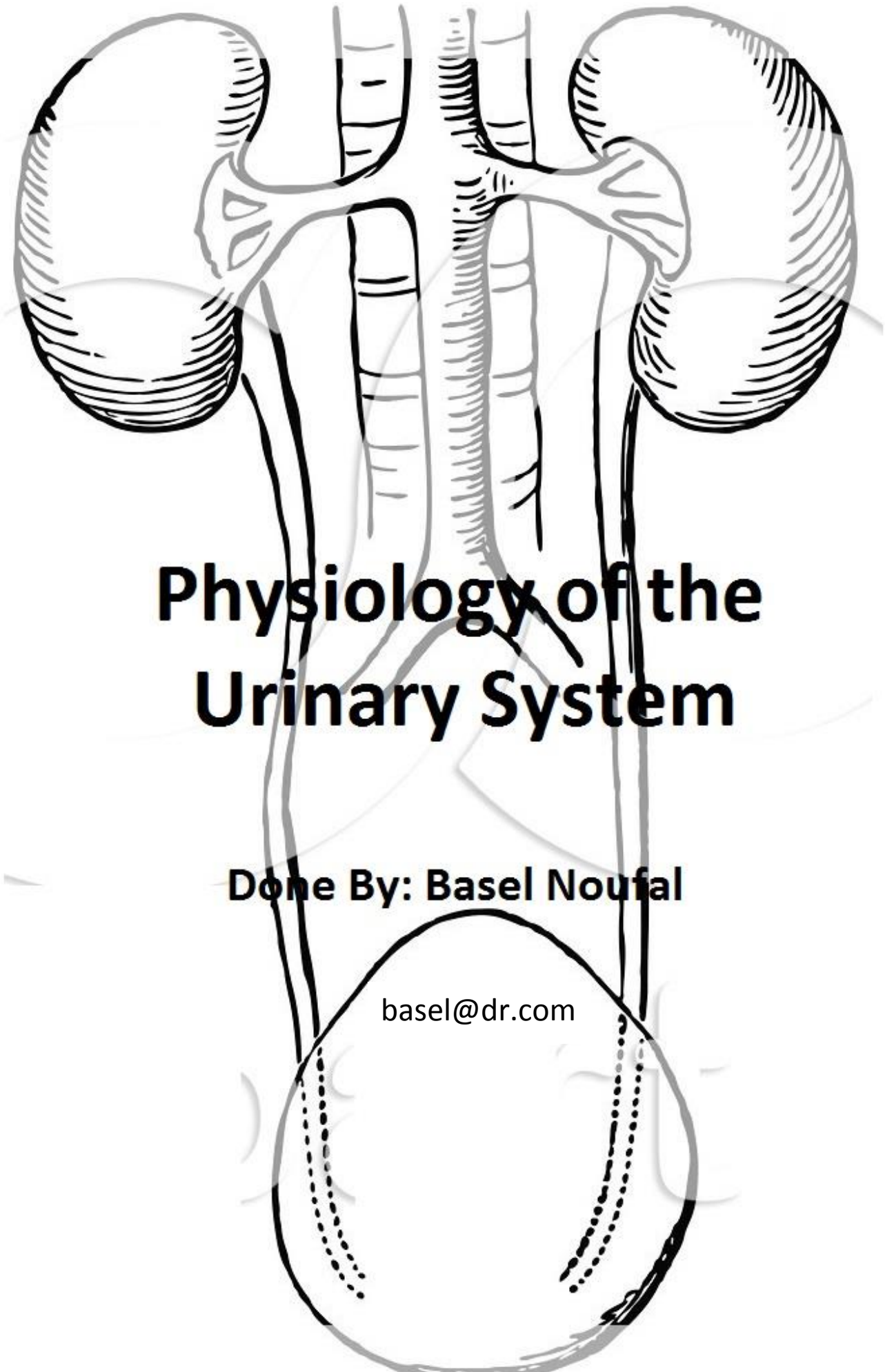

Physiology of the Urinary System

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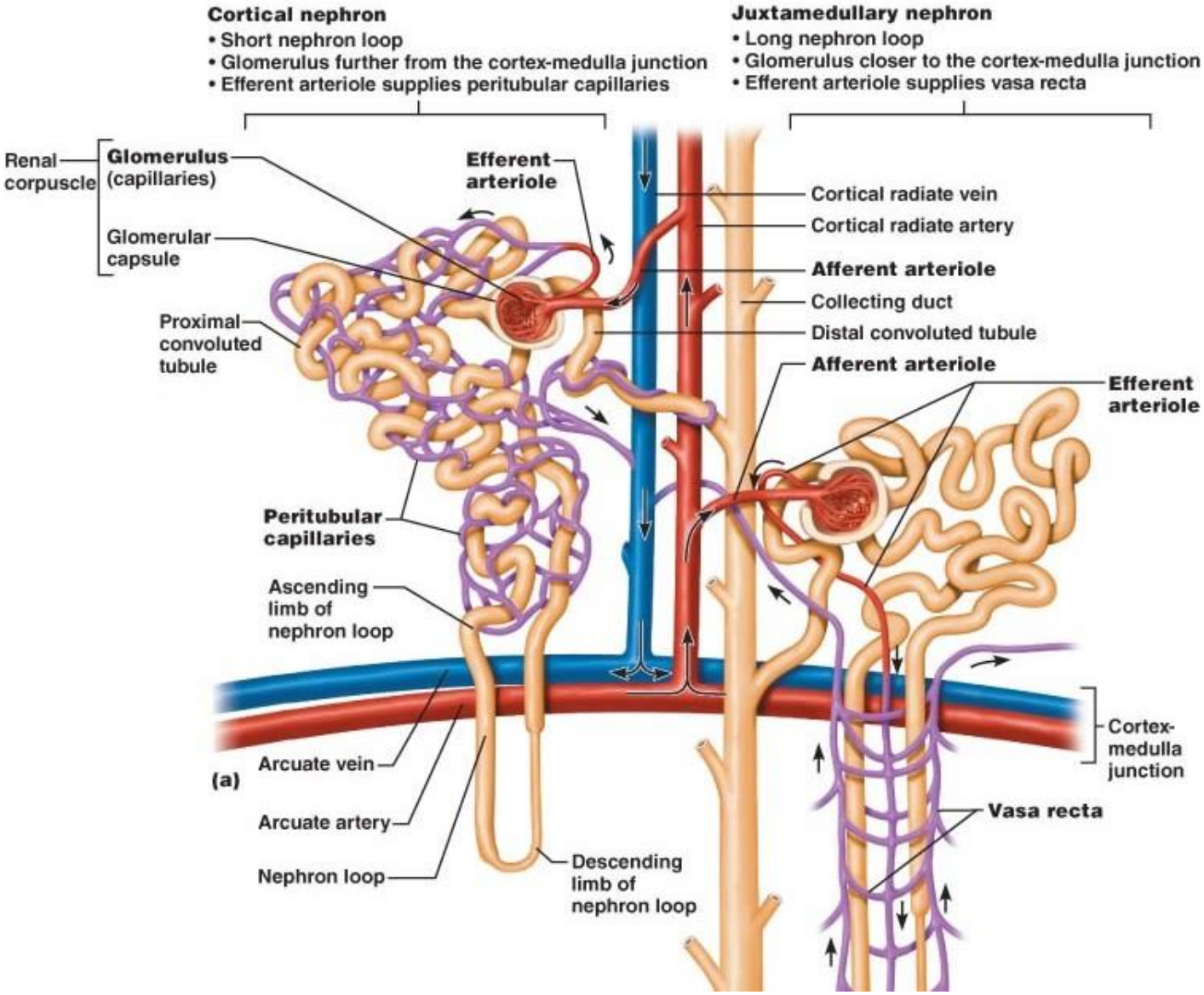
- **Functions of The Kidneys**
  - ❖ Remove waste products and foreign chemicals.
  - ❖ Control acid-base balance.
  - ❖ Control blood levels of electrolytes.
  - ❖ Regulate fluids volume of the body, and thus, blood pressure.
  - ❖ Secrete hormones such as erythropoietin, which is important for erythropoiesis, and without which, anemia develops.
  - ❖ Convert 25-hydroxycholecalciferol into 1,25-dihydroxycholecalciferol (calcitriol), the most active form of vitamin D.
  - ❖ Gluconeogenesis (conversion of non-sugar sources, particularly amino acids, into glucose).
- **Blood Supply of The Kidneys**



- ❖ The renal artery (the fifth branch of the aorta) enters the kidney through its hilum and divides many times to form segmental arteries, interlobar arteries, arcuate arteries, interlobular arteries (cortical radiate arteries).
- ❖ Interlobular arteries divide again into many afferent arterioles.
- ❖ Each afferent arteriole enters a glomerulus and divides to form the glomerular capillaries.
- ❖ The capillaries converge again to form efferent arterioles.
- ❖ Efferent arterioles leave the glomerulus and divide, once again, to form peritubular capillaries.
- ❖ Peritubular capillaries rejoin to form interlobular veins, arcuate veins, interlobar veins.
- ❖ Interlobar veins join to form the renal vein which leaves the kidney through its hilum.
- ❖ Note that the glomerular capillaries form the efferent arterioles, which divide again (instead of converging) to form other capillaries. This is known as the portal circulation.
- ❖ Vasa recta are peritubular capillaries that branch off the efferent arterioles of juxtamedullary nephrons (those nephrons closest to the medulla). They enter the medulla, and surround the loop of Henle.
- ❖ Each kidney contains one million nephrons; each of which is 6 cm long.
- ❖ The cortex contains the glomeruli of the nephrons, giving the cortex a granular appearance. In contrast, the medulla, which contains most of the length of the tubules, appears striated.

- **Renal Blood Flow (RBF)**
  - Defined as the volume of blood entering both kidneys per unit time.
  - Can be expressed as the volume of blood which supplies each milligram of the renal tissue per unit time.
  - Averages **1.25 L/min** or **4.2 ml/mg.min**. That is, **25%** of cardiac output.
  - High compared to other vital organs (**0.03** and **0.5** ml/mg.min for skeletal muscles and brain, respectively).
  - This high flow rate is consistent with the function of the kidneys, reconditioning of blood. That is, the composition of the blood is significantly modified as it passes through the kidneys.
  - However, unlike other tissues, O_2 and nutrients concentrations do not decrease significantly as the blood leaves the kidneys. This is indicated by the a-vO_2 which is relatively low (**1.4 ml/dl**) compared to **6** and **6.2** ml/dl for the skeletal muscles and the brain, respectively.

- **Renal Plasma Flow (RPF)**
  - Can be calculated using this equation: **RBF = RPF / (1-HCT).**
  - If the RBF is 1200 ml/min and HCT is 0.45, then RPF = 660 ml/min. RPF will be considered to be **625 ml/min** throughout the remainder of the text.
  - Maintaining the RPF within its normal range is very important; even a decrease in the RPF for a short time causes the blood to accumulate in the narrow loops of Henle, totally losing the function of the nephrons.
  - Practically, the **RPF** is measured first. Then, the **RBF** can be mathematically obtained, as discussed later.

Remember:

- The a-vO_2, or the arteriovenous oxygen difference, is the difference in oxygen concentration between arterial and venous blood. It is used as an indication of how much oxygen is extracted from the blood in capillaries.
- 55% of the blood is plasma (fluids and solutes and suspended particles) while 45% is formed of suspended cells. This 45% is termed hematocrit (HCT) or packed cell volume (PCV).

- **Renal Clearance**
  - Defined as the volume of plasma completely cleared of a substance per unit time.
  - Refers to the volume of plasma necessary to supply the amount of a substance excreted in urine per unit time.
  - Excretion rate is the amount of a substance excreted in urine per unit time. It is calculated by multiplying urine flow rate by urine concentration of the substance (U_s x V).
  - Urine flow rate (urine output) is the volume of urine excreted per unit time.
  - To understand the concept of clearance, assume that 60 grams of a substance were dissolved in a glass containing 1 liter of water, and after a minute, half of the substance (30 grams) were removed from the solution. This is equivalent to having two glasses of water; one contains 60 grams dissolved in 0.5 liter of water, and the second contains 0.5 liter of water without any of the substance. This means that the clearance of the substance is 0.5 L/min (i.e., 0.5 liter can be isolated after a minute keeping the amount of the substance in the other 0.5 liter the same).

- Renal clearance can be stated mathematically as follows:

$$C_s \times P_s = V \times U_s$$ where:

C_s: clearance rate of the substance. P_s: Plasma concentration of the substance.

V: urine flow rate (urine output). U_s: Urine concentration of the substance.

- If the plasma concentration of a substance is 10 mg/ml, and 25 ml of urine were collected within a minute. The concentration of the substance in the urine was 7 mg/ml. Then C_s = 25 ml/min x 7 mg /ml / 10 mg/ml = 17.5 ml/min.

- **RPF Measurement**
  - Amount excreted = amount filtered + amount secreted – amount reabsorbed
  - A special substance (paraaminohippuric acid or **PAH**) is almost completely excreted. Therefore, since all the blood entering the kidneys will be cleared of PAH, the clearance of PAH is the RPF. Thus, using the clearance equation, and substituting RPF for C_s:

$$\mathbf{RPF \times P_s = V \times U_s}$$

  - PAH is **90%** excreted. This, in fact, is the maximum percentage achievable because **10%** of blood entering the kidneys does not participate in urine formation. Rather, it supplies the renal tissue with the necessary oxygen and nutrients. Thus, 90% of blood entering the kidneys (true renal plasma flow) is the **effective renal plasma flow**.
  - PAH clearance was measured to be 585 ml/min, which is the effective renal plasma flow. Thus, the RPF = 585/0.9, or around 650 ml/min.

- Filtration is a passive process (i.e., no transporters are needed to move substances (including water) across capillaries membranes.
- What determine the amount filtrated are the permeability of the membrane for the substance, its concentration gradient across the membrane and the time the substance remains in the glomerular capillaries. *This implies that as the concentration gradient increases, filtration rate increases linearly and unlimitedly*.
- Secretion, on the other hand, is an active process (i.e., transporter-dependent). Therefore, increasing the concentration gradient increases secretion rate *only to a limit.* Above the limit, further increase in concentration gradient does not increase secretion rate *because all the transporters have been occupied (i.e., saturated).* The transport rate at which secretion (or reabsorption, as explained later) rate reaches is maximum is designated T_{max} (transport maximum).

  - The T_{max} of PAH transporters is **80 mg/min**. Therefore, to measure the RPF accurately, PAH reaching the peritubular tubules per minute must not exceed 80 mg. Otherwise, less than 90% would be excreted, and thus the RPF would be underestimated.

- In fact, even an amount just less than 80 mg (e.g., 75 mg) would result in inaccurate RPF estimation because although not all the transporters would be occupied, *the probability for each PAH molecules to bind to a transporter would be exceedingly low*, and those PAH molecules which escape and do not bind to their transporter would be returned to the venous blood rather than excreted leading to underestimation of RPF.
- Therefore, with very low plasma concentration, most of the PAH in the urine (80%) is secreted actively by the transporters in the tubules. Only 20% is filtered and not reabsorbed. However, with much higher plasma concentrations only 80 mg/ml is excreted in the urine by secretion, and the rest is the filtered PAH. In this case, PAH clearance would approach the GFR rather than RPF.
- The difference between predicted excretion rate for PAH (assuming all PAH molecules bind to their transporter) and actual excretion rate is called splay. It is high at high PAH concentrations (i.e., just less than T_{max}) and approaches zero at lower concentrations.
- **Note:** the last two points will be further explained when glucose reabsorption is discussed.

❖ **Example:** If the plasma concentration of a substance is **2500 mg/ml** and **60%** of the substance passing through the kidneys is filtered and **10%** secreted while **20%** reabsorbed. Assuming infinitive T_{max} for the substance transporters, calculate the clearance and the RPF (per minute) given that during the next 24 hours, **720 ml** of urine were collected, and the substance concentration in urine was **100 mg/ml**.

- V = 720 ml/day = 0.5 ml/min Us = 100 mg/ml Pc = 2500 mg/ml
- Cs = (V x U) / Ps = 0.5 x 100 / 2500 = 0.02 ml/min
- Excreted = 60 + 10 -20 = 50%
- Since T_{max} is much higher than the concentration of the substance reaching peritubular capillaries, the splay phenomenon does not affect the accuracy of the calculated RPF. Thus, RPF = 0.02 / 0.50 = 0.04 ml/min. (half of the RPF was cleared)

• Glomerular Filtration

- First step of urine formation.
- Large amounts of plasma diffusing passively into Bowman's capsule.

❖ Filtration is a bulk flow of fluids with the dissolved solutes. It is driven by the pressure gradient across the membrane.

❖ Diffusion is the movement of particles down their electrochemical gradient.

- The composition of the filtrate is essentially similar to that of the plasma. However, glomerular capillaries, like most other capillaries are not permeable to proteins. Also, calcium and fatty acids are not freely filtered since they are partially bound to plasma proteins.

- **Note:** Most systemic capillaries have an arterial end where plasma is filtered and a venous end where most filtered plasma is reabsorbed. Plasma in glomerular capillaries *is only filtered and not reabsorbed*. Another exception is gastrointestinal capillaries which function only to reabsorb nutrients.

• Glomerular Filtration Rate (GFR)

- The volume of plasma filtered from the glomerular capillaries to Bowman's capsules per unit time.
- Determined by the balance between starling forces and the capillary filtration coefficient **(K_f)**, which is different for different substances and is the product of the permeability of the capillary for the substance and the surface area of the capillary provided for filtration.
- The permeability of the capillary for a substance, in turn, is determined by the molecular weight and the charge of the substance.

Substance	Molecular Weight	Filterability
Water	18	1.0
Sodium	23	1.0
Glucose	180	1.0
Inulin	5,500	1.0
Myoglobin	17,000	0.75
Albumin	69,000	0.005

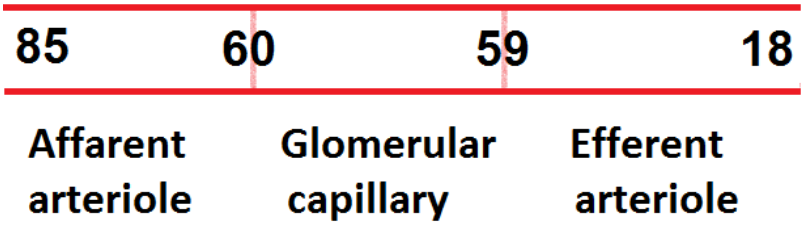
❖ Cations are more readily filtered than anions because the endothelium and the basement membrane are negatively charged and thus, repel anions. Cations with a molecular weight less than 70,000 kDa are readily filtered. In contrast, anions need to be much smaller in order to pass through the capillaries. Albumin, for example, despite its relatively low molecular weight (70,000 kDa), does not cross due to its highly negative net charge. However, Cl^-, yet negatively charged, readily crosses due to its very low molecular weight.

❖ Water is filtered freely across glomerular capillaries and thus, the filterability of water is said to be 1. The filterability of other substances ranges from 0 to 1 and is determined relative to that of water.

- The RPF averages 625ml/min; the glomerular filtration rate is 125 ml/min. Dividing these numbers yields 0.2, which is the filtration fraction.

• Starling Forces

1. **Pc**: the hydrostatic pressure generated by the pumping force of the heart. It averages 60 mm Hg in the glomerular capillaries.



- Note that Pc decreases markedly as blood passes through the arterioles, indicating high blood flow resistance in these vessels.

2. **π_c**: the colloid osmotic pressure generated by the impermeable proteins in the plasma. Since 20% of plasma passing through the capillary is filtered, impermeable proteins concentration increases as they pass along the length of the capillaries from 28 mmHg to 36 mm Hg. Thus, the average **π_c** is approximately 32 mm Hg.

3. **P_i:** the hydrostatic pressure generated by the interstitial fluid. It averages 18 mm Hg

4. **π_i:** the colloid osmotic pressure of the interstitial fluid. Since filtered plasma is free of proteins, it equals zero.

- **Note**: in other organs, π_i plays an important role in determining filtration rate. This depends on the permeability of the capillaries to proteins. In the liver interstitium, for instance, π_i is very high due to the very high permeability of the liver capillaries to plasma proteins.

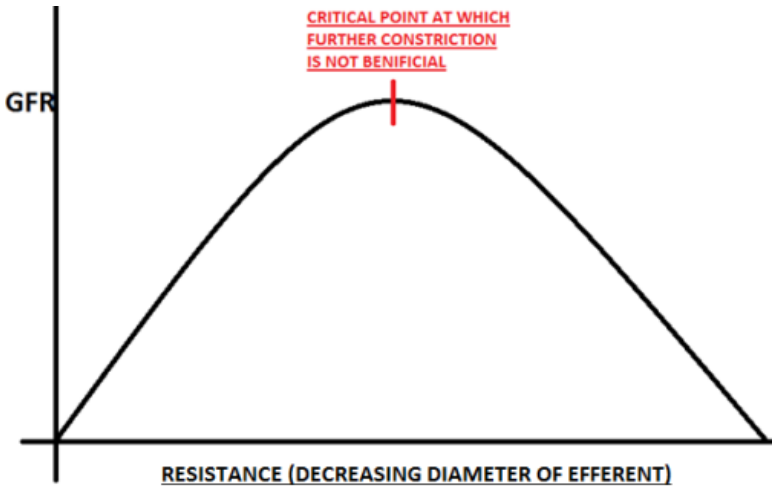
- P_c favors filtration while P_i and π_c favor reabsorption. Therefore, net filtration pressure equals **60 – 18 – 32, or 10 mm Hg**.

- Any obstruction to urine flow (e.g., in prostate hypertrophy and stone formation) increases the hydrostatic pressure inside the Bowman's capsule, decreasing the GFR.

- **Note:** From the glomerular capillaries, 180 L/day are filtered. In contrast, only 20 L/day are filtered throughout the rest of the body. This is attributable to the relatively low P_c in other body capillaries.

- Normal GFR must be maintained. Increased GFR as a result of an increase in P_c or an increase in the permeability of the capillaries, for example, results in generalized edema; decreased GFR causes waste products to accumulate in the plasma.

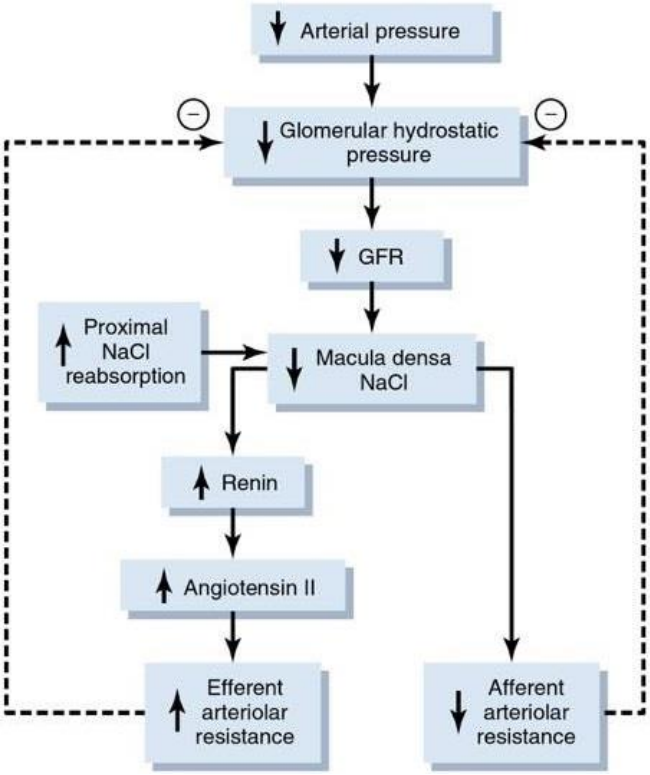
- P_c can be physiologically increased or decreased markedly by changing efferent and afferent arterioles diameters. Constricting the afferent arteriole causes decreased blood reaching the capillaries, hence decreased P_c and filtration rate. Also, constricting the efferent arterioles causes blood to accumulate in the capillaries. This results, at the beginning, in increase in P_c and filtration rate. However, after a while, fluid leaving the capillaries will leave behind very concentrated proteins (i.e., high π_c**)** which would oppose further filtration.



INCREASING RESISTANCE OF EFFERENT AND ITS EFFECT ON GFR

- Thus, any drug that, directly or indirectly, constricts the afferent arterioles can decrease the GFR. NSAIDs for example inhibit the formation of prostaglandins, which normally dilate the afferent arterioles. Therefore, kidney functions must be monitored regularly whith NSAIDs administration, especially for chronic diseases; Even a slight incrase in creatinine level after the administration of such drugs may necessaite stopping the drug, especially in old-aged patients, whose GFR is already declining.

- The GFR is not affected by a small increase or decrease in mean arterial pressure (MAP). Otherwise, even an MAP of 90 mm Hg, which is not far from normal value (93 mmHg), would decrease P_c to 50 mmHg. This would, in turn, stop filtration.



- How does the body maintain constant GFR despite the huge daily fluctuations in MAP? There are two physiological mechanisms that function to *autoregulate* GFR and RPF. To answer the question, the juxtaglomerular complex must be discussed first.

- This complex consists of 2 types of cells:
  - Macula densa which is a group of specialized epithelial cells located in the initial portion of the distal tubule.
  - Juxtaglomerular cells which are located in the walls of the efferent and afferent arterioles. They are the major storage sites for the enzyme, renin.

- Decreased GFR slows the flow rate in the loop of Henle causing increased reabsorption of Na^+ and Cl^- in the ascending loop of Henle, thereby decreasing their concentrations at the macula densa cells. This initiates a signal from the macula densa, which:
  - Decreases resistance to blood flow in the afferent arterioles raising P_c and helping returning GFR toward normal.
  - Increases renin release from the juxtaglomerular cells.

- Renin enzymatically cleaves angiotensinogen forming angiotensin I, which is further cleaved by a converting enzyme in the lungs to form angiotensin II.

- Angiotensin II helps to correct the GFR by
  - Potently constricting the arterioles of the body increasing MAP.
  - Constricting the efferent arterioles increasing P_c in the glomerular capillaries and helping returning GFR toward normal.
  - Directly increasing the reabsorption of Na^+ from the nephron, especially the proximal tubules.

- Increasing aldosterone secretion from the adrenal glands. This increases Na^+ and water reabsorption, as discussed later.

- Constricting the efferent arterioles is not only important to increase P_c in the capillaries but also to decrease P_c ahead of the efferent arterioles (i.e., peritubular capillaries). This causes the hydrostatic pressure in these vessels to decrease allowing the opposing forces to increase reabsorption, thereby conserving body fluids.

- With these mechanisms, the GFR changes only a few percentage points, even with large fluctuations in MAP between the two limits (75 and 160 mm Hg).

- **Remembr**: Since only 0.5% of fluids passing through most other systemic capillaries are filtered, π_c does not change significantly throughout the capillaries (i.e., remains 28 mm Hg)

- **Remeber**: albumin, *and not globulins*, is the main protein which determines $\boldsymbol{\pi_c}$ although plasma concentration of albumin is only slightly higher than that of globulins. The reason for this is that the molecular weight of albumin is much less than that of globulins, 70,000 and 200.000, respectively. This means that 1mg/ml of albumin would contain more molecules than similar concentration of globulins. Since *osmolality is determined by the number of particles*, albumin exerts more oncotic pressure than globulins at same concentrations.

• Filtered Load

- If a substance is freely filtered in the kidneys (i.e., its filterability is 1), then the filtration rate of that substance is referred to as the filtered load and is calculated as follow:

Filtered load (amount per unit time (mg/min)) = **GFR x plasma concentration**

• Measuring GFR

- Inulin is a small exogenous substance (5,000 kDa) that is freely filtered (i.e., its filterability is 1). What special about this substance, making it suitable for measuring GFR, is that it is *neither absorbed nor secreted.* In other words, *Inulin that is filtered is that excreted*. Thus, the clearance of inulin is, in fact, the GFR.
  Mathematically: **$GFR \times P_s = V \times U_s$.**

- Note that PAH used to measure RPF is completely excreted as it passes through the kidneys. However, *most of the excreted amount is secreted and not filtered*. Thus, the clearance of PAH *is the RPF rather than the GFR*.

- Inulin, like PAH, is an exogenous substance. This makes it only suitable to measure GFR for research, and not routine clinical, purposes.

- Another substance that is endogenous and used as a clinical test to measure the GFR is creatinine which is a waste product of muscular metabolism.
- Creatinine is freely filtered but around 10% of the excreted amount is secreted and not filtered. This overestimate the GFR. However, in plasma, 10% of creatinine is bound to proteins, and thus not filtered. This underestimates GFR. Since GFR will be 10% overestimated and 10% underestimated, measured GFR is almost the actual GFR.
- **Note:** when measuring plasma creatinine concentration, the free and bound forms are measured.
- Plasma concentration of creatinine is 1 mg/dl. Therefore, filtered load of creatinine can be calculated by multiplying 1 mg/dl by GFR which is 125 ml/min. This yields 1.25 mg/min or 1.8 g/day.
- Creatinine excretion (and formation) rate is also 1.8 g/day. It is not much affected by muscular activity and a range between 1.5 and 2 g/day is considered normal.
- Note that the plasma creatinine concentration reflects the GFR. If, for instance, measured to be 3 mg/dl, then one can tell that this 3-fold increase is a result of a decrease in GFR to 1/3, though does not determine exact GFR.
- Using the equation of clearance, the clearance of creatinine or **GFR = (U_{cr} x V) / P_{cr}.**
- Plasma and urine concentrations of creatinine are easily measured. In contrast, urine volume over the day is not always compatible due to the patients' incompliance, especially elderly and children. Therefore, two equations were estimated to obtain GFR (ml/min/1.73m^2) without the need to collect urine. The first is used to estimate GFR for adults and the second for children:

- **GFR = (140 – age) x ideal body weight x 1 (for males) or 0.85 (for females) / (72 x P_{cr}).**
- **GFR = K x height (cm) / P_{cr}, where K depends on muscle mass which varies with child age.**

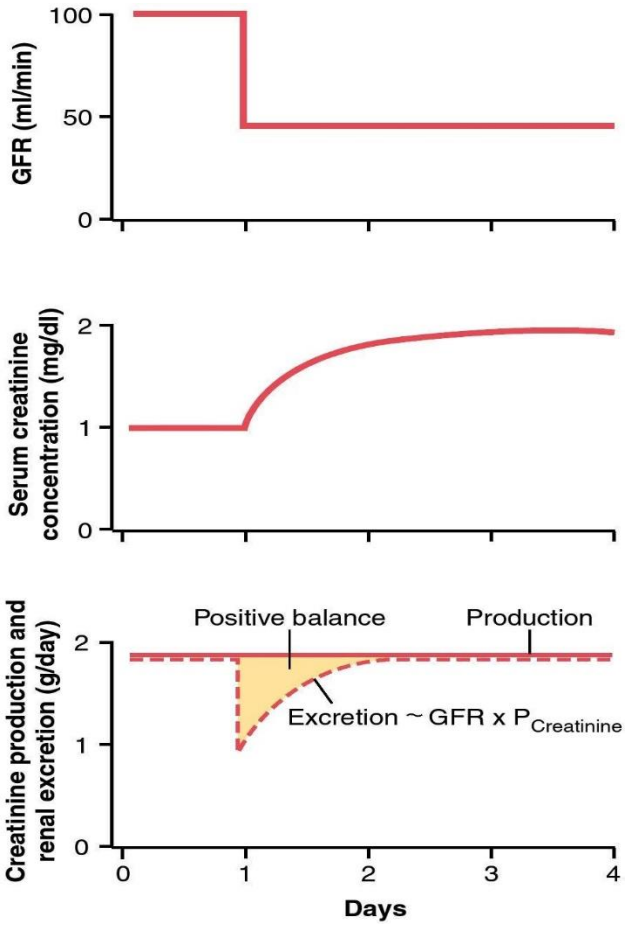
- Note that the accuracy of GFR measurement using these equations is around 95%. However, in end-stage renal failure, these equations cannot be used since, in this case, GFR approaches zero and thus, excreted amount is the secreted, not filtered; thus, creatinine clearance overestimates GFR.
- Renal diseases are classified according to the percentage of GFR to normal:
  - **50-99%:** decreased renal preserve. Usually asymptomatic with normal urea and creatinine levels.
  - **20-49%:** renal insufficiency. Urea and creatinine levels are elevated, and usually, accompanied with anemia and hypertension. However, the patient survives with low salt and protein diet.

- **5-19%:** renal failure. External intervention is needed.
- **<5%:** end-stage renal failure. The patient must undergo hemodialysis and kidney transplant.

- Also, the severity of the renal diseases can be determined by the following classification:
  - **60-89%:** mild; **30-59%:** moderate; **15-29%:** severe; **<15%:** end stage.

❖ If GFR suddenly decreases by 50%, the kidneys will transiently filter and excrete only half as much creatinine, causing accumulation of creatinine in the body fluids and raising plasma concentration.

❖ Plasma concentration of creatinine will continue to rise until the filtered load of creatinine (P_{Cr} × GFR) and creatinine excretion (U_{Cr} × V) return to normal and a balance between creatinine production and creatinine excretion is re-established but at the expense of elevated plasma creatinine concentration.

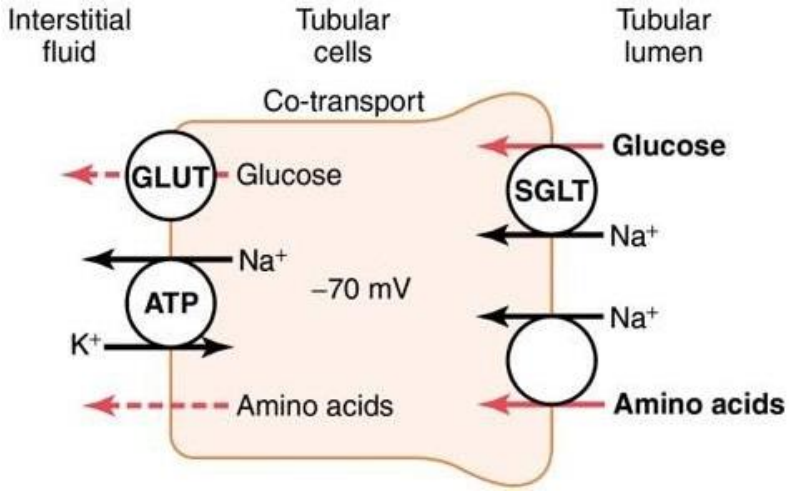


- **Renal Reabsorption**
  - Occurs in all parts of the renal tubule; the proximal tubule, the loop of Henle, the distal tubule, the collecting tubule, and the collecting duct.
  - Filtered substances, including water, move back to the peritubular capillaries from the tubular lumen. Absorbed substances either exit the lumen by diffusing between cells (paracellular pathway) through the tight junctions, which are not really tight, especially in the proximal tubule, or enter the cells first, either actively or by diffusion.
  - Almost 180 L/day are reabsorbed; a decrease by 10% increases urine output from 1.5 to 20 L/day (almost a 13-fold increase).

- Unlike glomerular filtration, reabsorption is highly selective.
- Tubular reabsorption includes passive and active mechanisms.
- Some substances, such as glucose and amino acids, are completely reabsorbed from the tubules.
- Many of the ions in the plasma, such as sodium, chloride, and bicarbonate, are also highly reabsorbed, but their rates of reabsorption and urinary excretion are variable, depending on the needs of the body.
- Waste products, such as urea and creatinine, conversely, are poorly reabsorbed from the tubules and excreted in relatively large amounts.

• Glucose Reabsorption

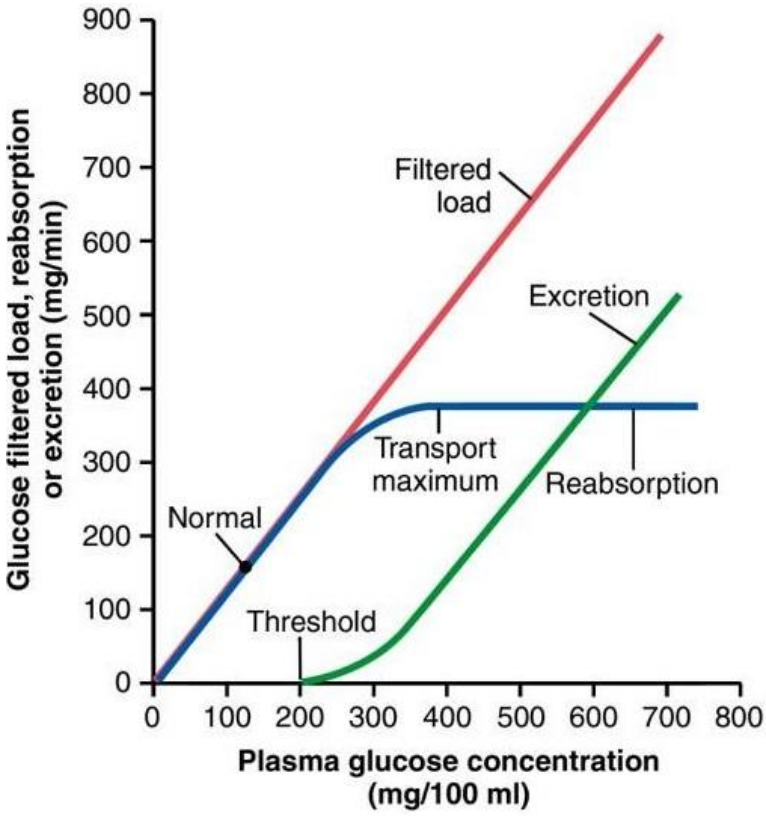
- Fasting blood glucose level normally ranges between 70 and 110 mg/dl. The average level will be considered to be 100 mg/dl.
- Glucose filtered load = GFR (125 ml/min) x glucose level (100 mg/dl), or 125 mg/min.
- None of the filtered glucose is normally excreted. This means that the rate of glucose reabsorption is also 125 mg/min.
- Glucose is reabsorbed in the proximal tubules by secondary active co-transport mechanism in exchange with Na^+.
- Specific carrier proteins (SGLT2 and SGLT1) in the brush border of the proximal tubular cells combine with a sodium ion and a glucose molecule at the same time moving glucose into the cell cytoplasm against its concentration gradient.



- SGLT1 has higher affinity but lower capacity than SGLT2.
- After entry into the cell, glucose exits across the basolateral membranes by diffusion to enter the interstitium, driven by the high glucose concentration in the cell and *facilitated by specific transport proteins (GLUT2 and GLUT1).*
- Sodium ions are transported out of the cell by the Na^+/K^+ ATPase pump, which opposes the effect of SGLT in increasing intracellular Na^+ concentration, and thus maintains high extracellular Na^+ level relative to its intracellular level. Therefore, the reabsorption of

glucose depends on energy expended by the primary active Na^+/K^+ ATPase pump in the basolateral membrane.

- The capacity of SGLT for glucose transportation (T_{max}) is 375 mg/min. This suggests that a blood glucose level below 300 mg/dl would not cause glucose to appear in the urine (see the additional box in the next page for justification). However, because of the splay phenomenon described earlier, glucose levels higher than 180 mg/dl do result in excretion of glucose.
- This glucose level, at which glucose starts to appear in urine, is termed renal threshold.
- The presence of detectable glucose in urine is termed glycosuria.



- Therefore, if glucose plasma concentration is elevated to 170 mg/dl, for instance, the kidneys can still reabsorb all the filtered glucose molecules, thereby keeping high blood glucose level. In contrast, if, hypothetically, T_{max} were 160 mg/dl, for example, then the kidneys would not be able to reabsorb glucose at plasma levels perhaps higher than 110, thereby getting rid of excess glucose and keeping plasma concentration within normal range.
- With good understanding of the previous point, we can conclude that *the higher* ***T_{max} to normal plasma level*** *ratio of a substance is, the less the role of the kidneys in maintaining normal plasma concentration of that substance (i.e., excretion of excess amounts).*
- A good example of a substance that has low T_{max} to plasma level ratio is phosphate. Phosphate plasma concentration is around 1 mM (i.e., its filtered load is 1 mol/L x 125 ml/min, or 0.125 mmol/min), and T_{max} equals 0.1mM/min.
- What determines the difference in actual and theoretical thresholds? The affinity of the transporters to the transported substance. An infinitive affinity would diminish the effect of splay.

- Glycosuria can be nephrogenic or diabetogenic. Usually, measuring blood glucose level is differential. High level confirms diabetogenic glycosuria; normal level confirms nephrogenic glycosuria (e.g., low number or affinity of the glucose transporters). Nephrogenic glycosuria is benign (i.e., good prognosis), as it does not progress.

In these two pages, the concepts discussed so far are reviewed and applied. I recommend filling these two tables with the italic numbers without help.

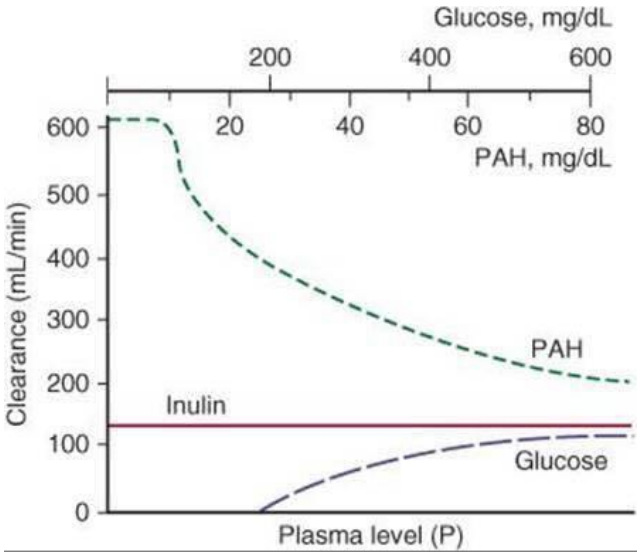
$P_{glucose}$ (mg/dl)	Mg/min entering kidneys	Filtered load (mg/min)	Reabsorbed (mg/min)	Excretion rate (mg/min)	Clearance (ml/min)
100	*625*	*125*	*125*	*0*	*0*
640	*4,000*	*800*	*375*	*425*	*66*
5,000	*31,250*	*6,250*	*375*	*5,875*	*118*
100,000	*625,000*	*125,000*	*375*	*124,625*	*124.6*

P_{PAH} (mg/dl)	Mg/min entering kidneys	Filtered load (mg/min)	Secreted (mg/min)	Excretion rate (mg/min)	Clearance (ml/min)
5	*31.25*	*6.25*	*25*	*31*	*620*
30	*187.5*	*37.5*	*80*	*117*	*390*
100	*625*	*125*	*80*	*205*	*205*
10,000	*62,500*	*12,500*	*80*	*12,580*	*125.8*

- Note that:
  - mg/min entering kidneys = RPF in dl/min (6.25 dl/min) x P
  - Filtered load = GFR in dl/min (1.25 dl/min) x P
  - Reabsorbed = T_{max} (if P to T_{max} ratio is large).
  - Excretion rate = filtered load – reabsorbed, in case of glucose and filtered load + secreted, for PAH.
  - Clearance = excretion rate / P

  - Combining these equations together, at high concentrations:
    - Clearance = (GFR x P – T_{max}) / P, for glucose.
    - Clearance = (GFR x P + T_{max}) / P, for PAH.
  - Assuming infinitive affinity of the transporters for glucose and PAH,
    1) Calculate the maximum plasma concentration of glucose below which glucose does not appear in the urine (theoritical renal threshold), knowing that T_{max} is 375 mg/min.
       - For glucose not to appear in the urine, glucose clearance must be zero. Thus, 0 = (1.25 x P – 375) / P. That is, P = 300 mg/dl.
    2) Calculate the maximum plasma concentration of PAH below which PAH is not reabsorbed , knowing that T_{max} is 80 mg/min.
       - For PAH not to be reabsorbed, PAH clearance must be 6.25 dl/min (i.e., RPF). Thus, 625 = (1.25 x P – 80) / P. That is, P = 16 mg/dl.

- Note that the clearance of glucose increases and that of PAH decreases as their plasma levels increase to eventually approach (but never equal) GFR (which is also inulin clearance) at very high theoretical concentrations.
- Note that inulin clearance equals GFR at any plasma concentration of inulin. This is because all inulin reaching the kidneys is passively filtered.
- Thus, theoretically, these substances can be used as glomerular markers (like inulin) to estimate GFR if injected in very high concentrations.



- **Amino Acids Reabsorption**
  - Like glucose, amino acids are completely reabsorbed from the renal tubules by secondary active co-transport mechanism. Amino acids first bind to special carrier proteins in the brush border to enter the cells with sodium ions. Then, following a concentration gradient, they passively exit the cells at the basolateral membrane to enter the interstitial fluid. Sodium gradient is maintained by Na^+/K^+ pump.
  - Each carrier protein is specialized to reabsorb one type of the three types of the amino acids; acidic, neutral and basic.
  - A special carrier transports cystine. A defect in this carrier causes cystine to be excreted in urine (cystinuria) and can result in the formation of kidney stones.

- **Water Reabsorption**
  - Of the 125 ml of water filtered per minute, 124 ml are reabsorbed. Thus, the percentage of water reabsorption is almost 99.3%. On average, Only 1 ml/min is excreted.
  - The tubular reabsorption of water is by osmosis (i.e., is a passive process).
  - 65% of water is reabsorbed from the proximal tubule; 15% from the descending limb of the loop of Henle; none from the ascending limb of the loop of Henle; 10% from the distal tubule. The rest 9.3% is reabsorbed from the collecting tubule in the presence of ADH. Since reabsorption of water from the collecting ducts is controlled by ADH, it is this segment of the lobules which determines the osmolarity of the urine.
  - To measure water reabsorption, Inulin is injected, and its concentration is measured by a special technique called micropuncture technique by which a micropipette (25 µm) is inserted in different parts of the nephron. Since inulin is neither reabsorbed nor

secreted, it can be used to determine where and how much water is reabsorbed in different parts of the nephron by observing inulin dilution as it passes through these parts.

- For example, it has been found that inulin concentration in the late proximal tubule was three times that in Bowman's capsule. This implies that two thirds of the water has been reabsorbed by the time it passed through the proximal tubule.
- Also, the concentration of inulin did not change as the blood passed through the ascending part of the loop of Henle. Thus, it is known that water is not reabsorbed as it passes through the ascending loop of Henle.
- Note that the concentration of inulin cannot decrease while flowing distally through the tubules because inulin is not reabsorbed and water is not secreted.
- This method is also used to test how drugs, or even endogenous hormones, affect reabsorption of water (e.g., ADH had no effect on water reabsorption from the loop of Henle.)
- With water reabsorption in different parts of the nephron known, this method can be used to estimate the reabsorption of other substances.
- For example, Na^+ concentration in the plasma has been found to be similar to its concentration in the late proximal tubule. Knowing that two thirds (67%) of water is reabsorbed, it is easy to conclude that also, 67% of the Na^+ is reabsorbed as it pass through the proximal tubule.

- **Segmental Clearance**
  - The volume of fluid completely cleared of a substance as it passes through a certain segment of the nephron per unit time. It is calculated mathematically as follows:

$$C_s = \frac{V \times T_s}{P_s}$$, where

    - Ts is the concentration of the substance in the end of the segment.
    - Ps is the concentration of the substance in the beginning of the segment.
    - V is the fluid flow rate.

  - For example, if the concentration of a substance in Bowman's capsule was 8mg/ml. After a minute, 100 ml have passed through the proximal tubule and the concentration was measured to be 4 mg/ml, then Cs = 4mg/ml / 8mg/ml x 100ml/min, or 50ml/min
  - Segmental clearance can be compared to that of inulin in a specific segment by dividing the substance clearance by inulin clearance.

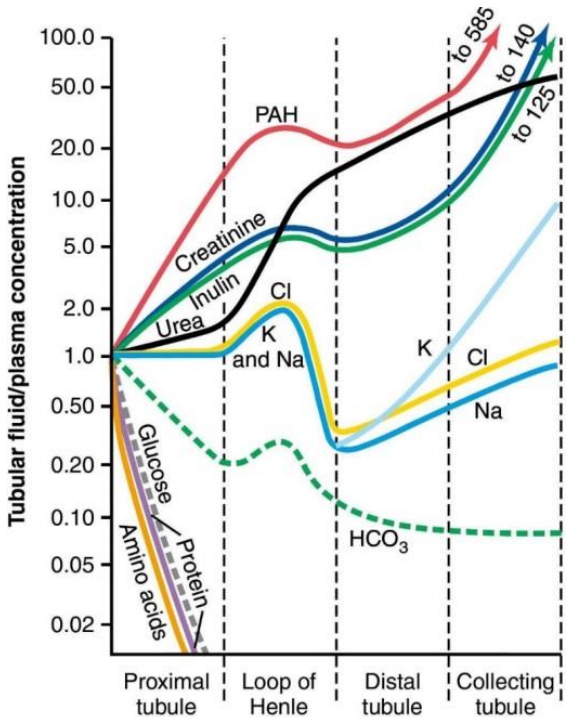
$$\frac{C_x}{C_{in}} = \frac{\frac{T_x \times V}{P_x}}{\frac{T_{in} \times V}{P_{in}}}$$

Since the flow rate is the same:

$$\frac{C_x}{C_{in}} = \frac{\frac{T_x}{P_x}}{\frac{T_{in}}{P_{in}}}$$

- If $C_x/C_{in} = 1$ in a specific segment, this suggests that X is, like inulin, neither reabsorbed nor secreted. However, this is not necessarily true; X might have been reabsorbed and secreted again through this segment. Because of this, short segments give more accurate conclusions.
- If $C_x/C_{in} = 2$, this means that X was secreted in the segment.
- C_{sodium} / C_{in} in the proximal tubules is 1/3. This is because 2/3 of Na^+ is reabsorbed from this segment.

- This graph shows the changes in average concentrations of different substances at different points in the tubular system relative to the concentration of that substance in the plasma and in the glomerular filtrate. A value of 1.0 indicates that the concentration of the substance in the tubular fluid is the same as the concentration of that substance in the plasma. Values below 1.0 indicate that the substance is reabsorbed more avidly than water, whereas values above 1.0 indicate that the substance is reabsorbed to a lesser extent than water or is secreted into the tubules.



- Note, especially, the following:
  - Glucose and amino acids are completely absorbed within the proximal tubules.
  - Inulin concentration at the end of the proximal tubules is 3 times that in the plasma. This is because two thirds of water is reabsorbed by the end of the proximal tubules, as noted earlier. Also, inulin clearance is 125 ml/min (which is GFR)
  - Creatinine clearance is 140ml/min (slightly higher than GFR)
  - PAH clearance is 585ml/min (90% of renal plasma flow, or effective renal plasma flow)
  - Na^+, K^+ and Cl^- concentrations remain constant until the end of the proximal tubules. This is because, like water, two thirds of these ions are reabsorbed from the proximal tubules.

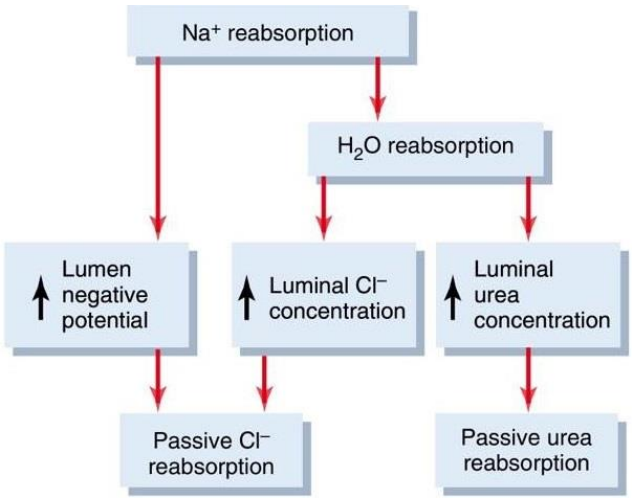
- **Sodium Homeostasis**

  - Importance of sodium in our bodies:
    - Contributes to the osmolarity of the plasma.
    - Important for muscles contraction.
    - Important to control fluids volume.
    - Important for concentrating the urine.
    - Important for secondary active transport of many substances.

  - Na^+ plasma concentration is around 140 mM; its intracellular concentration is 14 mM.
  - Filtered load of Na^+ is 125 ml/min x 140 mM, or 17.5 mmol/min, or 25,200 mmol/day.
  - Na^+ daily intake averages 150 mmol/day. The excretion rate is the same. Most of the excreted Na^+ is excreted by the kidneys; only 5 mmol/day is excreted by other pathways, such as through the GI secretions or by sweating.
  - The kidneys are the major organs in maintaining Na^+ homeostasis.
  - 99.4% of filtered sodium is reabsorbed.
  - To understand how Na^+ and K^+ are reabsorbed. The following facts must be kept in mind:

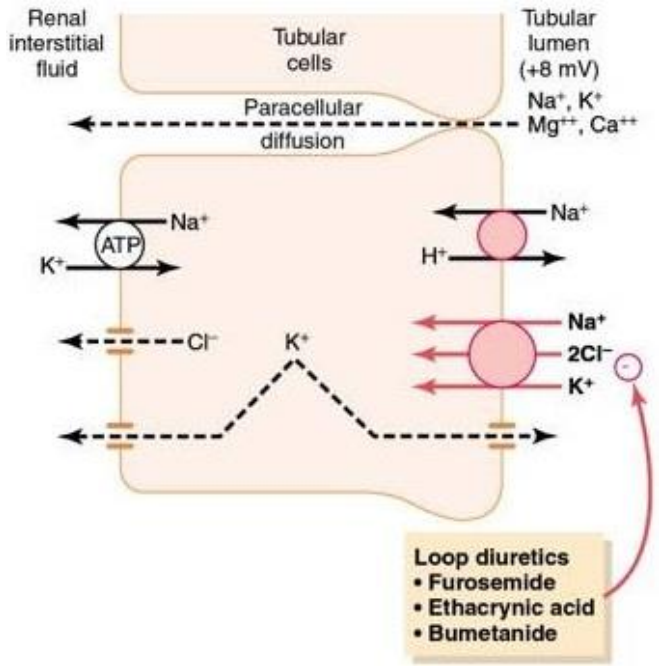
  - First, the proximal tubules, distal tubules and the first part of the collecting ducts are located in the cortex of the kidneys while the descending and ascending loops of Henle, and the second part of the collecting ducts are located in the medulla.
  - Second, the interstitium surrounding the proximal and distal tubules is iso-osmolar. In contrast, the osmolarity of the interstitium increases with descending toward the tip of the loop of Henle to reach 1400 mOsm/L. Then, with ascending again toward the cortex, the interstitium becomes hypo-osmolar with an osmolarity of 100 mOsm/L.

  - This means that water reabsorption is favored in the descending loop of Henle. In contrast, Na^+ reabsorption is favored in the ascending loop of Henle.
  - Many important substances are reabsorbed or secreted passively, depending on Na^+ transport as shown in the figure:



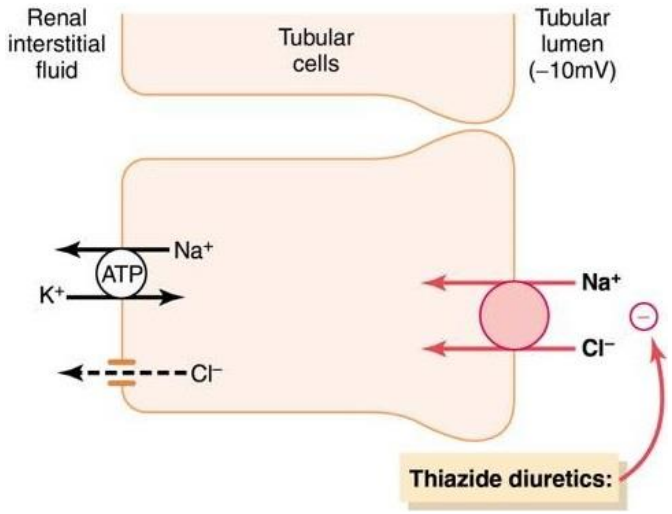
- **Na^+ Reabsorption from the Proximal tubules**
  - Almost 65% of the filtered Na^+ and the filtered water are reabsorbed from the proximal tubules. Na^+ is either co-transported, in the first half on the proximal tubule, with glucose and amino acids, or counter-transported with H^+, as discussed later.
  - In the second half of the proximal tubule, however, most organic solutes are reabsorbed. Thus, in this half, Na^+ is mainly coupled with Cl^- reabsorption whose concentration increases as water is reabsorbed.
  - Note that because water permeability of the proximal tubules is high, water reabsorption keeps pace with Na^+ reabsorption, thereby maintaining constant Na^+ and K^+ reabsorption along the length of the proximal tubule. This is in contrast to organic solutes (glucose, amino acids and HCO_3^-) whose concentration decrease significantly that they are almost completely reabsorbed by the end of the first half of the proximal tubule. Therefore, the proximal tubular fluid is iso-osmolar.

- **Na^+ Reabsorption from the Loop of Henle**
  - Composed of functionally three distinct segments; thin descending limb, thin ascending limb and thick ascending limb.
  - In the descending loop of Henle, water is reabsorbed by osmosis and Na^+ is not reabsorbed. This causes 15% increase in Na^+ concentration.
  - In the thick ascending loop of Henle, 25% of Na^+ and K^+, and not water, are reabsorbed. This is achieved by Na^+/K^+ ATPase pumps in the epithelial basolateral membrane and $1Na^+$, $2Cl^-$, $1K^+$ co-transporter in the luminal membrane, which uses the potential energy released by downhill diffusion of Na^+ into the cell to drive the reabsorption of K^+ into the cell against its concentration gradient.



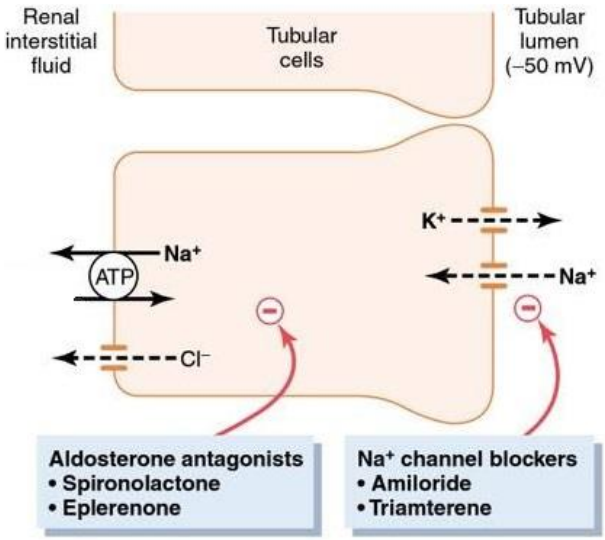
  - Also, there is significant paracellular reabsorption of K^+ and other cations owing to the slight positive charge of the tubular fluid relative to the interstitial fluid.
  - Since the thick ascending loop of Henle is almost totally impermeable to water, the reabsorption of these ions causes the tubular fluid to become diluted (i.e., hypo-osmolar) as it flows to the distal tubule.
  - Note that loop diuretics (furosemide, ethacrynic acid and bumetanide) inhibit $1Na^+$, $2Cl^-$, $1K^+$ co-transporter.

- **Na^+ Reabsorption from the Early Distal Tubule**
  - Avidly reabsorbs Na^+ and K^+ but not water
  - About 5% of the filtered load of Na^+ is reabsorbed.
  - Na^+/Cl^- co-transporter in the luminal membrane transport Na^+ and Cl^- from the lumen into the cell. Then, Na^+/K^+ ATPase pump maintains low intracellular Na^+ concentration by transporting Na^+ to the interstitium which is coupled to transport of K^+ from the interstitium into the cell.



  - **Note:** thiazide diuretics, which are widely used to treat hypertension and heart failure, inhibit this Na^+/Cl^- co-transporter.

- **Na^+ reabsorption from the Late distal and Cortical Collecting Ducts**
  - Composed of two types of cells; principal cells and intercalated cells.
  - Principal cells reabsorb Na^+ by special channels and secrete K^+. Na^+/K^+ ATPase pump in the basolateral membrane maintains low intracellular Na^+ concentration and high intracellular K^+. K^+ transported into the cell diffuses down its concentration gradient through its channels into the tubular lumen.
  - **Note:** This pump is the major site of action for K-sparing diuretics. Spironolactone and eplerenone antagonize aldosterone in principal cells, thereby inhibiting stimulation of Na^+ reabsorption and K^+ secretion. Amiloride and triamterene block luminal Na^+ and K^+ channels which inhibits Na^+ entry into the cell. Thus, decreasing Na^+ available to be reabsorbed by Na^+/K^+ pump at the basolateral membrane. This also decreases K^+ entry into the cell, and thus, K^+ secretion.



  - **Keep in mind:** Intercalated cells secrete H^+ against large concentration gradient (900x) and reabsorb HCO_3^- and K^+. This H^+ ATPase plays a key role of acid-base regulation of body fluids, as discussed later.

- **Reabsorption from the Medullary cortical ducts**
  - The reabsorption of water from the collecting ducts is variable. ADH causes the permeability of the collecting ducts to water to increase, thereby increasing

reabsorption of water and concentrating urine. On average, 4% of Na^+ is reabsorbed from the collecting ducts.

- Unlike late distal tubules and cortical collecting ducts, medullary collecting ducts are permeable to urea. This helps to raise the osmolarity in the interstitium surrounding these ducts, as discussed later.

- Eventually, less than 1% of Na^+ is excreted.

• Regulation of Na^+ and Water Reabsorption

o Antidiuretic Hormone (ADH)

❖ ADH is synthesized in the hypothalamus (85% by the supraoptic nuclei and 15% by the paraventricular nuclei). It is stored in the posterior pituitary.

❖ Its release is stimulated by:

- Osmoreceptors in the hypothalamus, an increase by 1% in the osmolarity of plasma increases ADH release.
- Volume receptors, a decrease by 10% in ECF volume increases ADH release. ❖
- Pressure receptors, decreased blood pressure (as in bleeding) also leads to ADH release.

❖ ADH binds to specific V_2 receptors in the late distal tubules, collecting tubules, and collecting ducts. The stimulation of this receptor eventually increases the density of water channels (aquaporin) at the luminal and basolateral sides of the cells membranes.

❖ In peripheral (nephrogenic) diabetes insipidus where ADH receptors do not respond to ADH, and in central diabetes insipidus, where ADH secretion is decreased or absent, urine output is very high (polyuria) and the urine is very hypo-osmolar.

o GFR

❖ Increased Na^+ plasma level increases ECF volume and blood pressure and, at the same time, decreases the capillaries oncotic pressure. These, in turn, increase GFR, and consequently, excess Na^+ and water excretion. This effect on increased blood pressure on Na^+ and water excretion is called pressure natriuresis and diuresis, respectively.

o Aldosterone

❖ Aldosterone increases Na^+ reabsorption while at the same time increasing K^+ secretion by stimulating the Na^+/K^+ ATPase pump on the basolateral side of the principal cells of the late distal tubules and cortical collecting.

❖ Aldosterone also increases the sodium and potassium permeability of the luminal side of the membrane by increasing the number of their channels.

❖ Aldosterone effects are somewhat delayed since it induces genes transcription to generate new channels and new enzymes which participate in ATP formation.

- **Note:** In the absence of aldosterone, as occurs with adrenal destruction or malfunction (Addison's disease), there is marked loss of sodium from the body and accumulation of potassium. Conversely, excess aldosterone secretion, as occurs in patients with adrenal tumors (Conn's syndrome), is associated with sodium retention and decreased plasma potassium concentration due, in part, to excessive potassium secretion by the kidneys.

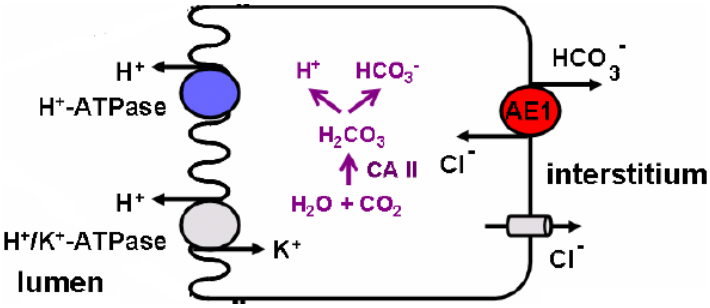
- **Atrial Natriuretic Hormone (Factor or peptide)**
  - Secreted from the cardiac atrial cells in response to stretch (i.e., increased plasma volume)
  - It directly decreases Na^+ reabsorption at the distal tubules and inhibits aldosterone secretion. Also, it dilates the afferent arterioles to increase GFR and Na^+ excretion.

Potassium Homeostasis

- Maintaining normal plasma concentration is essential to life.
- The membrane potential of cells is largely determined by the equilibrium potential of potassium. This is due to the relatively higher permeability of the cellular membranes to potassium ion than sodium and chloride ions.
- The equilibrium potential of an ion is the membrane electrical potential at which net flow of the ion across the membrane is zero. In other words, it reflects the net charge difference required to stop net diffusion of the ion despite the presence of a concentration gradient which would, otherwise, drive the ion from the area of high concentration to the area of low concentration.
- Thus, it is apparent that as the permeability of the ion increases, its equilibrium potential also increases and vice versa. For example, the equilibrium potential of an ion which is not permeant at all would be zero.
- Another factor that does affect the equilibrium potential is the concentration gradient which this difference in electrical potential is supposed to maintain.
- The equilibrium potential can, therefore, be calculated using Nernst equation as follows
  - **E = -61 log (P x [in] / P x [out]),** where: [out] and [in] are the concentrations of the ion outside and inside the cell, respectively; P is the permeability of the membrane to the ion. Canceling P:
  - $$E = -61 \log \frac{[in]}{[out]}$$
  - For K^+, E = -61 log(150/4), or -91.5mV.

- The membrane potential of cells of different tissues is not the same; it is -90 for cardiac ventricular cells. This means that K^+ is the only determinant of the membrane potential in these cells because K^+ channels are open while Na^+ and Ca^{2+} channels are closed.
- In hyperkalemia, [in]/[out] ratio decreases. That is, the membrane potential of heart cells becomes less negative (i.e., closer to the threshold). This increases the excitability of the cells but also, inactivates fast Na^+ channels. Thus, the transfer of the action potential becomes slow since its conduction becomes dependent on slow voltage gated Na^+ channels. These changes result in cardiac arrhythmias.
- In hypokalemia, the membrane potential becomes more negative; thus decreasing the excitability of the cells (i.e., paralysis).
- Potassium intake must be equal to potassium output. On average, daily potassium intake and excretion is 100 mEq/day, of which 95 mEq/day is excreted by the kidneys. Thus, with average urine output of 1.5 L, K^+ urine concentration calculates to be 65 mEq/day.
- Filtered load of potassium = GFR (180 L/day) x plasma concentration (4 mEq/L), or 720m Eq/day.
- 65% of K^+ is reabsorbed from the proximal tubules, 25% from the ascending loop of Henle. The rest 10% (72 mEq/day) are excreted. Thus to excrete 95 mEq/day, 25 mEq/day must be secreted.
- Therefore, K^+ excreted in urine is either the filtered, unabsorbed K^+, or the secreted K^+. Normally, the secreted amounts are small but can increase significantly with high ingestion of potassium.
- For the secretion of K^+ to take place, The following is required:
  - Na^+/K^+ pumps at the basolateral membrane of the distal tubular and cortical collecting epithelial cells (principal cells). These pumps transport K^+ from the interstitium into the epithelial cells against a concentration gradient.
  - K^+ channels at the luminal membrane of these epithelial cells. These channels transport K^+ from the epithelial cells into the tubular lumen with a gradient.
  - Flow of fluids in these tubules to maintain K^+ gradient in favor of secretion of K^+.
- **Note:** diuretics increase fluids volume in these tubules, and thus decrease K^+ concentration. This increases K^+ secretion and, in severe cases, causes hypokalemia.
- The normal range of plasma K^+ concentration is 3.5-5.5 mEq/L.
- A meal containing 50 mEq of potassium would raise ECF K^+ level from 4 to 7.5 mEq/L (because these 50 mEq would be dissolved in the ECF whose volume averages 14 L. 50/14 = 3.5 mEq/L is therefore, the increase in plasma potassium concentration).
- This increase is dangerous and can result in cardiac arrhythmias and cardiac arrest.

- **Factors affecting K^+ level**
  - Insulin secretion facilitates transport of K^+ from the ECF to the inside of the cells, where it has no harmful effects. The intracellular potassium is then gradually excreted. Thus, plasma concentration does not increase significantly at any moment.
  - In acidosis (increased H^+ concentration in the plasma), excess H^+ is secreted while reabsorbing K^+ at the same time by H^+/K^+ pumps of the intercalated cells. A decrease in ECF pH by 1 results in increased extracellular K^+ concentration by 1.7-2 mEq/L. Also, as a compensation for acidosis, cellular uptake of H^+ increases while secreting K^+ to maintain electroneutrality. This also, increases plasma K^+ concentration.



  - Note: in ketoacidosis, K^+ concentration is increased but with administration of insulin, K^+ level may drop down resulting in hypokalemia.
  - Hyperkalemia is the most potent stimulator of aldosterone release. Aldosterone decreases K^+ reabsorption, as discussed earlier.
  - Beta receptor stimulation increases K^+ uptake by cells. Alpha receptor stimulation decreases K^+ uptake by cell. Thus, doing severe exercise with concomitant administration of a beta blocker can cause severe hyperkalemia.

Calcium Homeostasis

- Remember: 98.9% of body calcium is in bones. 1% is in cells. 0.1% (1 mM or 10^{-3} mol/L) is in the ECF, of which 9% are complexed to anions, 41% are protein bound, 50% free ionized Ca^{++}.
- In alkalosis, more negative charges are available on proteins to be complexed to Ca^{++} causing hypocalcemia. The opposite occurs in acidosis.
- 65% of calcium is reabsorbed in the proximal tubules, 8% in the distal tubule, 25% in the thick ascending limb of Henle and 1%is eventually excreted.
- PTH and thiazide diuretics increase calcium reabsorption while loop diuretics decrease it.
- Remember: Parathyroid hormone increases tubular reabsorption of calcium in the distal tubules and the loops of Henle. It also inhibits phosphate reabsorption by the proximal tubule and stimulates magnesium reabsorption by the loop of Henle.

- **Obligatory Urine Volume**
  - A normal human must excrete about 700 mOsm/day.
  - If maximal urine concentrating ability is 1400 mOsm/L, the minimal volume of urine that must excreted is 700/1400, or 0.5 L/day. This is called the obligatory urine volume or output.
  - Oliguria is decreased urine output below the obligatory urine volume.
  - Some dessert animals can concentrate the urine to as high as 10,000 mOsm/L, This allows these organisms to survive without drinking water.

- **Changes of the osmolarity of the tubular fluid**

  - The Osmolarity is the number of osmoles (Osmol or Osm) per unit volume.
  - The osmole is the number of moles of solutes that contribute to the osmotic pressure in a solution. The osmolarity of 1 mol/L solution of glucose is the same as the osmolarity of 1 mol/L solution of Na^+, despite the large difference in the molecular weight between glucose and Na^+. The osmolarity of 1 mol/L of NaCl solution is 2 Osm since NaCl dissociates into Na^+ and Cl^+ in aqueous solution. Thus, NaCl solution attracts water twice as much as glucose solution of the same concentration does.
  - Since most cell membranes are permeable to water, the osmolarity of ICF and ECF is equal and averages 300 Osm/L.
  - The molecular weight of NaCl is 58.5 kDa. The osmolarity of 1 mol/L of NaCl solution is 2,000 mOsm/L. Thus a solution with an osmolarity of 300 mOsm/L has a concentration of NaCl = 9 g/L, or 0.9 g/100ml. Therefore, normal saline solution is 0.9%.
  - The molecular weight of glucose is 180 kDa. The osmolarity of 1 mol/L of glucose solution is 1,000 mOsm/L. Thus, a solution with an osmolality of 300 mOsm/L has a concentration of glucose = 50 g/L, or 5%.

  - **Proximal tubule**
    - As fluid flows through the proximal tubule, solutes and water are reabsorbed in equal proportions, so little change in osmolarity occurs; thus, the proximal tubule fluid remains isosmotic to the plasma, with an osmolarity of about 300 mOsm/L.

  - **Loop of Henle**
    - As fluid passes down the descending loop of Henle, water is reabsorbed by osmosis and the tubular fluid reaches equilibrium with the surrounding interstitial fluid of the renal medulla, which is very hypertonic—about two to four times the osmolarity of the

original glomerular filtrate. Therefore, the tubular fluid becomes progresively more concentrated as it flows into the inner medulla.

- **This high osmolarity of the medullary interstitial fluid is maintained by three complex mechanisms:**
  1. The thick ascending loop of Henle continuously pumps Na^+ into the surrounding interstitium without permeating water to be reabsorbed by osmosis (even in the presence of large amounts of ADH). This process is called the single effect, and when combined with flow of fluids from the descending to the ascending loops of Henle, it is called countercurrent multiplication and accounts for about 700 of the 1200 mOsm/L.
  2. The medullary collecting ducts facilitate reabsorption of urea which is found in high concentrations in these ducts. This is even more activated in the presence of ADH. The reabsorption of urea into the interstitium is passive and accounts for about 500 of the 1200 mOsm/L.
     - **Note** that 50% of the filtered load of urea is reabsorbed by the proximal epithelial cells but in the ascending loop of Henle, the distal tubules and the cortical collecting tubules, little urea is reabsorbed because these segments are impermeable to urea.
     - **Note**: urea reabsorbed from the medullary collecting ducts is secreted again into the ascending loop of Henle, and so on.
  3. The blood vessels which supply medullary cells (vasa recta) carry only 5% of the renal blood flow. This prevents the loss of the hypertonicity of the medullary interstitial fluid, though does not add to the osmolarity of the interstitial fluid.

❖ Factors than can depress the maximum concentrating ability of the kidney:
- Low protein diet (as in vegetarians) which decreases urea formation.
- Increased blood pressure or RPF, which increases medullary blood flow in the vasa recta, thereby washing out the solutes contributing to the hyperosmolarity of the interstitium.
- Diuretics, which decrease NaCl reabsorption losing the ability to concentrate the urine.

- **Distal and Collecting Tubules**
  - Additional reabsorption of Na^+ causes the tubular fluid to become even more dilute, decreasing its osmolarity to as low as 50 mOsm/L (In the absence of ADH).
  - The osmotic gradient alone cannot cause reabsorption of water from these segments; ADH is also necessary. It can increase urine osmolarity up to 1200 to 1400 mOsm/L. However, the average osmolarity of human urine is about 650mOsm/L; thus, the urine,

in general, is considered hypertonic (compared to the osmolarity of the plasma which averages 285 mOsm/L).

- **Measuring Urine Osmolarity.**
  - **Osmometer**
    - Each 1 Osm decreases the freezing point of a 1 kg (or 1 liter) water solution by 1.86 degrees. Thus, in case of aqueous solutions, such as plasma:
    - Freezing temperature = osmolarity x 1.86.
    - If the freezing point of a solution is -0.5 degrees, then the osmolarity = 0.5/1.86 = 0.269 Osm/L, or 269 mOsm/L.

  - **Urine Specific Gravity**
    - A measure of the weight of solutes in a given volume of urine.
    - Expressed in g/ml and, in humans, normally ranges from 1.002 to 1.028 g/ml, rising by 0.001 for every 35 to 40 mOsm/L increase in urine osmolarity.
    - The protein-free plasma specific gravity is 1.010. Isosthenuria refers to urine whose osmolarity equals 1.010; hyposthenuria and hypersthenuria refer to urine whose osmolarity is lower or higher than 1.010, respectively.
    - It is determined by the number and size of the solute molecules. This contrasts with osmolarity, which is determined only by the number of solute molecules in a given volume
    - Therefore, when there are significant amounts of large molecules in the urine, such as glucose, contrast dyes, or some antibiotics, urine specific gravity measurements may falsely suggest a very concentrated urine, despite a normal urine osmolality.

- **Regulation of pH**
  - The normal concentration of extracellular H^+ averages 40 nM, or 40 mEq/L. This can increase 4 times or decrease to one fourth (i.e., from 10 to 160nM) in abnormal conditions without causing death.
  - These values correspond to average normal pH of 7.4; range of pH at which life is possible of 6.8-8.0. **Remember**: pH = -Log $[H^+]$.
  - However, the lower limit of normal extracellular pH is 7.35 while the upper limit is 7.45.
  - pH lower than 7.35 is referred to as acidosis while pH higher than 7.45 is referred to as alkalosis.

- Maintaining an extracellular pH within its normal range is crucial to life; acidosis, for instance, can affect electrolytes balance causing hypercalcemia, hyperkalemia, hyperchloremia. The opposite occurs in alkalosis; hypocalcemia can cause convulsions of skeletal muscles, which can be fatal if the respiratory muscles are affected. Even more importantly, our enzymes activities are greatly decreased with even slight changes in pH.
- **The three means by which pH is strictly regulated are:**
  - Buffer systems, which function within seconds to correct any change in pH.
  - Lungs, which function within minutes.
  - Kidneys, whose function is delayed (hours to days) yet the most powerful.
- Acids formed in our bodies are either volatile or nonvolatile. Volatile acids are formed by CO_2 (H_2CO_3) and excreted by the lungs as CO_2; nonvolatile ones are those formed mainly from the metabolism of proteins, and thus cannot be excreted by the lungs.

• Buffer Systems

- Bicarbonate buffer: important ECF buffer.
- Phosphate and ammonia buffers: important renal tubular buffer.
- Proteins: important intracellular buffers. These are the most abundant buffers yet not the most effective

The intracellular pH is around 7.0 (i.e., H^+ is more concentrated inside the cells than in the ECF). Since the pK of Phosphate is 6.8, phosphate buffer is also effective in maintaining intracellular pH of 7.0 although proteins are much more concentrated in the cells, and thus more important regulators.

Note that H^+ in the ECF cannot permeate the cellular membranes. Therefore, proteins and phosphate inside the cells do not participate in buffering excess H^+ in the ECF.

• Bicarbonate Buffer System:

- Consists of carbonic acid (**H_2CO_3**) and bicarbonate (**HCO_3^-**)
- **H_2CO_3** is a weak acid formed by a reaction between CO_2 and H_2O catalyzed by carbonic anhydrase.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

- When a strong acid such as HCl is added, the increased H^+ released from the acid is buffered by HCO_3^- to form the very weak acid H_2CO_3.

- As a result, more H_2CO_3 is formed, causing increased CO_2 and H_2O production. This excess CO2 greatly stimulates respiration, which eliminates it from the ECF.
- When a strong base, such as NaOH, is added, the OH^- released from the NaOH combines with H_2CO_3 to form additional HCO_3^-. Thus, the weak base $HCO3^-$ replaces the strong base NaOH.
- As a result, the concentration of H_2CO_3 decreases (because it reacts with NaOH), causing more CO2 to combine with H2O to replace the H2CO3. This decreases CO2 levels in the blood which inhibits respiration and decreases the rate of CO2 expiration. Also, the rise in blood HCO_3^- is compensated for by increased renal excretion of HCO_3^-.
- Henderson-Hasselbalch equation

$$pH = pK_a + \log\frac{[A^-]}{[HA]}$$

In case of bicarbonate buffer system:

$$pH = 6.1 + \log\frac{HCO_3^-}{0.03 \times Pco_2}$$

- **Example:** calculate the pH of a solution containing 24 mM of HCO_3^- and 12mM of CO_2.
  pH = 6.1 + log (24/12) = 7.4
- **Example:** calculate the pH of a solution containing 0.25 mM of $H_2PO_4^-$ for each 1 mM of HPO_4^{2-}.
  pH = 6.8 + log (1/0.25) = 7.4
- These values are true for the buffer systems in our bodies, why pH calculated to be 7.4, the normal extracellular pH.

❖ Henderson-Hasselbalch equation:

$$pH = pK_a + \log\frac{[A^-]}{[HA]}$$

❖ In case of the bicarbonate buffer solution:

$$pH = pK + \log\frac{[HCO_3^-]}{[H_2CO_3]}$$

❖ Since H_2CO_3 concentration in the plasma is difficult to measure, and since CO_2 concentration is proportional to it:

$$pH = pK_a + \log\frac{[HCO_3^-]}{[CO_2]}$$

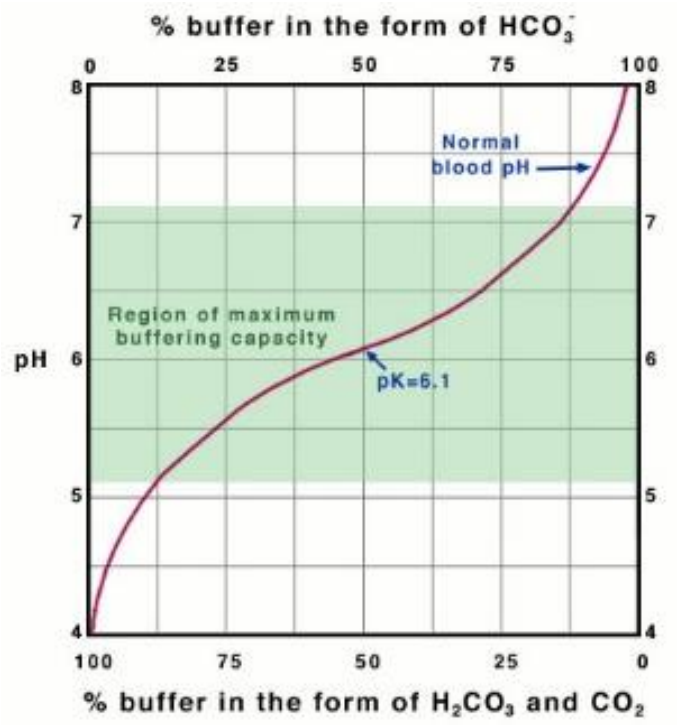
❖

❖ Most clinical laboratories measure the blood CO_2 tension (Pco_2). Since plasma concentration of CO_2 = Pco_2 x solubility coefficient, and since the pK for HCO_3^- is 6.1:

$$pH = 6.1 + \log\frac{HCO_3^-}{0.03 \times Pco_2}$$

- With good understanding of the effects of [HCO_3^-] and Pco_2 on blood pH, it is apparent that acidosis can be due to either decrease in plasma [HCO_3^-] or increase in Pco_2. Alkalosis, on the other hand, can be due to increased plasma [HCO_3^-] or decreased Pco_2.

- **Buffer Power**
  - The pH of the system is the same as the pK when each of the components (HCO_3^- and CO_2) constitutes 50% of the total concentration of the buffer system.
  - The buffer system is most effective in the central part of the curve, where the pH is near the pK of the system. This means that the change in pH for any given amount of acid or base added to the system is least when the pH is near the pK of the system.
  - The buffer system is still reasonably effective for 1.0 pH unit on either side of the pK, which for the bicarbonate buffer system extends from a pH of about 5.1 to 7.1 units.

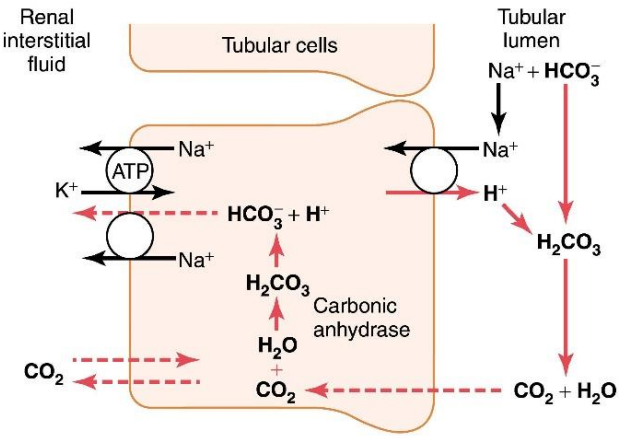


  - Beyond these limits, the buffering power rapidly diminishes. And when all the CO_2 has been converted into HCO_3^- or when all the HCO_3^- has been converted into CO_2, the system has no more buffering power.
  - The absolute concentration of the buffers is also an important factor in determining the buffer power of a system. With low concentrations of the buffers, only a small amount of acid or base added to the solution changes the pH considerably.
  - pK for bicarbonate buffer system is 6.1, which means that this system operates on the portion of the buffering curve where the slope is low and the buffering power is poor. Also, the concentrations of the two elements of the bicarbonate system, CO_2 and HCO_3, are not great.
  - Nevertheless, the bicarbonate buffer system is the most powerful extracellular buffer in the body. This apparent paradox is due mainly to the fact that HCO_3^- and CO_2, are regulated, respectively, by the kidneys and the lungs, as discussed later.

- **Renal Control of Acid-Base Balance**
  - The kidneys regulate extracellular pH by excreting acidic urine or basic urine.
  - Large numbers of HCO_3^- are filtered continuously into the tubules, and if they are excreted into the urine this removes base from the blood. Large numbers of H^+ are also secreted into the tubular lumen by the tubular epithelial cells, thus removing acid from the blood.
  - *Simply, if more H+ is secreted than* HCO_3^- *is filtered, there will be a net loss of acid from the extracellular fluid. Conversely, if more* HCO_3^- *is filtered than* H^+ *is secreted, there will be a net loss of base.*
  - The remainder of the discussion will show, in details, how H^+ is secreted and how HCO_3^- is filtered and reabsorbed.

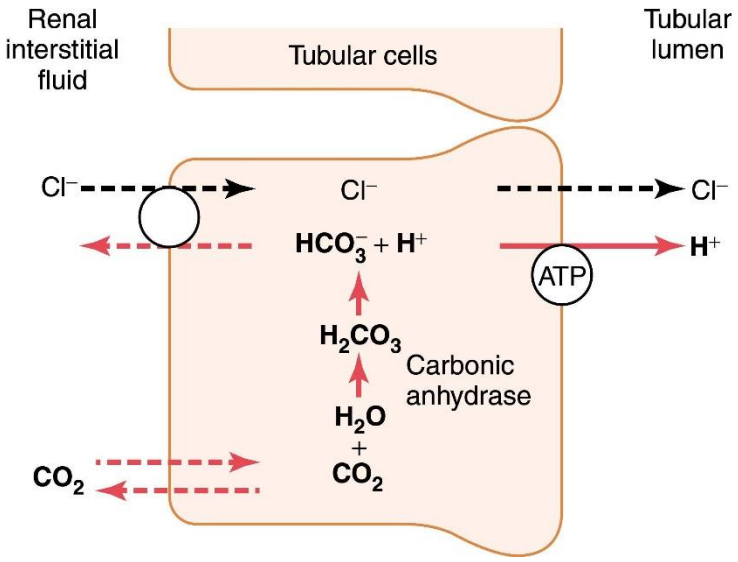
- **H^+ secretion and HCO_3^- reabsorption**
  - The filtered load of HCO_3^- is 180 L/day x 24 mEq/L, or 4320mEq/day. All bicarbonate filtered is reabsorbed.
  - Keep in mind, as explained later, that in any part of the tubule, *for each* HCO_3^- *reabsorbed from the tubules, an* H^+ *must be secreted into the tubules,*
  - 85% of bicarbonate reabsorption and H^+ secretion occurs in the proximal tubule; 10% in the thick ascending limb of the loop of Henle; about 5% in the distal tubule and collecting duct. Therefore, only 1 mEq/day is excreted in urine.
  - Reabsorption of HCO_3^- and secretion of H^+ does not occur in the descending and ascending thin limbs of the loop of Henle.

- **H^+ secretion in the proximal tubule, thick ascending loop of Henle, and the early distal tubule.**
  - CO_2 either diffuses into the tubular cells or is formed by metabolism in the tubular epithelial cells.
  - CO_2, under the influence of the enzyme carbonic anhydrase, combines with H_2O to form H_2CO_3, which dissociates into HCO_3^- and H^+.
  - The H^+ is secreted from the cell into the tubular lumen by Na^+/H^+ counter-transporter which uses the energy released by transporting Na^+ downhill to secrete H^+ uphill (i.e., secondary active transport).



- At the same time, the formed HCO_3^- moves downhill across the basolateral membrane and is reabsorbed by the peritubular capillaries.
- The net result is that for each H^+ secreted, an HCO_3^- enters the blood.
- Drugs that inhibit carbonic anhydrase (e.g., some diuretic) decrease H^+ secretion and HCO_3^- reabsorption, thereby increasing the risk of acidosis.

- **H^+ secretion in the Late distal and collecting tubules**
  - HCO_3^- and H^+ are formed by the same reactions.
  - At the luminal membrane of the Intercalated cells of these segments, H^+ is secreted by H^+ ATPase, which uses the energy released by the breakdown of an ATP molecule to transport H^+ uphill (i.e., primary active transport).
  - **Note:** also at the luminal membrane, H^+/K^+ ATPase secretes H^+ and reabsorb K^+, both uphill. (refer to K^+ reabsorption section)



  - Tubular acidosis is of 4 types; type 1 results from decreased activity of this H^+/K^+ ATPase or H^+-ATPase (i.e., decreased secretion of H^+ from the distal and collecting tubules); type 2 results from decreased activity of the proximal Na^+/H^+ counter-transporter.
  - With type 1, H^+ gradient can only change pH from 7.4 to, minimally, 5.2. Therefore, type 2 is less dangerous than type 1.

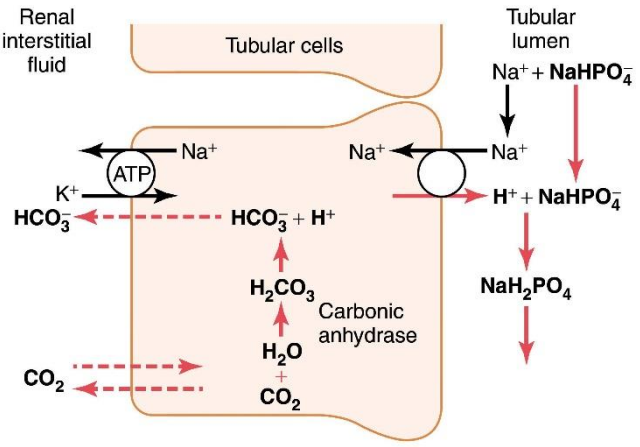
- **Reabsorption of Filtered HCO_3^-**
  - HCO_3^- does not permeate the luminal membrane. HCO_3^- in the tubular fluid combines with H^+ to form H_2CO_3 which dissociates into CO_2 and H_2O. CO_2 easily crosses the luminal membrane to enter the cell, where it recombines with H_2O to form H_2CO_3 which dissociates into HCO_3^- and H^+.
  - HCO_3^- transport at the basolateral membrane is facilitated by Na^+/HCO_3^- co-transporter in the proximal tubule and Cl^-/HCO_3^- counter-transporter in the late distal tubule, thick ascending loop of Henle and in the collecting tubules and ducts
  - Note again, that for each H^+ formed in the tubule, an HCO_3^- enters the blood although HCO_3^- which enters the blood is not the same HCO_3^- filtered.

- Also note that only 1 H^+ in the tubular cell is sufficient to reabsorb all HCO_3^- in the lumen. That is, there is no net secretion of H^+ in these segments. Refer to the figure in page 32.

- **HCO_3^- and H^+ titrate each other in the tubules**
  - H^+ secreted into the tubules is buffered by HCO_3^-, keeping tubular pH unchanged. After reabsorption of the 4320 milliequivalents of HCO_3^-, other buffers in the tubules bind to the excess H^+, as discussed later.
  - As noted earlier, for each H^+ secreted, HCO_3^- must be absorbed. Thus:
  - When there is an excess of HCO_3^- over H^+ in the urine, as occurs in metabolic alkalosis, the excess HCO_3^- cannot be reabsorbed; therefore, the excess HCO_3^- is left in the tubules and eventually excreted into the urine, which helps correct the metabolic alkalosis.
  - In acidosis, there is excess H^+ relative to HCO_3^- causing complete reabsorption of the HCO_3^-; the excess H+ passes into the urine.
  - To reabsorb 4320 mEq of HCO_3^- (the filtered load of HCO_3^-), 4320 mEq of H^+ must be secreted. Also, 80 mEq of H^+ produced by nonvolatile acids must be secreted each day. Summating these numbers, 4400 mEq of H^+ must be secreted into the tubules each day. Note that these 80 milliequivalents do not combine with HCO_3^- in the tubules because all HCO_3^- has been reabsorbed. However, still, for each H^+ secreted, an HCO_3^- enters the blood. This is explained next.

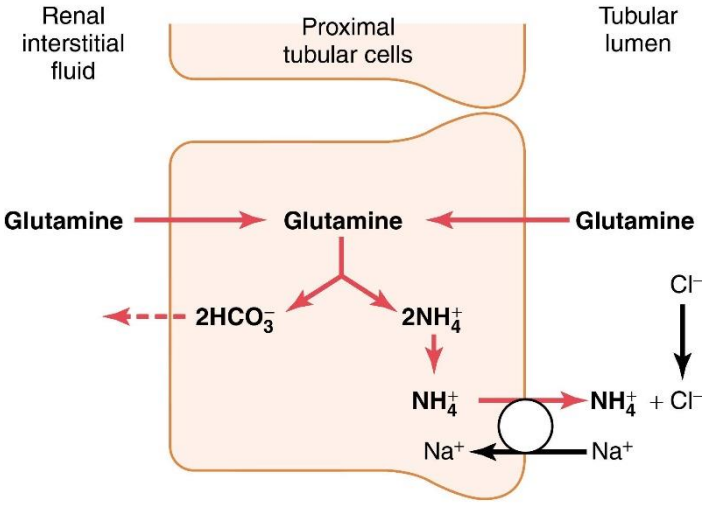
- **New HCO_3^- Generation**
  - The minimal urine concentration of H^+ that can be achieved by the previously discussed mechanisms is 0.03 mEq/L (i.e., minimal urine pH = -log (3×10^{-5}) = 4.5). Thus, for a person to excrete all the 80 mEq, 2667 liters of urine must be excreted per day!
  - Since CO_2 and H_2O are abundant in the epithelial cells, what limits urine pH? The reader, at this level, is expected to be able to answer this question easily. The answer is that the active transporters in the luminal membrane cannot achieve higher concentration gradient of H^+ between the inside of the cell and the tubular fluid. That is, a dynamic equilibrium is reached that new H_2CO_3 molecules are not formed in the cell.
  - **Note:** The primary H^+-ATPase can maximally establish a 900-fold concentration gradient of H^+ (i.e., decreases pH of the tubular fluid to -log ($10^{-7.4} \times 900$), or 4.5); the Na^+/H^+ counter-transporter, on the other hand, can maximally establish a fourfold gradient of H^+ (i.e., decreases pH to only 6.7).
  - How does the body manage to get rid of these 80 mEq of H^+ each day?
  - Excess H^+ in the tubules which does not react with HCO_3^- combines with tubular buffers, of which the most important are ammonia and phosphate buffers but not bicarbonate.

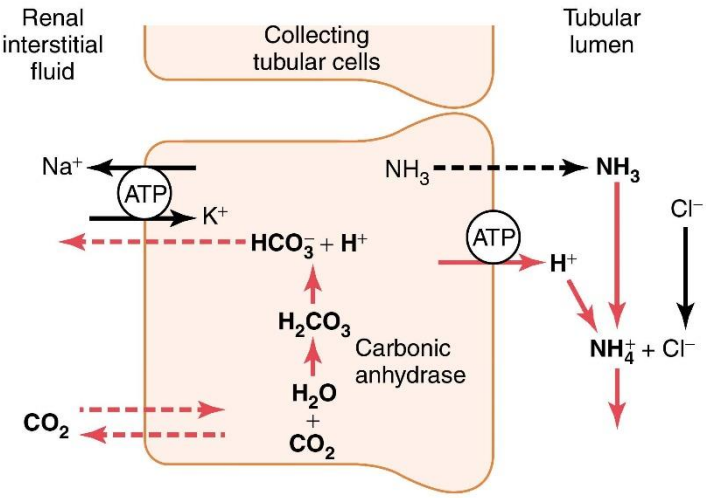
- **Phosphate Buffer**
  - H^+ reacts with HPO_4^{2-} in the tubule to form $H_2PO_4^-$ which is excreted in the urine.
  - Note that this causes new HCO_3^- in the cell to be formed. This will eventually enter the blood.
  - Therefore, it is apparent that whenever H^+ in the tubular fluid combines with a buffer other than HCO_3^-, a new HCO_3^- is added to the blood. This is a mechanism by which the kidneys can replenish the extracellular fluid stores of HCO_3^-.



  - The pK of phosphate buffer is 6.8; the urine is slightly acidic. Therefore, in the tubules, the phosphate buffer system functions at its most effective range of pH.
  - The filtered load of phosphate is 180 x 1.5, or 270 mg/day.
  - Normally, about 90% of the filtered phosphate is reabsorbed; only 30 mEq/day are available for buffering H^+.

- **Ammonia Buffer**





  - Glutamine enters the epithelial cells of the proximal tubules, thick ascending loop of Henle, and distal tubules.
  - In the cell, each molecule of glutamine is metabolized to ultimately form two NH_4^+ and two HCO_3^-. The $2NH_4^+$ are secreted into the lumen by a counter-transport mechanism in exchange for Na^+, which is reabsorbed. The $2HCO_3^-$ are considered new and are transported across the basolateral membrane, along with the reabsorbed Na^+.

- **Note:** In the collecting tubules, the addition of NH_4^+ to the tubular fluids occurs through a different mechanism. Here, H^+ is secreted by the tubular membrane into the lumen, where it combines with NH_3 to form NH_4^+, which is then excreted.
- The collecting ducts are permeable to NH_3, which can easily diffuse into the tubular lumen. However, the luminal membrane of this part of the tubules is much less permeable to NH_4^+; therefore, once the H^+ has reacted with NH_3 to form NH_4^+, the NH_4^+ is trapped in the tubular lumen and eliminated in the urine. For each NH_4^+ excreted, a new HCO_3^- is generated and added to the blood.
- One of the most important features of the renal ammonia buffer system is that it is subject to physiologic control as follows: An increase in extracellular fluid H^+ concentration stimulates renal glutamine metabolism and, therefore, increases the formation of NH_4^+ and new HCO_3^- to be used in H^+ buffering; a decrease in H+ concentration has the opposite effect.
- Normally, the amount of H^+ eliminated by the ammonia buffer system accounts for about 50% of the acid excreted and HCO_3^- generated by the kidneys.
- With chronic acidosis, glutaminase is stimulated and the rate of NH_4^+ excretion can increase to as much as 500 mEq/day increasing H^+ excretion and bicarbonate generation. This is the dominant mechanism which helps to correct chronic acidosis.

- **Net H^+ Excretion (or Net HCO_3^- Gain)**
  - **Net H^+ excretion = NH_4^+ excretion + urinary titratable acid - HCO_3^- excretion**
  - HCO_3^- excretion is calculated by multiplying urine flow rate by its urinary concentration.
  - The amount of excess H^+ is combined either with ammonia buffer or other buffers. To measure the amount of H^+ excreted conjugated with ammonia, NH_4^+ excretion is calculated, also, by multiplying the urinary flow rate by NH_4^+ urinary concentration.
  - To measure H^+ combined with phosphate and other less important buffers, such as citrate and urate, titratable acid value is determined by titrating the urine with a strong base (NaOH) to a pH of 7.4, which is the normal pH of both the plasma and the glomerular filtrate.
  - The number of milliequivalents required to return the pH to 7.4 equals the number of milliequivalents of H^+ added to the tubular fluid and combined with tubular buffers other than ammonia, why other than ammonia? Because pK of the reaction, $NH_3 + H^+ = NH4^+$, is 9.2. Thus, titration to a pH of 7.4 doesn't remove an H^+ from NH_4^+ (i.e., at this pH, most of the NH_3 is still bound to H^+).

- **Regulation of Renal tubular acid secretion**
  - As explained earlier, normally, the kidney tubules must secrete at least 4320 mEq/day of H^+ to reabsorb all the HCO_3^- that is filtered (4320 mEq/day), and there must be enough H^+ left over to be excreted as titratable acid or NH_4^+ to rid the body of the nonvolatile acids

  - In alkalosis, secretion of H^+ is reduced to a level that is too low to achieve complete HCO_3^- reabsorption, enabling the kidneys to increase HCO_3^- excretion. Titratable acid and ammonia are not excreted because there is no excess H^+ available to combine with nonbicarbonate buffers; therefore, there is no new HCO_3^- added to the blood in alkalosis.
  - In acidosis, the tubular H^+ secretion is increased sufficiently to reabsorb all the filtered HCO_3^- with enough H^+ left over to excrete large amounts of NH_4^+ and titratable acid, thereby contributing large amounts of new HCO_3^- to the total body extracellular fluid.

  - Aldosterone stimulates the secretion of H^+ by the intercalated cells of the collecting duct. Therefore, excessive secretion of aldosterone, as occurs in Conn's syndrome, can increase secretion of H^+ into the tubular fluid and, consequently, increase the amount of HCO_3^- added back to the blood. This usually causes alkalosis in patients with excessive aldosterone secretion. The tubular cells usually respond to a decrease in H^+ concentration (alkalosis) by reducing H+ secretion.
  - H^+ secretion is coupled to Na^+ reabsorption by the Na^+/H^+ exchanger in the proximal tubule and thick ascending loop of Henle. Therefore, factors that stimulate Na^+ reabsorption, such as decreased extracellular fluid volume, may also secondarily increase H^+ secretion.
  - Angiotensin II directly stimulates the activity of the Na^+/H^+ exchanger, increasing H^+ excretion.
  - Hypokalemia stimulates and hyperkalemia inhibits H^+ secretion in the proximal tubule.

- **Acid-Base Disorders**
  - **Metabolic acidosis**
  - Decrease in plasma HCO_3^- (e.g, by losing it, like in diarrhea (most common cause) or deep (pancreatic vomiting), consuming it, like in ketoacidosis, or renal inability to form it), which results in decreased filtration of HCO_3^-
  - Partial compensations by increased ventilation rate, which reduces Pco_2, and thus, H^+ formation; renal compensation by adding new HCO_3^- by the mechanism discussed earlier.

- **Respiratory acidosis**
  - Elevated extracellular fluid Pco_2, which stimulates H+ formation.
  - Associated with abnormalities that damage the respiratory centers or that decrease the ability of the lungs to eliminate CO2. For example, damage to the respiratory center in the medulla oblongata can lead to respiratory acidosis. Also, obstruction of the passageways of the respiratory tract, pneumonia, emphysema, or decreased pulmonary membrane surface area, as well as any factor that interferes with the exchange of gases between the blood and the alveolar air, can cause respiratory acidosis.
  - Compensation by addition of new HCO_3^- by the kidneys. The increase in HCO_3^- helps offset the increase in Pco2, thereby returning the plasma pH toward normal.

- **Respiratory alkalosis:**
  - Decrease in plasma Pco_2 caused by hyperventilation leads to a decrease in the rate of H^+ secretion. The decrease in H^+ secretion reduces the amount of H^+ in the renal tubular fluid.
  - Can be caused by severe hyperventilation (e.g., as a result of head trauma) or living at high altitudes. These conditions increase elimination of CO_2.
  - Compensation by increased excretion of HCO_3^-, which does not find available H^+ to react with in the tubule.

- **Metabolic alkalosis**:
  - Elevation in the extracellular HCO_3^- concentration.
  - Diuretics (except carbonic anhydrase inhibitors) increase H^+ excretion. Also, the loss of gastric acids and the administration of $NaHCO_3$ all lead to metabolic alkalosis.
  - Compensation by reduction in the respiration rate, which increases Pco_2; excess HCO_3^- excreted due to the unavailability of H^+ to react with in the tubule.

- **Mixed (complex) respiratory and metabolic disorder:**
  - In metabolic acidosis (decreased HCO_3^-), Pco_2 decreases by 1.2 mm Hg for every 1 mEq decrease in HCO_3^- concentration. If this decrease in a patient is higher than 1.2, then the patient develops respiratory acidosis. Conversely, if the decrease is lower than 1.2, the metabolic alkalosis is not sufficiently compensated for.
  - However, in metabolic alkalosis, Pco_2 increases by 0.7 mm Hg for every 1 mEq increase in HCO_3^- concentration. This is because the decreased Po_2, resulting from hypoventilation, itself induces the respiratory center to increase the ventilation rate back to normal (i.e., opposes the compensatory hypoventilation). In contrast, increased Po_2 accompanying hyperventilation does not inhibit the respiratory center.

- In respiratory acidosis and alkalosis, on the other hand, the compensation by the kidneys takes longer time to take place; for example,

- For every 10 mm Hg increase in Pco_2, HCO_3^- concentration increases by only 1 mEq/L in case of acute respiratory acidosis and 3.5 mEq/L in case of chronic respiratory acidosis.
- For every 10 mm Hg decrease in Pco_2, HCO_3^- concentration decreases by 2 mEq/L in case of acute respiratory alkalosis and 5 mEq/L in case of chronic respiratory alkalosis.

- **Anion Gap**
  - The concentrations of anions and cations in plasma must be equal to maintain electrical neutrality. Therefore, there is no real "anion gap" in the plasma. The cation measured is Na^+, and the anions are Cl^- and HCO_3^-.
  - Thus, $Cl^- + HCO_3^- +$ unmeasured anions = $Na^+ +$ unmeasured cations,
    rearranging the equation yields:
    unmeasured anions – unmeasured cations = anion gap = $Na^+ - Cl^- - HCO_3^-$. Therefore
  - The anion gap is the difference between unmeasured anions and unmeasured cations and is estimated as $[Na^+] – [HCO_3^-] – [Cl^-]$. Thus normal anion gap = 142 – 24 – 108 =10 mEq/L.
  - Ranges between 8 and 16 mEq/L.
  - Used mainly in diagnosing different causes of metabolic acidosis.
  - In metabolic acidosis, the plasma HCO_3^- is reduced. If the plasma sodium concentration is unchanged, the concentration of anions (either Cl^- or an unmeasured anion) must increase to maintain electroneutrality.

Increased Anion Gap (Normochloremia)	Normal Anion Gap (Hyperchloremia)
Diabetes mellitus (ketoacidosis)	Diarrhea
Lactic acidosis	Renal tubular acidosis
Chronic renal failure	Carbonic anhydrase inhibitors
Aspirin (acetylsalicylic acid) poisoning	Addison's disease
Methanol poisoning	
Ethylene glycol poisoning	
Starvation	

  - If plasma Cl^- increases in proportion to the fall in plasma HCO_3^-, the anion gap will remain normal. This is often referred to as hyperchloremic metabolic acidosis.
  - If the decrease in plasma HCO_3^- is not accompanied by increased Cl^-, there must be increased levels of unmeasured anions and therefore an increase in the calculated anion gap.

Disorder	$CO_2 + H_2O$	↔	H^+	HCO_3^-	Respiratory Compensation	Renal Compensation
Metabolic acidosis	↓ (respiratory compensation)		↑	**↓**	Hyperventilation	
Metabolic alkalosis	↑ (respiratory compensation)		↓	**↑**	Hypoventilation	
Respiratory acidosis	**↑**		↑	↑	None	↑ H^+ excretion ↑ HCO_3^- reabsorption
Respiratory alkalosis	**↓**		↓	↓	None	↓ H^+ excretion ↓ HCO_3^- reabsorption

Heavy arrows indicate *primary* disturbance.

- **Acute renal failure**
  - **Stages:**
    - **Risk**:
      - GFR decreases by more than 25% of the normal value.
      - Serum creatinine increase 1.5 times
      - Urine output less than 0.5 ml/kg/h for 6 hours.
    - **Injury**:
      - GFR decreases by more than 50% of the normal value.
      - Serum creatinine increase 2 times
      - Urine output less than 0.5 ml/kg/h for 12 hours.
    - **Failure:**
      - GFR decreases by more than 75% of the normal value.
      - Serum creatinine increase 3 times
      - Urine output less than 0.3 ml/kg/h for 24 hours.
    - **Loss:**
      - Persistent renal injury.
      - Complete loss of kidneys function for more than 4 weeks.
    - **End-stage renal failure**
      - Need for renal replacement therapy for more than 3 months.

  - **Causes:**
    - **Pre-renal:** those that decrease effective blood flow to the kidneys (e.g., MI, HF, bleeding, Conn's disease and hypotension). Without treatment, these progress into intrarenal acute injury with worse prognosis.
      - In pre-renal kidney injury, GFR decreases, and urea reabsorption increases; that is, urea to creatinine ratio rises. In contrast, in case of an intrarenal injury, this ratio decreases since the kidneys ability to reabsorb filtered substances, especially urea, is decreased.

- **Intrarenal:** those that cause a direct damage to the renal tissue (e.g., nephritis, nephrotoxic drugs, such as NSAIDs and some antibiotics). Kidney diseases can be cortical (glomerular), in which the filtration process is impaired, or medullary (tubular), in which the impairment involves reabsorption or secretion.
- **Post-renal:** those that obstruct urine flow (e.g., kidneys stones).

- **Indicators used to diagnose acute renal failure:**
  - Elevated plasma urea (normal range is 14-40 mg/dl). Since plasma urea level is affected by protein intake, creatinine level is more a sensitive test.
  - Elevated plasma creatinine (normal range is 0.6-1.4 mg/dl).
  - Oliguria (urine output below 300 ml/day.m^2) or anuria (urine output below 100 ml/day.m^2). *However, urine output may be normal, or even elevated, if the cause of the failure is intrarenal but the urine would be isosthenuric in this case, indicating inability of the kidneys neither to concentrate nor to dilute the urine.*

- **Recovery:**
  - The last kidney function to be regained after acute renal failure is concentrating urine.
  - This is because normal urine osmolarity requires intact hypothalamic function, posterior pituitary function, ascending limb function, collecting ducts response to ADH and hyperosmolar renal medullary interstitium.
  - To test urine concentrating ability, the patient is asked not to drink water at night. Then, urine samples are taken in the next morning at 8:00 AM, 8:30 AM and 9:00 AM. If the osmolarity of any of the samples is higher than 1000 mOsm/L, then the patient's kidneys functions are regained.