Renal Physiology

Functions

1. Regulation of Water & Electrolyte Balance
2. Excretion of Metabolic Waste Products
3. Excretion of Foreign Chemicals & Drugs
4. Regulation of Arterial Blood Pressure
5. Regulation of Erythropoiesis
6. Regulation of Vitamin D
7. Gluconeogenesis

Structure

- the kidneys are paired organs lying in the retroperitoneal space
- medial border = ***hilum*** → renal pelvis and ureter
- the renal pelvis subdivides into major ***calyxes***, then minor calyxes projecting to a renal ***pyramid***
- divided into outer ***cortex*** and inner ***medulla***
- medulla inner and outer zones are due to the boundary of thin segments (below)
- the cortex is granular, due to ~ 1-1.2 million ***nephrons*** per kidney
- the ***glomerulus*** is composed of capillary loops invaginated in Bowman's capsule
- glomerulus also contains ***mesangial cells*** in a central glomerular tuft
- these contain myofilaments and may contract altering GFR
- Bowman's capsule joins the proximal tubule, a single layer of epithelial cells lying on a basement membrane (BM)

- ***Filtration Barrier***

1. capillary endothelium - fenestrated
2. basement membrane - homogeneous glycoprotein/mucopolysaccharide
3. capsular epithelial cells, podocytes & foot processes

- the foot processes are covered with ***glycosialoproteins*** which partially occlude the slits
- these are ***negatively*** charged, hence present a charge-selective barrier
- there are also extremely thin diaphragms which bridge the slits at the BM

- ***Tubules***

1. proximal tubule (PT) * histologically 3 segments
   i. pars convoluta
   ii. pars recta
2. loop of Henle - 3 parts
   i. descending thin limb
   ii. ascending thin limb
   iii. ascending thick limb
3. macula densa - passing between arterioles of own glomerulus
4. distal tubule (DT) - also termed the late distal tubule
   i. distal convoluted tubule
   ii. connecting segment
   iii. initial collecting tubule

- ***Collecting System***

1. cortical collecting tubule
2. medullary collecting tubule
3. papillary collecting ducts

• these drain into a ***calyx*** of the renal pelvis, then to the ureter & bladder

- ***Blood Supply***

1. renal artery
2. interlobar arteries
3. arcuate arteries
4. interlobular arteries
5. afferent arterioles - parallel series from each interlobular artery
6. glomerular capillaries
7. efferent arterioles
8. peritubular capillaries - nonspecific

NB: interlobular, arcuate, interlobar and renal veins parallel arteries

- ***Regional Differences***

• nephrons are not homogeneous and vary according to the location of the glomerulus in the cortex,

a. superficial cortical nephrons - all ***short looped***

b. midcortical nephrons - mixture

c. juxtamedullary nephrons - all ***long looped***

• the efferent arterioles of the ***juxtamedullary glomeruli*** are long and form vascular bundles extending and branching into the medulla
• the descending ***vasa recta*** extend to the inner medulla, networking and forming a ***countercurrent exchanger*** with the ascending vasa recta
• the interstitial cells increase in size and number from cortex to papilla

Juxtaglomerular Apparatus

Def'n: = an aggregation of three cell types,

1. ***granular cells***
   - differentiated smooth muscle cells of ***afferent*** arterioles
   - secretory vesicles contain ***renin***
2. extraglomerular ***mesangial cells*** - continuous with the mesangium
3. ***macula densa cells***
   - terminal part of thick ascending limb LOH
   - probably control both renin secretion and GFR

NB: JGA has a rich ***sympathetic*** nerve supply

Intrarenal Hormone Synthesis

a. angiotensin II - intrarenally generated

b. prostaglandins - esp. PGE_2 and prostacyclin

c. kinins - lysyl bradykinin, bradykinin

d. erythropoeitin

e. Vit.D - hormone not a vitamin
- not synthesized but biotransformed

GLOMERULAR FILTRATION

• average values for mythical 70 kg male

a. GFR ~ 180 l/d ~ **125 ml/min**
   • this represents ~ 20% of plasma entering the glomerular capillaries

b. total plasma volume ~ 3.0 l → filtered ~ 60 times/day

c. urine production ~ 1-2 l/d → ~ 99% of filtrate is reabsorbed

Composition of Filtrate

• the filtration barrier is freely permeable to water and crystalloids, MW ≤ 30,000
• however, is virtually impermeable to colloids
• small quantities, mainly of albumin escape → ~ 50 mg/l
• as proteins are not filtered, the filtrate electrolyte concentrations vary slightly from plasma water due to the ***Donnan effect***
• further, any crystalloid protein bound is only partially filterable
• small proteins are filterable, eg. myoglobin, Hb, light chains etc
• there is no hindrance to molecules of MW < 7,000, (~ 4-8 µm)

Filtration Barrier

1. endothelial fenestrae
2. basement membrane
3. slit diaphragms, and
4. slits

• the entire path is ***extracellular***
• the greatest restriction is at the ***basement membrane***, through the hydrated spaces between glycoprotein chains
• some high MW molecules traverse the basement membrane and lodge in the slit diaphragms
• these are possibly taken-up by ***endocytosis*** and broken down
• in addition to steric hindrance, ***electric charge*** is important
• the cell coats of the endothelium, basement membrane, and cell coats of the podocytes are all ***polyanions*** → at any MW, ***anions*** are selectively restricted

• this is not critical for plasma crystalloids
• the "leaky" glomeruli of certain disease states results from loss of the polyanion coat

Net Filtration Pressure

Def'n: = algebraic sum of opposing ***hydraulic*** and ***osmotic*** forces (Starling)

$$NFP = (P_{GC} + \pi_{BC}) - (P_{BC} + \pi_{GC})$$

NB: as there is virtually no protein, $\pi_{BC} \sim 0$, therefore

$$NFP \sim P_{GC} - (P_{BC} + \pi_{GC})$$

- both hydraulic and oncotic pressures change along the length of the glomerular capillary

Arteriolar		Afferent	Efferent
P_{GC}	mmHg	60	58
P_{BC}	mmHg	15	15
π_{GC}	mmHg	**21**	**33**
NFP	mmHg	**24**	**10**

- the above figures are for dogs but are probably similar for man
- in all mammals other than the dog, $P_{GC} \leq 60$ mmHg and the NFP may reach ***filtration pressure equilibrium***
- this likely to occur if the GC hydrostatic pressure is low, eg. hypovolaemia
- increases in π_{GC} are greater than predicted from the filtration fraction due to the non-linear osmotic activity of albumin
- GFR is also dependent on ***hydraulic*** $\mathbf{H_2O}$ ***permeability*** and ***surface area***

GFR = hydraulic permeability x SA x NFP

- the first two combined to give the ***filtration coefficient*** ($\mathbf{K_F}$)

GFR = K_F x NFP

- as the NFP ~ 10-24 mmHg and GFR = 180 l/d, so K_F is very high

K_F = GFR / NFP = 125 ml/min / 17mmHg
= 7.4 ml/min/mmHg

- this is due in part to the large SA, but mainly to the high permeability, which is 10-100 times greater than non-renal capillaries

Direct Determinants of GFR		
$GFR = K_F \times (P_{GC} - P_{BC} - \pi_{GC})$		
K_F [1]	• contraction/relaxation of mesangial cells alters SA & K_F → proportional changes in GFR	
P_{GC}	• increase renal a. pressure • decrease afferent aa. resistance • increase efferent aa. resistance	increase GFR
P_{BC}	• increase intratubular pressure	decrease GFR
π_{GC}	• increase plasma oncotic pressure → sets initial π • decrease total renal plasma flow → determines rate of rise of π	decrease GFR

[1] effects of changes in K_F may be greatly reduced where NFP reaches ***filtration pressure equilibrium***, as GFR results from only a part of available SA anyway

Tubular Reabsorption

- qualitatively a different process from GF, the later being ***bulk flow***
- relatively little bulk flow occurs across the tubular epithelium due to,

1. small filtration pressures, and
2. low hydraulic permeability

Transport Mechanisms

1. ***simple diffusion*** SD
   - the result of an appropriate ***electrochemical gradient*** = **∂[EC]**
   - as this occurs through cell membranes
   - lipid solubility is a major determinant
2. ***simple facilitated diffusion*** SFD
   - this also down an EC gradient but is dependent upon interactions with specific membrane proteins, either channels or carriers
   - most important for non-lipid soluble molecules
   - displays the characteristics of specificity, saturability and competition
3. ***secondary active transport*** SAT
   - two or more molecules interact with specific membrane proteins and are translocated across the cell membrane
   - the substance undergoing SAT is transported against its EC gradient
   - the energy for this is derived from the simultaneous movement "downhill" of the other molecule (usually Na^+ in the PT)
   - may represent either co-transport or countertransport, depending on the direction of movement of the respective moieties
4. ***primary active transport*** PAT
   - transport via interaction with specific membrane proteins but requiring energy from membrane-bound ATP'ase which forms part of carrier protein
   - this also displays the characteristics of specificity, saturability and competition
5. ***endocytosis***
   - important for uptake of macromolecules
   - requires ATP for the splitting-off of the membrane, therefore technically is a form of PAT

▪ ***Mechanisms in Reabsorption***

• except for a few substances which follow simple diffusion through the tight junctions between cells, all other reabsorbed substances must cross two plasma membranes,

a. the luminal (or apical) membrane, and

b. the basolateral membrane

• the basement membrane must also be traversed but this is functionally ***inert***
• ***bulk flow*** or simple diffusion into the peritubular capillaries is the final common step for all reabsorbed substances
• intracellular $[Na^+]_i$ is maintained low by the basal Na^+/K^+-ATPase, Na^+ being reabsorbed from the lumen by simple facilitated diffusion
• the continued net movement of Na^+ depends entirely on ***basolateral transport***
• a large number of solutes are transported with Na^+ via the following mechanisms,

a. Na^+ entry *per se* by SFD

b. cotransport with glucose or organic acids = SAT

c. countertransport with intracellular H^+ = SAT

d. cotransport with Cl^- = SAT

e. Na^+ following Cl^- diffusing through tight junctions

NB: for glucose, amino acids, phosphate etc., transport is a secondary active process, the energy being derived from the basal Na^+/K^+-ATPase

▪ ***Transport Maximum***

• where reabsorption requires specific membrane proteins, these display ***saturability***

→ the maximal tubular transport capacity $\mathbf{T_{Max}}$

• for glucose, T_{Max}

a. male ~ 375 mg/min

b. female ~ 300 mg/min

• the former being equivalent to a $[Gluc]_{pl}$ ~ 180-200 mg/100 ml (**10.0 - 11.1 mmol/l**)

• glucose, or any similar solute, is excreted before the T_{Max} is reached → ***splay***
• this is due to,

a. system kinetics are the same as enzyme systems
   - ie. maximal activity is substrate-dependent & T_{Max} is not seen until the [Gluc] > T_{Max}

b. the nephron population is non-homogeneous

Tubular Secretion

• as for reabsorption, the initial step is simple diffusion/bulk flow from the peritubular capillaries to the interstitial fluid
• from there, solute gains entry to the tubule either by SD through tight junctions, or by active transport through the cells
• in contrast to glomerular filtration, SD from capillaries is an equilibrium process
• therefore, ***protein-bound*** substances (inc. drugs) may undergo secretion
• removal from proteins being by ***mass action***
• there are several ***low specificity*** systems for organic anions and cations
• these are analogous to the reabsorptive systems being,

a. active - ie. T_{Max} limited
b. competitive

Bidirectional Transport

• rarely, if ever, does purely unidirectional movement occur, the net transport of solute being the sum of opposing fluxes
• the epithelium is not completely impermeable to crystalloids, therefore bidirectional diffusion always occurs
• active reabsorptive processes create a concentration gradient for passive secretion
• active secretory processes create concentration gradient for passive reabsorption

NB: since ***back-diffusion*** is solely the result of the gradient created by the active pumping, the net flux is always in the active direction

• the "leak" component of epithelial "pump-leak" systems is the major determinant of maximal δ[EC] achievable
• for most mineral ions & organic molecules the major path of "leak" is paracellular, not transcellular, depending on the characteristics of the junctional complexes,

1. PT → "leaky"
   - high water permeability
   - actively transports large quantities of solute
   - low maximal δ[EC]
2. DT & CT → "tight"
   - low water permeability
   - slow rates of transport of solute
   - high maximal δ[EC]

Tubular Metabolism

• traditionally glomerular filtration, tubular reabsorption and tubular secretion are considered as the basic renal processes, however, metabolism is also important for many substances
• eg. synthesis of HCO_3^- and ammonia from glutamine

RENAL CLEARANCE

Def'n: the ***clearance*** of a substance is the ***volume*** of plasma from which the substance was completely cleared by the kidneys ***per unit time***, (units = vol. plasma/time), where,

$$C_X = \frac{U_X \times V}{P_X}$$

NB: clearance of "x", C_X = ***GFR*** when the substance "x" meets the following criteria,

i. freely filterable at the glomerulus
ii. not reabsorbed by tubules
iii. not secreted by tubules
iv. not synthesised by tubules
v. not broken-down by tubules

• these are met by the polysaccharide ***inulin***, so C_{IN} = GFR
• however, inulin does not occur naturally in the body and requires several hours of infusion to reach steady state concentration
• therefore, ***creatinine*** is used to ***estimate*** GFR

• ***creatinine*** is formed from muscle ***creatine*** and is released at approximately a constant rate, therefore blood [Cr] changes little per 24 hrs
• however, Cr is ***secreted*** by the tubules and ***overestimates*** GFR by small amount
• for freely filterable substances, when,

a. $C_X < C_{IN}$ → net tubular reabsorption
b. $C_X > C_{IN}$ → net tubular secretion

• if "x" is significantly protein-bound, in order to compare C_X with C_{IN}, the freely filterable fraction of "x" must be used, not the total $[x]_{pl}$

• ***para-aminohippurate*** PAH, undergoes tubular secretion and is freely filterable
• at low $[PAH]_{pl}$, virtually all PAH escaping filtration is secreted by the tubules, therefore all plasma supplying nephrons is cleared of PAH
• about 10-15% of total renal plasma flow (TRPF) supplies non-secreting portions of the kidney
• thus, C_{PAH} actually measures ***effective renal plasma flow*** (ERPF) and this is ~ 85-90% of TRPF

NB: this only applies at a low $[PAH]_{pl}$, at higher levels the T_{Max} is exceeded

• the radiographic contrast ***Diodrast*** is handled similarly to PAH

• ***urea*** is freely filterable but ~ 50% is reabsorbed (R: 40-60%)
• therefore is less accurate than creatinine as an estimate of GFR

Fractional Excretion

Def'n: fractional excretion, FE_X, is the mass of "x" excreted as a fraction of the total mass filtered, where

$$FE_X = \frac{U_X \times V}{GFR \times P_X}$$

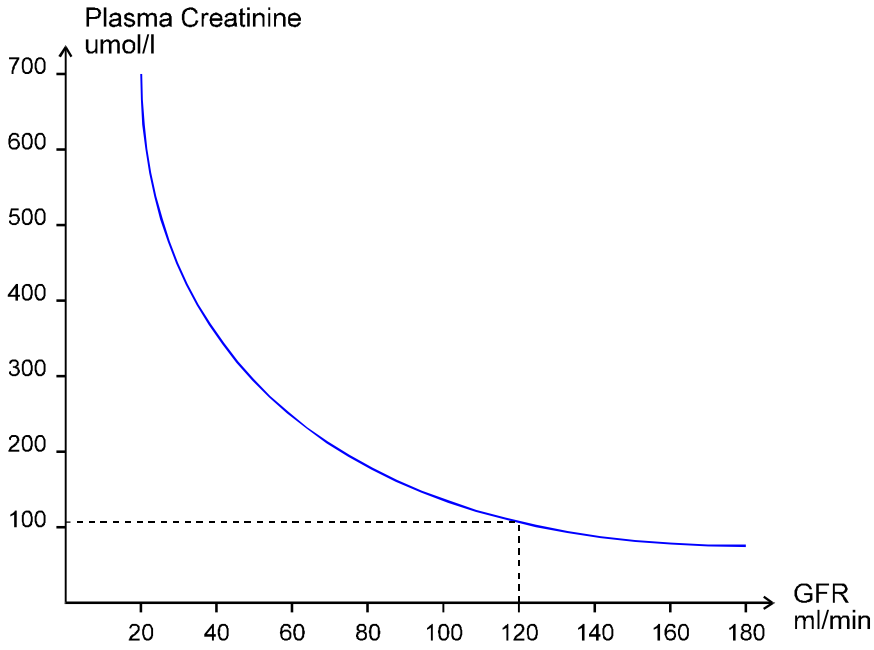
- and if,

1. $FE_X < 1.0$ → net tubular reabsorption
2. $FE_X > 1.0$ → net tubular secretion

- also, when C_{IN} is used to calculate GFR, FE_X becomes C_X/C_{IN}

$$FE_X = \frac{U_X \times V/P_X}{U_{IN} \times V/P_{IN}}$$

- although ***creatinine clearance*** is a good estimate of GFR, the ***plasma creatinine*** is often used as a clinical indicator of GFR
- as most excreted Cr gains entry to tubule by filtration and a negligible amount is secreted, there is an ***inverse*** relationship between $[Cr]_{pl}$ and GFR



- this is not completely accurate because,

a. some Cr is secreted by the tubules
b. $[Cr]_{pl}$ at normal GFR varies between individuals
c. Cr production may not remain constant

- ***urea*** also serves as indicator but is less accurate due to variable reabsorption

Handling of Organic Substances

▪ ***Reabsorption of Organic Nutrients***

• these include,

i. glucose
ii. amino-acids
iii. Krebs cycle intermediates
iv. water soluble vitamins
v. lactate, acetate, β-hydroxybutyrate, etc.

• main site is the ***proximal tubule***
• they share the following characteristics,

a. reabsorption is an ***active*** process against a δ[EC]
b. the "uphill" step is across the ***luminal membrane***, usually via cotransport with Na^+
c. they cross the basolateral membrane by simple facilitated diffusion
d. the manifest T_{Max}'s which are well above the amounts normally filtered
e. manifest ***specificity***
   • ie. there are a large number of different carriers, though, closely related solutes may share carriers, (eg. AA's), allowing for ***competition***
f. transport is inhibited by various drugs and disease processes

▪ ***Proteins and Peptides***

• the main site is the ***proximal tubule***, but the mechanisms are significantly different
• the amounts filtered are very small, ~ 0.02% of $[Alb]_{pl}$ → 10 mg/l in urine
• a normal GFR → loss of 1.8 g/day in the absence of reabsorption (normal loss ≤ 100 mg/d)
• the reabsorptive process is easily saturated when the filtered load is increased in disease states
• the initial step is attachment to specific ***binding proteins*** on the luminal membrane, followed by ***endocytosis***
• endocytosed vesicles merge with lysosomes and proteins are broken-down to low MW fragments and AA's
• the later are released from the basolateral membrane to the interstitium
• many other plasma proteins, though their plasma concentrations are lower, are filtered to a far greater degree due to a lower MW, eg. GH (MW ~ 20,000) is 60% filterable
• relatively large fractions of these smaller proteins are filtered and degraded in tubular cells

→ the kidneys are a major site of ***protein catabolism***

• even smaller, linear polypeptides such as angiotensin are completely filterable and are catabolised into AA's within the tubular lumen by ***peptidases*** on the luminal membrane
• fragments and AA's are then reabsorbed

Urea

• highly permeable and crosses most biological membranes with ease
• freely filterable so $[urea]_{BC} \sim [urea]_{pl}$
• as water and solute are reabsorbed in the PT, so the luminal [urea] increases
• this creates a concentration gradient and urea diffuses from the lumen to the interstitium and peritubular capillaries

NB: this is a ***passive*** process totally driven by the reabsorption of water

• urea is ***actively secreted*** into the straight PT and loop, so that the [urea] at the start of the DT is increased to ~ 2x the initial $[urea]_{BC}$
• the source of this secreted urea is reabsorption from the collecting ducts, not the peritubular capillaries
• urea continues to be reabsorbed in the DT and cortical collecting tubule but only small amounts due to the low permeability $\rightarrow$ further increased [urea]
• most of the urea entering DT reaches the medullary CTs which, because of their high permeability to urea and extensive water reabsorption, reabsorb large amounts of urea, which then enters interstitium
• this is the source of the urea entering the loops above

• the net overall handling of urea $\rightarrow$ ***reabsorption* ~ 60%**

a. ~ 50% due to PT reabsorption
b. ~ 10% by the remainder of the nephron during cyclic travel

• this figure decreases to ~ 40% when urine flow is high, ie. water reabsorption is low
• the reabsorption range is ***constant*** irrespective of $[urea]_{pl}$, ie. there is no effective T_{Max}

Proximal Secretion of Organic Anions

• the PT secretes large numbers both exogenous and endogenous anions
• many are freely filterable, others are extensively protein-bound
• for the later, ***PT secretion*** constitutes the only significant path for excretion
• active PT secretory path(s) exhibit ***low specificity***, therefore are able to eliminate a wide range of anions
• the PT handles ***glucuronate*** and ***sulphate conjugates*** from liver metabolism
• the mechanism for secretion of PAH = active transport into the PT cell then facilitated diffusion at the luminal membrane

NB: not known if basal entry is primary active transport or secondary process

• as many ions share the same carriers there is ***competition*** for transport
• transport is also saturable with respective T_{Max}'s for anions
• most anions, cf. PAH, undergo ***no*** net reabsorption anywhere along the nephron

• a few select anions undergo active tubular absorption distal to the PT, eg. urate
• plasma ***urate*** is mainly ionised, therefore not protein bound and is freely filterable
• it undergoes both active tubular secretion and reabsorption, both in the PT
• usually tubular secretion < reabsorption so the net effect → ***reabsorption***
• the ***secretory*** rate is regulated to maintain a normal plasma urate
• most subjects with a high $[urate]_{pl}$ excrete less for any given plasma urate
• elevation is rarely caused by decreased GFR or excessive reabsorption

■ ***Proximal Secretion of Organic Cations***

• the system is analogous to that for anions,

a. relatively non-specific
b. exhibit T_{Max}'s
c. exhibit competitive inhibition

• these systems are essential for the excretion of cations extensively bound to plasma proteins and not filterable at the glomerulus

■ ***Passive Diffusion of Weak Organic Acids & Bases***

• many organic ions are ionised forms of weak acids and bases
• the non-ionised fraction undergoes passive diffusion along a δ[EC], the major determinants for which are the ***luminal pH*** and the pK'_A of the acid/base

1. acidification of the lumen, by mass action
   → reabsorption of weak acids & secretion of weak bases
2. alkalinisation of the lumen, by mass action
   → reabsorption of weak bases & secretion of weak acids

NB: as the major $\delta[H^+]$ occurs in ***distal tubule*** segments, these are the main site for pH-dependent passive diffusion

■ ***In Summary***

• the excretion of a weak acid or base reflects the amount,

a.	filtered at the glomerulus	- GFR - filterable fraction
b.	secreted actively in the PT's	- [plasma] - T_{Max}
c.	which diffuses passively	- urine flow - luminal pH - pK_a' for acid/base

RENAL HAEMODYNAMICS

1. total renal blood flow, ***TRBF*** **~ 1100 ml/min**
   ~ 20-25% of CO (5000 ml/min)
   ~ 90% to cortex
2. total renal plasma flow, ***TRPF*** **~ 605 ml/min** (Hct ~ 0.45)
3. the ***filtration fraction*** **~ 20%** (GFR = 125 / 605 ml/min)

• the low medullary flow is due to high resistance of the ***vasa recta***
• low flow is required to maintain the medullary concentration gradient
• kidney mass ~ 1% of total body weight, ∴ total flow is well in excess of metabolic needs

Autoregulation and Mean Arterial BP

• renal blood flow is virtually constant over the BP range 80-180 mmHg due to autoregulation
• this is the case for both RBF and GFR
• the ***afferent arterioles*** are the major site of regulation of renal vascular resistance
• effectively blunting significant changes in glomerular capillary pressure
• these effects are seen in both isolated and transplanted kidneys, therefore the mechanisms are totally ***intrarenal***, factors including

1. myogenic mechanism
2. tubulo-glomerular feedback
3. sympathetic control
4. angiotensin II
5. intrarenal baroreceptors
6. macula densa
7. renal SNS
8. intrarenal prostaglandins

▪ ***Myogenic Mechanism***

• smooth muscle contraction in response to stretch/tension
• one possible mechanism is distortion of the plasma membrane and opening of Ca^{++} channels with a subsequent increase in $[Ca^{++}]_i$

▪ ***Tubulo-Glomerular Feedback***

• primarily regulates ***GFR***, changes in RBF being secondary
• increased renal artery pressure →

1. increased P_{GC}
2. increased NFP & GFR
3. increased flow in PT, loop & ***macula densa***

• cells of the ***macula densa*** detect the increased rate of flow, possibly in response to increased luminal $[Na^+]$, $[Cl^-]$, or osmolality
• these cells release several vasoconstrictor mediators

a. angiotensin II | permissive effects
b. prostaglandins |
c. ***adenosine*** → afferent arteriolar ***constriction***

• regulation of GFR prevents large changes in solute & water excretion with changes in arterial BP
• autoregulation may also blunt the effects of defective solute reabsorption in the PT, whereby GFR may be reduced below normal to compensate (ie. loop diuretics)

NB:
1. autoregulation is not perfect, changes of renal artery pressure do cause changes in RBF and GFR, these are merely limited
2. autoregulation is virtually absent at mean arterial pressures **< 70 mmHg**
3. despite autoregulation, RBF & GFR can be significantly altered by the sympathetic nervous system and the renin-angiotensin system

▪ ***Sympathetic Control***

• there is a rich SNS supply to kidney, afferent > efferent aa.
• SNS tone is reflexly mediated by aortic and carotid sinus ***baroreceptors***

a. a***-receptors*** → vasoconstriction
b. β-receptors are present but these are relatively inert, such that adrenaline causes only vasoconstriction

• as both afferent & efferent aa. are innervated, ↑ SNS activity causes a greater reduction in RBF than GFR, efferent tone raises P_{GC} but adds series resistance to the total renal vascular resistance
• ↑ SNS tone immediately raises BP, but also decreases Na^+ & H_2O excretion via GFR and increases tubular reabsorption of Na^+ & H_2O
• this ↑ SNS tone can also be mediated by ***venous baroreceptors*** in the atria and great veins,

NB: this response is actually > arterial receptors

• other reflexes can also increase SNS outflow,

a. peripheral chemoreceptors - hypoxia
b. CNS - exercise, emotional, hypercarbia

- ***Angiotensin II***

• powerful constrictor of both afferent & efferent aa. (some dispute afferent)
• decreases RBF > GFR→ - ***GFR/RBF ratio*** as does the SNS
• causes contraction of ***mesangial cells***, therefore reduces K_F & GFR
• serum concentration is directly determined by renal ***renin*** release, which in turn is under the control of four mechanisms (see below)
• the final common pathway is ***decreased*** **[Ca^{++}]** in the cytosol stimulating renin release

- ***Intrarenal Baroreceptors***

• renin secreting granular cells act as baroreceptors, measuring pressure in the late afferent aa.
• secretion is inversely related to arteriolar pressure

- ***Macula Densa***

• may be the same mechanism as tubulo-glomerular feedback (? unlikely)
• renin secretion is inversely related to ***mass*** of NaCl reaching the MD
• this may be the cellular concentration, not the luminal, but these are directly proportional

- ***Renal Sympathetic NS***

• produces both indirect and direct effects

a. indirect → afferent aa. constriction and decreased aa. pressure
→ decreased NaCl load leaving the loop & reaching the macula densa

b. direct → β_1-receptors on the granular cells

• sensitivity of the direct effect >> the indirect

- ***Angiotensin II***

• direct ***negative feedback*** to granular cells
• other inputs affecting renin release include ADH, K^+, and Ca^{++}, all of which ***decrease*** release
• renally synthesised prostaglandins, *PGE_2 & PGI_2, stimulate renin release

- ***Prostaglandins***

• in addition to indirect effects through renin release, the PG's are more important as direct vasoactive agents on renal vasculature
• PGE_2 & PGI_2 are potent ***vasodilators***, especially on the afferent aa.
• their best documented role is to dampen the effects of increased SNS tone and angiotensin II, both of which cause increased PG synthesis in addition to vasoconstriction

> ***NB:*** thus, both autoregulation and renal PG's minimise any reduction in RBF & GFR induced by changes in BP, sympathetic activity, and angiotensin II

• animals given inhibitors of PG synthesis and exposed to hypovolaemia rapidly develop severe renal damage
• another effect of PG's seen in shock is the preferential increase in ***medullary blood flow***, leading to "wash-out" of the medulla and a decreased concentrating ability
• the kidneys also produce vasoconstrictor PG's, eg. TXA_2, but no physiological role has been found
• these are increased in certain disease states, eg. obstruction and drug induced renal failure, where vasoconstriction is pathological

- ***Other Factors***

a. ***ADH*** results in vasoconstriction only when present in high concentration
b. ***dopamine*** is a renal vasodilator and there is some evidence for sympathetic dopaminergic neurones
c. intrarenally produced ***kinins*** are potent vasodilators
d. ***adenosine*** is a vasoconstrictor, as opposed to its effects in other tissues

SODIUM, CHLORIDE AND WATER

• renal mechanisms for NaCl and water cope with wide ranges of intake and excretion,

a. salt intake from 50 mg to 25 g/d

b. water excretion from 400 ml to 25 l/d

• NaCl & H_2O are freely filterable at the glomerulus, there is extensive tubular reabsorption but ***no*** tubular secretion
• Na^+ reabsorption is driven by the basolateral Na^+/K^+-ATP'ase and is responsible for the major energy expenditure in kidney

Mechanisms of Sodium Reabsorption

a. Na^+ entry per se by SFD

b. Na^+ cotransported with glucose or organic acids = SAT

c. Na^+ countertransported with intracellular H^+ = SAT

d. Na^+ cotransported with Cl^- = SAT

e. Na^+ following Cl^- diffusion through tight junctions

- ***Site of Action in Nephron***

a. Proximal Tubule
   i. pars convoluta - a, b, c
   ii. pars recta - a, e

b. Loop of Henle
   i. ascending thin limb ?
   ii. ascending thick limb - d

c. Distal Tubule
   i. distal convoluted tubule?
   ii. connecting segment - a, c, ?d
   iii. initial collecting tubule - a, c, ?d

d. Collecting Tubule - a, c, ?d

- ***Mechanisms Driving Reabsorption of Other Solutes***

NB: reabsorption of Na^+ drives the reabsorption of other solutes by,

i. creates a lumen-negative transepithelial ***potential*** difference
ii. creates an ***osmotic*** gradient favouring reabsorption of water
→ subsequent concentration of luminal solute
iii. ***cotransport*** of organic nutrients, $HPO_4^=$, Cl^- etc.
iv. ***countertransport*** of H^+ ions achieving HCO_3^- reabsorption

• the final common path for reabsorption = ***bulk flow*** from the interstitium to the ***peritubular capillaries***, in accordance with,

$$P_{Net} = (P_{Int.} + \pi_{PC}) - (P_{PC} + \pi_{Int.})$$

• P_{Net} virtually always favours the net movement ***into*** capillaries
• the P_{PC} is generally quite low, ~ 10-15 mmHg, due to passage through two resistance circuits
• the oncotic pressure, π_{PC}, is higher than plasma due to colloid concentration during passage through the glomerulus

NB: although P_{Int} constitutes a part of the driving force for the final step in reabsorption, it also represents a "back-pressure" preventing the movement of fluid from the tubular lumen, creating ***back-diffusion*** through tight junctions

NaCl Coupling

- chloride is coupled to sodium by two mechanisms,
    1. concentration gradient
        - the concentration gradient created by passive movement of water in response to Na^+ reabsorption
    2. electrical gradient δV
        - in all nephron segments, excluding the ascending thick limb LOH and the later portions of the proximal tubule, the lumen is ***negative*** c.f. interstitium
        - the lumen negative δV varies from 0 to -4 mV in the PT, to -40 to -60 mV in portions of the late distal tubule
        - the major determinant of the δV, as for the concentration gradient, is the active transport of Na^+
        - therefore, in the PT where the δV is small, the concentration gradient is the main driving force for reabsorption
        - in the DT, where δV is large, this is the main driving force for reabsorption

- movement of Cl^- across the ***basolateral membrane*** is by simple facilitated diffusion
- this occurs due to two factors,
    1. $gCl^-_{BL} > gCl^-_L$
    2. $\delta V_{BL} > \delta V_L$

▪ ***Proximal Tubule***

- reabsorbs **~ 65%** of filtered sodium and water, plus organic nutrients etc.
- the PT is ***highly permeable*** to water
- the maximum osmotic gradient ~ 1 mosmol/l, water reabsorption keeping pace with solute
- therefore, the $[Na^+]$ remains virtually constant through the PT, whereas the ***mass*** of Na^+ is reduced by 65%
- late in the PT, some Na^+ is also reabsorbed by ***simple diffusion*** and ***solvent drag***
- early in the PT, HCO_3^- is the major anion reabsorbed with Na^+
- this is mainly by countertransport with H^+
- Cl^- initially lags behind and the concentration gradient is established by water reabsorption,

    $\rightarrow$ ~ 90% of HCO_3^- is reabsorbed in the PT

- accordingly, in the middle and late PT, Cl^- is the major anion with Na^+
- the passive reabsorption of Cl^- down its δ[EC] in the PT may be great enough to establish a (+)'ve δV gradient across the late PT
- this gradient accounts for the driving force for passive Na^+ reabsorption in this segment
- at the end of the PT,
    1. luminal osmolality ~ plasma osmolality
    2. the concentration of Cl^- is higher
    3. the concentration of HCO_3^- is lower

• ***osmotic diuretics*** are substances which are freely filterable at the glomerulus but undergo no tubular reabsorption
• therefore, by adding solute to the PT they retard the passive reabsorption of water, thus causing the late PT [Na^+] to fall
• occurring simultaneously with the active reabsorption of Na^+, there are large fluxes of Na^+ in both directions through the "leaky" junctions of the PT
• normally there is no significant net diffusional flux as there is no significant δ[EC]
• in the presence of an osmotic agent, a concentration gradient is formed favouring the passive diffusion of Na^+ into the lumen
• these diuretics, such as glucose and ketone bodies in diabetes, or mannitol, induce the excretion of large quantities of NaCl and water, in part by this proximal effect but also by effects in the loop of Henle

▪ ***Loop of Henle***

• reabsorbs a further 25% of the filtered NaCl plus 15% of filtered water
• the descending limb of Henle ***does not*** reabsorb NaCl
• the entire ascending limb of Henle does,

a. thin ALH → ? mechanism but movement of NaCl is well established
b. thick ALH → cotransport of Cl^- & Na^+ (basal Na^+/K^+-ATPase)

• drugs that inhibit transport of Cl^- in the ALH therefore also inhibit Na^+ reabsorption producing diuresis
• the ALH, unlike the PT, reabsorbs considerably more solute than water, therefore delivers ***hypotonic*** urine to the distal tubule
• the decrease [Na^+] is greater than the decrease in osmolality due to the addition of ***urea*** to lumen in the ALH

▪ ***Distal Tubule & Collecting Duct***

• NaCl reabsorption continues along the DT & CT so that the final urine contains ~ 1% of the filtered mass
• Cl^- reabsorption is mainly passive due to the δ[EC], but there is also a small component actively transported with Na^+ and possibly K^+

• H_2O permeability of the early DT, ie. distal convoluted tubule, is extremely ***low*** and ***not*** subject to physiological control
• accordingly almost no water is reabsorbed in this segment

• H_2O permeability of the late DT, ie. distal tubule and initial collecting tubule, is also extremely low, however under the influence of ***ADH*** becomes highly water permeable

• further removal of solute in the EDT presents the LDT with ***markedly hypotonic*** urine containing even less Na^+
• removal of Na^+ continues in the LDT and collecting system, so that the final urine may contain virtually no Na^+

• water reabsorption is governed by the permeability of luminal membrane under the influence of ADH,

1. high [ADH]→ mass diffusion of water
   urine is ~ iso-osmotic to the medulla
2. low [ADH] → limited diffusion of water
   large volume of dilute urine
   virtually no H_2O reabsorbed after the loop of Henle

• ADH in physiological concentration has virtually ***no effect*** on Na^+ transport
• ***ADH receptors*** on the basolateral membrane activate ***adenylate cyclase*** & cAMP within the cell which leads to increased permeability of the luminal membrane
• ? insertion or phosphorylation of membrane protein channels
• ADH exhibits local (-)'ve feedback due to the induction of medullary synthesis of ***prostaglandins***
• the later oppose the ADH induced generation of cAMP
• altered PG synthesis may therefore account for the altered tubular responsiveness seen in various disease states
• eg. in hypovolaemic shock, increases in PGE may be associated with high output renal failure
• other factors may also alter tubular responsiveness to ADH, eg. adrenal insufficiency produces a tendency to hyper-responsiveness to ADH
• in the LDT and CT passive diffusional fluxes are very low due to the relative impermeability of cell junctions → "tight" epithelium

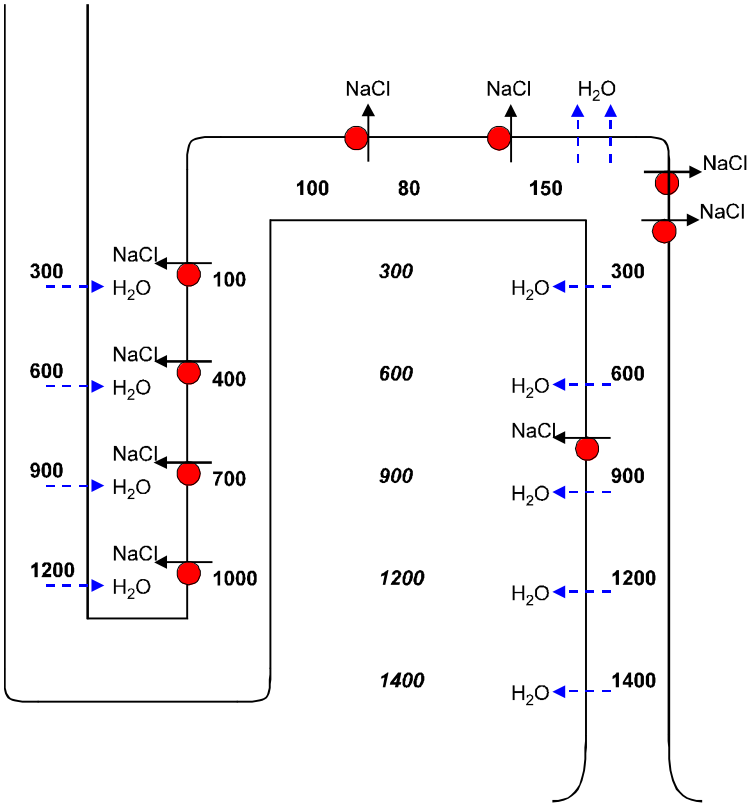
The Medullary Countercurrent System

• the maximal concentration of urine produced **~ 1200-1400 mosmol/l**
• urea, sulphate, phosphate and other waste **~ 600 mosmol/d**

→ ***obligatory water loss*** ~ (600 mosmol/d)/(1300 mosmol/l)
~ 0.46 l/d

• concentration of the renal medulla by the countercurrent multiplier effect of the ***loop of Henle*** relies on the following characteristics,

1. the ALH is not homogeneous, structurally or functionally →
   - very thin from the bend until the outer medulla where the thick limb begins
   - remains very impermeable to water over the entire length
   - thick ALH actively transports NaCl
   - relatively permeable to NaCl
     → maximum ***transluminal gradient*** **~ 200 mosmol/l**
2. the DLH (late PT not included)
   - does not actively transport Na^+ or Cl^-
   - is highly permeable to water over its entire length
   - is relatively impermeable to ions



• at each horizontal level, the medullary interstitium is concentrated by the transport of solute from the ALH
• as the DLH is freely water permeable → water passively leaves the tubule concentrating the luminal contents
• these two processes proceed at each horizontal level so that the final concentration of solute deep in the medullary interstitium is
~ 1200-1400 mosmol/l
• the gradient at each horizontal level across the ALH remains at only 200 mosmol/l, while that across the DLH is near zero
• therefore, the osmolality in the ALH is always ***less*** than that of the DLH
• the fluid leaving the thick ALH for the DT is ~ 200 mosmol/l below plasma,
ie. **~ 100 mosmol/l**

NB: the concentration gradient of 200 mosmol/l established by the ALH, is effectively multiplied by the countercurrent flow;
active ion transport in the ALH is the essential component of the entire system

• the highest concentration at the tip of the loop depends on three factors,

a. the ***length*** of the loop
b. the strength of the ***ion pump***
c. the degree of water ***impermeability*** of the ALH

• the former determines the vertical concentration gradient, the later the horizontal
• the real function of the countercurrent system is to concentrate the ***medullary interstitium***, so that under the influence of ***ADH*** the luminal contents of the medullary collecting ducts becomes iso-osmotic to 1400 mosmol/l

• the action of ADH proximal to the medullary collecting ducts, (ie. late DT and cortical CT), is essential to reduce the volume of urine presented for final concentration
• the gradient would otherwise be "washed-out" by a high medullary flow
• in steady-state, NaCl from the ALH and water from the DLH are removed from the interstitium by ***bulk flow*** into the peritubular capillaries

• ***urea*** may be involved in the movement of NaCl from the thin ALH, however is far more important in determining the maximal concentrating ability of the kidney,

→ ~ 50% of solute in the medulla is urea, not NaCl

• the [urea] rises along the DT, cortical + outer medullary CTs due to solute and water reabsorption and impermeability to urea
• the high [urea] presented to the inner medullary CT then equalises with the interstitium, raising the interstitial osmolality further
• the absence of urea would necessitate far greater transport of NaCl from the ALH to achieve 1400 mosmol/l
• ***urea***, unlike NaCl from the ALH, ***does not*** cause net movement of water, or alter the concentration of any other solute, it merely diffuses along it's own concentration gradient, thereby increasing total osmolality of interstitium

• the ***vasa recta*** form a ***countercurrent exchange*** system, having descending and ascending branches running in close proximity to the loop and each other
• solute in the descending branch becoming progressively more concentrated toward the medulla and vica-versa

NB: the concentration of vessel contents prevents excessive loss of solute from the medulla by ***diffusion***, and subsequent loss of cortical-medullary gradient, it does not prevent the movement of solute and water by ***bulk flow***

• thus, the net salt and water entering the interstitium from the loops and CT's is removed and the concentration gradient is maintained

■ ***Factors Affecting Concentrating Ability***

• decreased concentrating ability occurs early in most forms of renal disease → ***isosthenuria***
• changes in renal ***medullary blood flow*** will alter the gradient by either removing too much, or too little water ± solute
• destruction of the loops by disease or drugs
• decrease in NaCl pumping by the ALH, either due to ***tubular disease***, drugs, or a marked reduction in ***GFR*** reducing NaCl delivery to loop
• a large increase in ***flow*** through the loop, eg. an osmotic diuresis, "washes-out" the gradient preventing final concentration of urine

- ***In Summary***

1. in the PT ~ 65% of filtered NaCl and water are reabsorbed but the urine remains iso-osmotic
2. in the loop, water is reabsorbed from the DLH but a greater amount of NaCl is removed from the ALH, so that ***hypo-osmotic*** fluid enters the DT
3. the early DT is always impermeable to water, therefore further dilutes the urine, this, with the ALH are referred to as "diluting segments"
4. from the late DT, plasma ADH determines water permeability of the tubule,
   i. low [ADH]
      - little water reabsorbed, therefore these segments contribute to the production of a large volume of dilute urine

   ii. high [ADH]
      - by the end of the cortical CT's the urine is again iso-osmotic, and is further concentrated through the medulla to 1400 mosmol/l (containing ~ no NaCl)
5. although NaCl in the medullary interstitium is essential for the concentrating ability of the kidney, the final urine may contain virtually none, the excreted solute being urea, creatinine, urate, K^+, etc.
6. the excretion of large quantities of Na^+ is always accompanied by the excretion of large amounts of water, however, the excretion of large amounts of water does not necessitate the excretion of Na^+, decreased $[ADH]_{pl}$ not significantly altering Na^+ transport

REGULATION OF EXTRACELLULAR VOLUME

CONTROL OF SODIUM

NB: as Na^+ is actively pumped from cells and the intracellular $[Na^+]$ is low, so the total extracellular fluid volume depends primarily upon the mass of extracellular Na^+, which in turn correlates directly with the ***total body*** **Na^+**

• there are Na^+-sensitive receptors in the body, (adrenal cortex, macula densa and the brain), but these are less important in Na^+ regulation as these respond to the $[Na^+]$, not the total mass of Na^+ in the body
• total ECF volume is not monitored directly, its components, the ***intravascular*** and ***interstitial*** volumes are
• as Na^+ is not secreted by the tubules,

$$Na^+ \text{ excretion} = (GFR \times [Na^+]_{pl}) - Na^+ \text{ reabsorbed}$$

• although $[Na^+]_{pl}$ may alter significantly in disease states, in most physiological states it is relatively constant
• therefore, control of excretion is via two variables, ***GFR*** and ***Sodium Reabsorption***, the later being quantitatively more important

Control of GFR

Direct Determinants of GFR		
$GFR = K_F \times (P_{GC} - P_{BC} - \pi_{GC})$		
K_F[1]	• contraction/relaxation of mesangial cells alters SA & K_F → proportional changes in GFR	
P_{GC}	• ↑ renal a. pressure • ↓ afferent aa. resistance • ↑ efferent aa. resistance	↑ GFR
P_{BC}	• ↑ intratubular pressure	↓ GFR
π_{GC}	• ↑ plasma oncotic pressure → sets initial π • ↓ total renal plasma flow → determines rate of rise of π	↓ GFR

[1] effects of changes in K_F may be greatly reduced where NFP reaches ***filtration pressure equilibrium***, as GFR results from only a part of available SA anyway

- ***Regulation of Glomerular Capillary Pressure***

• because of ***autoregulation***, arterial pressure changes over the normal physiological range have only small direct effects on GFR
• ***hypovolaemia*** will, however, produce reflex changes initiated from aortic arch, carotid sinus, venous and atrial baroreceptors causing an increase in ***SNS/PNS activity*** resulting in renal arteriolar constriction, lowering P_{GC} and GFR
• ***renin*** secretion would also be stimulated increasing ***angiotensin II*** enhancing vasoconstriction
• both the amount of sodium filtered and the amount excreted would be reduced, protecting the body Na^+ mass
• hypervolaemia will produce the opposite effects
• these changes in Na^+ excretion are partially offset by ***glomerulo-tubular balance***

- ***Regulation of Plasma Protein Concentration [PP]***

• sweating or diarrhoea will lead to hypovolaemia, but will also increase plasma oncotic pressure, thereby reducing NFP and GFR
• conversely a large salt intake would increase ECF and transiently lower the [PP], thus increasing GFR
• hypovolaemia from ***haemorrhage*** produces an inappropriate situation
• a reduced vascular compartment followed by a net movement of protein free fluid from the ICF
→ a reduction in plasma oncotic pressure, tending to raise GFR
• these effects are overridden by reflex changes in SNS/PNS tone

- ***Regulation of Glomerular Filtration Coefficient K_F***

• catecholamines and angiotensin II are known to reduce K_F, either directly or via an intrarenal mediator causing contraction of mesangial cells

Control of Tubular Sodium Reabsorption

▪ *Glomerulotubular Balance GTB*

• this is one reason for the lesser importance of alterations of the filtered load of Na^+
• the absolute reabsorption of solute and water in the PTs, and probably the loops of Henle and DTs, varies directly with the GFR
• that is, the percentage of filtrate reabsorbed proximally remains at ~ 65%
• this requires no external neural or hormonal input, occurring in the ***isolated kidney***

NB: the effect is to blunt large changes in Na^+ excretion secondary to changes in GFR, however, δGFR still does affect Na^+ excretion, as,

i. the absolute quantity of Na^+ leaving the PT does alter
ii. GTB is not perfect, reabsorption ***does*** change with GFR

• therefore,

1. ***autoregulation*** prevents large changes of GFR with δBP
2. ***GTB*** prevents large changes in Na^+ excretion with δGFR

NB: 2 lines of defence against profound haemodynamic alterations of Na^+ excretion

▪ *Aldosterone*

• this is the single most important controller of Na^+ balance
• produced in the adrenal cortex, in the ***zona glomerulosa*** and stimulates Na^+ reabsorption in the late DTs and the CTs (probably not in the medulla)
• proximal to this site of action, over 90% of the filtered Na^+ has already been reabsorbed
• therefore, the total quantity of Na^+ reabsorption dependent on aldosterone is ~ 2% of the filtered load, viz.

$$\begin{aligned} 2\% \text{ of } (GFR \times [Na^+]_{pl}) &= (0.02)(180 \text{ l/d})(145 \text{ mmol/l}) \\ &= 522 \text{ mmol/d} \\ &\sim \mathbf{30 \text{ g NaCl/d}} \end{aligned}$$

• aldosterone also stimulates Na^+ transport in other epithelia,

i. sweat glands
ii. salivary glands
iii. the intestine

• similarly, the effect is to reduce the luminal $[Na^+]$
• like other ***steroids***, aldosterone combines with a ***cytosolic receptor***, migrates to nucleus, increases synthesis of specific ***mRNA*** with subsequent ***protein synthesis***
• the mode of action of this protein may involve,

i. ? activation of luminal Na^+-channels
ii. increased $[Na^+]_{ICF}$
iii. a 2° increased activity of basolateral Na^+/K^+-ATPase

- there is also a direct effect on activity of Na^+/K^+-pump which occurs over a longer time span
- this effect takes ~ 45 mins, due to the requirement for ***protein synthesis***

> ***NB:*** therefore, decreases in Na^+ excretion occurring in minutes, eg. orthostatic, are ***not*** due to aldosterone

- four direct inputs to the adrenal regulate aldosterone secretion,

1. angiotensin II → most important factor
2. ↑ plasma $[K^+]$ → stimulation
3. ↑ plasma $[Na^+]$ → inhibition
4. ACTH → permissive

- the effects of $[Na^+]$ are minor in humans, $[K^+]$ being far more important
- ACTH stimulates release when present in high concentrations, but more importantly is ***permissive*** for other factors within the physiological range
- however, ACTH secretion is not keyed to Na^+ balance
- other possible factors in release include β-endorphin, β-lipotropin and dopamine
- the former two are secreted with ACTH as products of POMC

- ***angiotensin II*** is by far the most important controller of aldosterone secretion in Na^+ balance
- accordingly, aldosterone secretion is largely determined by the release of ***renin***, which is also determined by four factors,

1. intrarenal baroreceptors → ↑
2. the macula densa → ↑
3. the renal sympathetic NS → ↑
4. angiotensin II → ↓

Factors Other Than Aldosterone Influencing Tubular Na^+ Handling

▪ ***Atrial Natriuretic Factor***

- 26 AA peptide hormone (sources range 24-28)
- synthesised from a 126 AA prohormone in atrial secretory granules
- released in response to atrial stretch/wall tension
- plasma half life, $t_{½\beta}$ ~ 3 mins
- clearance ~ 34 ml/kg/min
- maximal natriuresis is less than that seen with frusemide, however ANF is ~ 100 times as potent
- receptors are concentrated in cortical glomeruli
- the postulated second messenger is ***cGMP***
- ? there is no direct effect upon Na^+ transport, or the Na^+/K^+-ATPase
- neither amiloride nor prostaglandin inhibitors have an effect upon its actions
- ANF effects include,

a.	systemic vasodilatation	- transient hypotension IVI - predominantly venodilatation - decreases cardiac preload
b.	increases GFR:RBF ratio	- efferent vasoconstriction - afferent vasodilatation - increases filtration fraction - increases salt delivery to DT
c.	increases K_F	
d.	increases MBF:CBF ratio	
e.	decreases plasma renin	- direct & indirect
f.	decreases plasma aldosterone	- direct & indirect
g.	increases urinary excretion of	- Na^+, Cl^-, K^+ - Ca^{++}, $HPO_4^=$, Mg^{++}
h.	increases urine volume	

▪ ***Effects of Angiotensin II***

1. adrenal cortex - increased secretion of ***aldosterone***
2. kidneys
   i. arteriolar constriction decreasing GFR but increasing GRF:RPF ratio
   ii. direct tubular effect increasing Na^+ reabsorption
3. vascular smooth muscle - increased tone
4. CNS/PNS - facilitation of sympathetic activity
   - stimulates secretion of ADH
   - stimulates thirst

NB: all of which favour ***retention*** of salt and water and elevation of BP

- ***Additional Factors***

1. ***intrarenal physical factors***
   - the ***interstitial hydraulic pressure***, while favouring the final bulk flow of reabsorbed solute & water into the capillaries, also produces back-diffusion and when elevated is associated with a reduced overall level of fluid reabsorption
   - the two main factors governing this pressure are the peritubular hydraulic and oncotic pressures
   - the peritubular oncotic pressure varies directly with the filtration fraction, the ***GFR:RPF ratio***
   - this ratio increases as most mediators of renal vessel constriction affect both afferent and efferent aa.
   - these physical factors affect reabsorption only in the PT where large diffusional fluxes occur, and are probably only important in large alterations of ECF volume
2. ***distribution of RBF***
   - nephrons are not a homogeneous population, redistribution of flow to postulated "high-reabsorption" nephrons would affect Na^+ balance
3. direct tubular effects of ***catecholamines***
   - renal SNS tone and circulating adrenaline have direct action on tubular cells enhancing Na^+ reabsorption, definitely in the PT, ? others
4. direct tubular effects of ***angiotensin II***
   - same c.f. CA's, in addition to stimulation of aldosterone and its intrarenal vascular effects, has direct effect on tubular cells enhancing reabsorption
   - also like the CA's, the effect is seen in the PT but ? other segments

e. other humoral agents
   - cortisol, oestrogen, growth hormone, and insulin enhance reabsorption
   - parathyroid hormone, progesterone, and glucagon decrease reabsorption

- ***Summary of Sodium Regulation***

- control of Na^+ excretion is via GFR and Na^+ reabsorption
- the later is controlled principally by the renin-angiotensin-aldosterone system but also by the SNS
- SNS activity is important in,
  i. control of aldosterone via the renin-angiotensin system
  ii. determination of intrarenal vascular factors & GFR
  iii. direct action on tubular function

- despite these functions, the denervated kidney maintains Na^+ balance
- reflexes that control these inputs are BP-regulating and initiated most often by changes in arterial ± venous pressure
- CVS function depends on plasma volume, which is a component of ECF volume, the later reflecting the mass of Na^+ in the body
- these reflex systems maintain Na^+ balance within **~ 2%** in normal individuals despite marked variations in intake and loss

Disordered Sodium Balance

▪ *Secondary Hyperaldosteronism*

• characterised by persistent Na^+ retaining reflexes, (decreased GFR, increased aldosterone, etc.), despite progressive overexpansion of the ECF and the development of oedema
• increased aldosterone is secondary to elevated ***renin*** via reflex control
• classically seen in,

1. hepatic cirrhosis
2. congestive cardiac failure
3. nephrotic syndrome

▪ *Primary Hyperaldosteronism*

• Na^+ retention does occur initially but ***escape*** occurs and Na^+ balance returns to normal
• elevated ECF volume initiates Na^+ losing responses $\rightarrow$ increased ANF, increased GFR etc.
• the net effect of which is to deliver an increased load of Na^+ to the collecting ducts, beyond their reabsorptive capability, thereby increasing excretion

NB: that is, persistent Na^+ retention can not be initiated by an abnormality of only one of the pathways controlling balance

REGULATION OF OSMOLALITY & ECF VOLUME (ADH)

• although protection of the ECF is linked to the Na^+ mass, the ability of water to follow Na^+ reabsorption is dependent on secretion of ADH
• therefore, decreases in plasma volume reflexly increase both aldosterone and ADH
• ADH, or ***arginine vasopressin***, is an ***nonapeptide*** synthesised by discrete neurones in the ***supraoptic*** > paraventricular nuclei of the hypothalamus
• axons terminate in the ***posterior pituitary*** from where ADH reaches the blood
• synthesised as a large and inactive ***prohormone + neurophysin + glycopeptide***
• these are stored in granules and split into ADH-neurophysin during passage from the perikaryon to the terminal bulbs
• neurophysin also binds ***oxytocin***
• newly synthesised hormone appears in the posterior lobe within ~ 30 mins of a stimulus such as haemorrhage
• mechanism of vesicle release = depolarisation $\rightarrow$ Ca^{++} influx $\rightarrow$ ***exocytosis***

NB: some neurones also terminate in the external zone of the median eminence, from where ADH enters the adenohypophyseal portal circulation and acts as an ***ACTH releasing factor*** (CRF)

• ADH undergoes rapid enzymatic cleavage *in vivo*, mainly in the liver and kidney
• the elimination half life, $\mathbf{t_{b½}}$ **~ 17 to 35 mins**

• deamination at the 1AA reduces its susceptibility to peptidases and substitution of d-Arg for l-Arg at the 8AA reduces pressor activity,

$\rightarrow$ ***desmopressin*** (DDAVP)

NB: there are two main physiological mechanisms for release,

■ ***Hyperosmolality***

• Verney (1947) showed that a **~ 2%** increase in osmolality in the hypothalamus produced a sharp antidiuresis on dogs
• current candidates for the ***osmoreceptors*** of the hypothalamus are,

a. the organum vasculosum of the lamina terminalis (OVLT)
b. the subfornical organ (SFO)

• both of which are ***outside*** the blood brain barrier
• the threshold for secretion is **~ 280 mosmol/l**
• individuals vary but below this level ADH is virtually undetectable
• above this level [ADH] rises sharply and ***linearly*** with plasma osmolality
• there is also evidence for a direct functional interaction between neural centers for ***thirst*** and ADH secretion
• drinking decreases ADH release before any change of plasma osmolality

Volume Depletion

• haemorrhage, Na^+ depletion, or other acute causes of decreased ECF volume, irrespective of plasma osmolality, cause release of ADH
• secretion appears to come from a readily releasable "pool" of hormone, which ~ 10-20% of ADH in the pituitary
• subsequent release is at a slower rate
• other chronic conditions in which ***effective circulating volume*** is reduced are also associated with elevated levels of ADH,

i. CCF
ii. cirrhosis with ascites
iii. hypothyroidism
iv. excessive diuresis
v. adrenal insufficiency

• receptors include the baroreceptors of the LA, pulmonary veins, carotid sinus and aortic arch
• the afferent pathways are in the vagus and glossopharyngeal nn.
• secretion of ADH is under tonic ***inhibitory*** (GABA) control of the baroreceptors
• secretion in response to hypoxia, nausea and pain may also be mediated by receptors in the carotid sinus and aortic arch
• ***iso-osmotic*** contraction of the ECF produces little secretion **< 10%** change, after which [ADH] increases rapidly and ***exceeds*** the response due to osmolar stimulation
• levels produced under these circumstances are high enough for ADH to have direct pressor effect on vascular smooth muscle

Other Mediators of ADH Release

• mechanisms for which there is good evidence for stimulation of release include,

a. angiotensin II - synthesised by brain as well as peripherally
b. dopamine
c. endogenous opioids, pain/"stress"
d. hyperthermia
e. hypoxia
f. nausea
g. drugs - either stimulate or inhibit secretion

Stimulat^n:	Inhibit^n:
tricyclics	ethanol
vincristine, vinblastine	phenytoin
loop diuretics	glucocorticoids
cyclophosphamide	mineralocorticoids
colchicine	
chlorpropamide	

• release is inhibited by GABA → inhibitory interneurone is GABA'ergic
• ***prostaglandins*** may play a role in both osmotic and volumetric release of ADH

Renal Effects of ADH

- after release, the circulation $t_{\beta½}$ ~ 17 to 35 mins
- removed by enzymatic cleavage and receptor binding in smooth muscle
- smooth m. and hepatic receptors = **V_1** ***receptors*** and act via phosphoinositol phosphate and Ca^{++}
- **V_2** ***receptors*** in the kidney act via adenylate cyclase & cAMP
- water reabsorption in the cortical CT and beyond is governed by permeability of the luminal membrane under the influence of ADH:

1. high [ADH]- mass diffusion of water, urine iso-osmotic to medulla
2. low [ADH] - limited diffusion of water, large V of dilute urine
   - virtually no H_2O reabsorbed after loop of Henle

- achievable osmolality,

a. minimal ~ 50 mosmol/kg
   - as much as 15% of filtered water may appear in the urine (15% of 180 l/d = 27l)

b. maximal ~ 1200-1400 mosmol/kg
   - corresponding to the medullary interstitium

- proposed sequence of events,

a. **V_2** ***receptors*** on basolateral membrane activate adenylate cyclase
b. increase in $[cAMP]_i$
c. activated cAMP-dependent protein kinase ± phosphoprotein phosphatase
d. microtubules and microfilaments important in ADH response
e. aggregation of proteins at luminal membrane
f. ? insertion or phosphorylation of membrane protein channels
g. increased permeability of luminal membrane

- ADH in physiological concentration has virtually ***no effect*** on Na^+ transport
- ADH may promote Na^+ and water retention by a reduction in GFR secondary to contraction of afferent arterioles and mesangial cells
- ADH exerts local (-)'ve feedback due to induction of medullary synthesis of ***prostaglandins***, the later opposing ADH induced generation of cAMP
- altered PG synthesis may therefore account for the altered tubular responsiveness seen in various disease states
- eg. hypovolaemic shock associated high output renal failure

- ***Non-Renal ADH Effects***

• volume depletion may produce a high [ADH] with direct pressor effects on vascular smooth m.
• its effects on the heart are indirect → reduced coronary flow and reflex alterations in SNS/PNS
• also contracts smooth m. of the GIT and uterus
• increases ***Factor VIII*** concentrations in haemophilia and von Willebrand's disease, therefore may be used prophylactically during surgery
• increases platelet activity in renal failure, post-transfusion etc
• may play a role in regulation of ICP by altering the permeability of the arachnoid villi to water
• possible role as a neurotransmitter, eg. CRF in the pituitary

DDAVP Desmopressin

• chemically modified ADH = **1-deamino-8-*d*-arginine vasopressin**,

a. deamination results in resistance to plasma and hepatic proteases
   • resultant long plasma half life, $\mathbf{t_{½b}}$ **~ 76 minutes**

b. *d*-arginine greatly reduces vasoactive properties

• the duration of drug effect ~ 8-20 hrs
• intranasal bioavailability ~ 10%
• the dose for central DI is 10-40 µg/d nasally, or 1-4 µg/d IM
• for children the dose is ~ ¼ to ½ this amount

• for the procoagulant effects, an infusion of 0.4 µg/kg in 100 ml of NaCl, over 20 mins is usually sufficient to raise VIII:C, VIIIR:Ag and decrease the SBT
• further doses may be given 12 hrly as required
• indicated for haemophilia A and von Willebrand's disease

NB: but ***not*** for type II von Willebrand's disease, as platelet aggregation may be induced

Summary of ADH Effects		
Receptor Subtype	Second Messenger	Physiological Effects
V_1	IP_3 / Ca^{++}	• vasoconstriction especially coronary, mesenteric & skin • glycogenolysis
V_2	cAMP protein kinase ± phosphoprotein phosphatase	• increased DT/CD H_2O permeability • increased renal PGE_2 (opposes above) • increased PRA • tachycardia • facial flushing • lowered BP • increased PGI_2 • increased fibrinolytic activity (tPA) • increased Factor VIII related antigen • increased Factor VIII coagulant activity • increased von Willebrand factor multimers
V_3	??	• baroreceptor modulation • ? behavioural effects

RENAL REGULATION OF POTASSIUM BALANCE

▪ ***Functions***

1. ***resting membrane potentials***
   - the $[K^+]_{ECF}$ is closely regulated due to the primary importance of K^+ in neuromuscular excitability
   - the resting membrane potential being predominantly determined as follows

$$E_M = -61.5 \log \frac{[K^+]_o}{[K^+]_i}$$

   i. increasing $[K^+]_o \rightarrow$ decreases E_m
   ii. decreasing $[K^+]_o \rightarrow$ increases E_m

   - changes in ICF $[K^+]$ having only a small effect
   - acute changes having a greater effect than chronic, as with the latter both ECF & ICF levels are likely to move in the same direction

2. ***total body osmolality***
   - total body osmolality is related to the total exchangeable Na^+ & K^+ and TBW
   - changes in either total body Na^+_E or K^+_E may result in changes in plasma osmolality, viz.

$$[Na^+]_{pl} \sim \frac{Na^+_E + K^+_E}{TBW}$$

3. influences excitable tissues - neural
   - cardiac
   - smooth & skeletal muscle
4. intracellular osmotic pressure and electroneutrality
5. protein synthesis ~ 1 mmol/g of protein intake

▪ ***Regulation of ECF Potassium Concentration***

- ~ 98% of total body K^+ is intracellular due to the action of the membrane bound Na^+/K^+-ATPase
- thus, the ECF $[K^+]$ is a function of 2 variables,

   a. total body K^+
   b. ECF/ICF distribution

- due to relatively small extracellular component, even small shifts in internal balance can markedly alter the extracellular $[K^+]$
- such shifts are under physiological control, particularly in ***muscle & liver***, and these offset alterations of extracellular $[K^+]$

• the major factors in this control are,

1. ***adrenaline***
   - results in a net movement of K^+ into cells
   - mediated by β_2-adrenergic receptors
   - predominantly muscle & liver
   - important during exercise or major trauma
2. ***insulin***
   - at physiological concentration, insulin exerts a tonic permissive effect
   - promotes entry into muscle, liver and other tissues
   - more importantly, elevated plasma [K^+] stimulates insulin release, promoting its own entry into cells
3. ***glucagon***
   - counteracts effects of insulin
   - increases K^+ secretion in the late DT & CT
4. ***aldosterone***
   - DT of the nephron is the main site of action
   - increases secretion, ? independent of Na^+
   - facilitates net movement of K^+ into cells, esp. with chronic elevated total body K^+
   - this is independent of renal handling of K^+

NB: other factors that affect the balance of internal K^+ are not linked to homeostasis of the internal environment but do affect K^+ significantly, of these ***plasma*** **[H^+]** is the most important

- ***<u>Modifying Factors</u>***

1. acid-base status
2. Na^+/K^+-ATP'ase activity
3. Gibbs-Donnan effect
4. non-absorbable anions in the urine
5. diuretics
6. ECF volume & its effects on urine output
7. intestinal secretion

Basic Renal Mechanisms

• K^+ is freely filterable at the glomerulus, though, the urine [K^+] may be slightly less than plasma due to a ***Donnan effect***
• final urinary [K^+] represents only **~ 10-15%** of the filtered fraction
• therefore, tubular reabsorption predominates, but it can be demonstrated under certain conditions that the tubules actively secrete K^+
• K^+ handling shows ***heterogeneity*** between short & long looped nephrons

a. in the convoluted PT ~ 50% the filtered mass is reabsorbed
   - primarily by ***passive diffusion*** driven by the δ[EC] created by water reabsorption
   - additional small amount due to ***solvent drag***

b. in the pars recta of the PT and DLH → K^+ ***secretion*** predominates
   - occurs primarily by ***diffusion*** due to the high interstitial $[K^+]_i$ in the medulla

c. in the ALH → passive reabsorption is again the dominant process
   - this is so effective in ***short-looped nephrons*** that the amount of K^+ entering the DT is only ~ 10% of filtered mass

NB: 1. short-looped nephrons, the PT reabsorbs 50% and the ALH another 40%, ***plus*** the mass secreted into the pars recta and DLH
2. long-looped nephrons also show reabsorption in the ALH, but the quantity is unknown, certainly < 40%

• the early DT plays little if any role in K^+ handling

NB: the ***late DT*** and ***cortical CT*** are able to ***both*** reabsorb and secrete K^+, both processes being active (see below)

• the medullary CT usually manifests net reabsorption, this K^+ providing the high $[K^+]_i$ driving diffusion into the straight PT and DLH
• thus there is a recycling of K^+ from distal to proximal tubular segments analogous to that described for urea

▪ ***Important Generalisations***

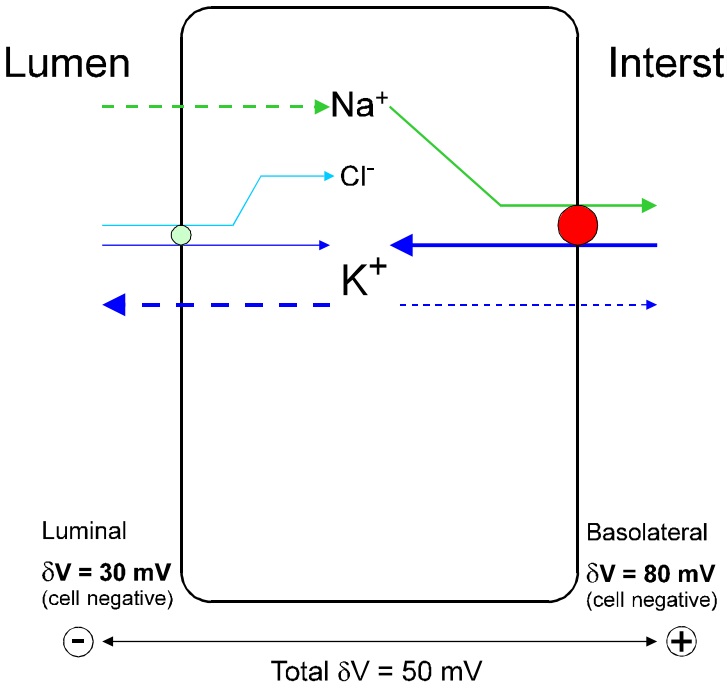
1. the transport processes in the PT and loop are relatively unchanged by increases or decreases in total body K^+; therefore, the total mass of K^+ delivered to the DT is always a small fraction of the filtered mass
2. physiological regulation of K^+ excretion is achieved mainly by altering K^+ transport in the ***distal tubule*** and ***cortical collecting tubule***
3. the major process regulated in these segments is the rate of K^+ ***secretion***
4. the effects on K^+ excretion mediated by the DT and cortical CT are so great that the effects of changes in the filtered load (GFR x $[K^+]_{pl}$) may be ignored

- ***Exceptions***

• under certain conditions, reabsorption in the PT and ALH may be decreased and the delivery of a large quantity of K^+ to the distal site may overwhelm reabsorptive processes, these include,

1. osmotic diuretics
2. loop diuretics
3. uncontrolled diabetes / DKA
4. psychogenic polydipsia
5. recovery phase of ATN
6. post-obstructive diuresis

Mechanism of Distal Potassium Secretion



• the critical event is the active entry of K^+ from the interstitium, via the basolateral membrane Na^+/K^+-ATPase, providing a high intracellular $[K^+]$
• backward diffusion is far less than diffusion into the lumen due to the low gK^+ of the basolateral membrane
• the concentration gradient is opposed by the luminal membrane potential, E_L = 30 mV, cell (-)'ve, however, the overall δ[EC] favours secretion
• in addition to basolateral gK^+ being lower, the E_{BL} = 80 mV cell (-)'ve, therefore, K^+ pumped into the cell favours net secretion
• the high luminal gK^+ is due to the presence of specific K^+ channels

• the presence of ***specific channels*** accounts for DT secretion, c.f. the PT which also has a high intracellular $[K^+]$ but low luminal gK^+ and an unfavourable electrical gradient

• the ability of these segments to manifest ***reabsorption*** relies on the presence of an active luminal pump, (probably cotransport with Cl^-)
• this pump is always operating, but at a low rate, and therefore opposes secretion
• thus, when the activity of the basolateral pump is reduced, the tubule may show net reabsorption due to the unopposed action of the luminal pump
• this luminal pump may also be physiologically regulated, but this is far less significant than regulation of the basolateral Na^+/K^+-ATP'ase

- ***In Summary***

• the fundamental step in secretion is the high intracellular [K^+] created by the basolateral pump
• passive luminal diffusion depends on,

1. opposing luminal E_M
2. luminal membrane gK^+
3. luminal [K^+] gradient

Homeostatic Control of Distal Secretion

• cells of the adrenal cortex are sensitive to ?extracellular [K^+], more likely their internal [K^+]
• increases in [K^+] increase the secretion of ***aldosterone*** which acts on the distal segments by,

1. increasing the activity of the basolateral Na^+/K^+-pump
   - coincident with its action enhancing Na^+ reabsorption in the same segments
2. increasing the luminal permeability to K^+

• the increased K^+ secretion induced by these changes occurs quite rapidly
• if plasma [K^+] remains high for several days, potassium ***adaptation*** occurs and the ability of the distal segments to secrete K^+ is markedly increased
• occurs mainly as a result of an increased number of basal pumps (? & luminal channels)
• low plasma [K^+] has the directly opposite effects

NB: K^+ secretion is not the only factor governed by aldosterone secretion,
→ Na^+ and H^+ also being influenced

Other Factors Influencing Potassium Homeostasis

• K^+ balance is affected by a large number of factors ***not*** designed to maintain homeostasis
• most important are the plasma [H^+] and altered renal Na^+ handling, especially due to ***diuretics***

- ***Acid-Base Changes***

• the existence of an ***alkalosis***, either metabolic or respiratory in origin enhances K^+ ***excretion***
• these stimulatory effects appear to be mediated by,

a. ↑ [K^+] in distal tubular cells, ***alkalosis*** → ↑ basolateral entry of K^+
b. inhibited distal K^+ reabsorption
   - the distal luminal pump requiring co-transport with Cl^- which is reduced in alkalosis

• ***respiratory acidosis*** and certain types of metabolic acidosis do tend to cause the opposite effects but only in the acute stages (< 24 hrs)
• in most forms of ***metabolic acidosis*** other factors ***enhance*** K^+ excretion
• even those forms that have an acute phase of K^+ retention, ultimately come to manifest increased K^+ excretion

Altered Renal Sodium Handling

• K^+ excretion is virtually always found to be ***enhanced*** when urinary Na^+ excretion is increased in the following situations,

a. high NaCl dietary intake

b. saline infusion

c. osmotic diuresis

d. loop diuresis

NB: increased excretion is due to enhanced distal tubular ***secretion***, although there is some contribution of reduced PT reabsorption

• all of these situations lead to an increased ***volume*** of fluid flowing through the distal segments, thereby reducing the rise in the luminal $[K^+]$ and enhancing diffusion from the tubule

NB: these effects are ***not*** seen with a water diuresis with a ***low ADH***, as the site of action of ADH is largely ***distal*** to the sites of K^+ secretion

• similarly a reduced flow of fluid in the distal segments tends to inhibit K^+ secretion
• further, in low flow states, luminal $[Na^+]$ becomes very low and causes the membrane to become hyperpolarised (cell more negative c.f. lumen)
• despite this tendency, in salt depletion and the diseases of ***secondary aldosteronism*** with oedema, K^+ secretion may be relatively ***unchanged*** due to the stimulatory effect of aldosterone

NB: these later conditions generally manifest normal rates of K^+ excretion, in contrast to ***primary aldosteronism*** where the elevated aldosterone and normal delivery of fluid to distal segments leads to ***severe*** K^+ depletion

RENAL REGULATION OF HYDROGEN-ION BALANCE

- ***Sources of Hydrogen Ion Gain or Loss***

1. CO_2
   - net production **~ 15,000 to 20,000 mmol/d**
   - doesn't contribute to net gain of H^+ as these are excreted by lung
2. non-volatile, fixed acids
   - includes sulphuric and phosphoric acids (generated from the catabolism of proteins and other organic molecules), lactic acid and keto-acids
   - in normal "Western" diets the net production **~ 40-80 mmol/d**
   - in vegetarians there may be net production of alkali
3. gastrointestinal secretions
   - vomitus containing a large $[H^+]$
   - other GI secretions have a high $[HCO_3^-]$, therefore net loss $\rightarrow$ H^+ gain
4. urine
   - kidneys normally excrete the 40-80 mmol of fixed acids generated per day
   - kidneys also adjust their H^+ excretion to adjust for any net loss or retention of CO_2 by the lungs, or any increase in metabolic generation of fixed acid

Buffering

- essential for preventing any large change from the normal **$[H^+]$ ~ 39 nmol/l**
- the normal daily acid load of 40-80 mmol would cause a profound change in plasma pH
- the only important ***extracellular*** buffer is the ***bicarbonate*** system,

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

- the major ***intracellular*** buffers are proteins and phosphates
- these systems are, however, in equilibrium and although 50-90% of buffering is intracellular, the assessment of HCO_3^- provides a reliable index,

$$pH = 6.1 + \log \frac{[HCO_3^-]}{0.0301 \times P_{aCO_2}}$$

- from the ***Henderson-Hasselbalch equation***, regulation of pH may be achieved by regulation of both CO_2 and HCO_3^-

- the kidneys function by two processes,

1. variable reabsorption of filtered HCO_3^-
2. addition of new HCO_3^- to plasma

Bicarbonate Reabsorption

NB: Filtered HCO_3^-/d = GFR x $[HCO_3^-]_{pl}$ (ie. freely filterable)
~ 180 l/d x 24 mmol/l
~ 4320 mmol/d

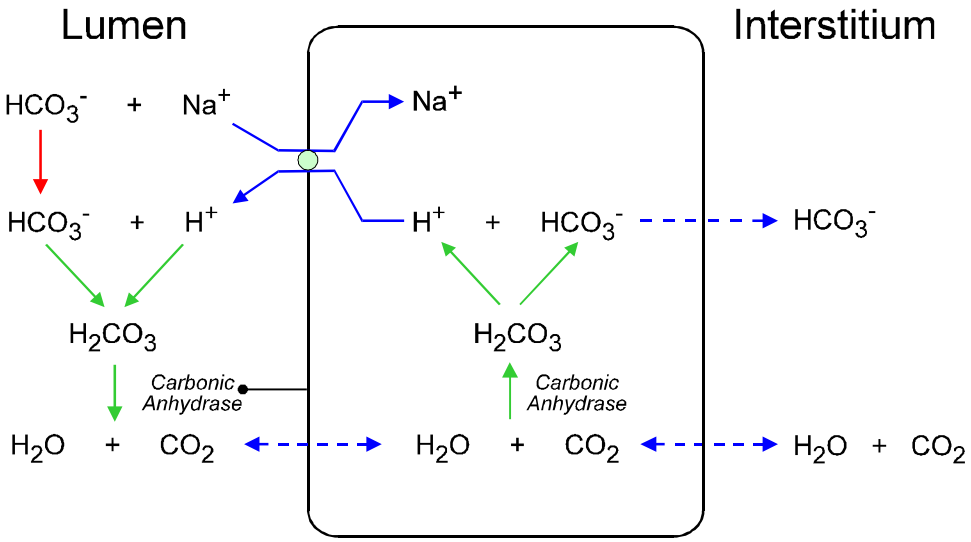
- excretion of this load of bicarbonate is equivalent to adding over 4000 ml of 1N acid to the body
- reabsorption of HCO_3^- is a conservation process and essentially ***none*** appears in the urine
- minimal ***passive*** reabsorption occurs for HCO_3^- because,

a. luminal and basolateral ***permeability*** is low, c.f. Cl^-

b. ***active transport*** processes are dominant and eliminate the δ[EC]

- the mechanism for reabsorption of HCO_3^- involves secretion of H^+ into the lumen
- this is generated within the cell from CO_2 and water by ***carbonic anhydrase*** (CA), the generated H^+ destined for the lumen and the HCO_3^- entering the peritubular plasma by facilitated diffusion
- the luminal membrane also contains CA and filtered HCO_3^- combines with the secreted H^+ and is converted to CO_2 and water which are free to diffuse into the tubular cell

NB: therefore, the filtered HCO_3^- ***does not*** itself enter peritubular plasma



- **H^+** ***secretion*** varies in different portions of the nephron,

a. proximal tubule → counter-transport with Na^+

b. distal segments → primary luminal H^+-ATP'ase pump

- these secreted H^+ ions are ***not*** excreted in the urine, but are reabsorbed as H_2O and CO_2
- therefore they ***do not*** constitute acid excretion, this being the case for any H^+ that combines with HCO_3^-
- the process of H^+ secretion and HCO_3^- reabsorption occurs throughout the nephron with the exception of the DLH
- in the **PT ~ 80-90%** of filtered bicarbonate is reabsorbed, the remainder normally being reabsorbed in the ALH, DT and CT

• the presence of ***luminal CA*** in the PT accounts for very large quantities of carbonic acid formed
• the later segments lack luminal CA, therefore distal conversion of $H_2CO_3 \rightarrow CO_2 + H_2O$ occurs slowly and often after urine has left the nephron
• therefore urine PCO_2 may be greater than plasma under certain conditions

Renal Excretion of Acid

• this is synonymous with "addition of new bicarbonate to plasma"
• secreted H^+ combining with luminal HCO_3^-, effects HCO_3^- reabsorption, ***not*** acid excretion
• secreted H^+ combining with ***urinary buffer*** is excreted in the urine and the generated HCO_3^- represents "new" bicarbonate entering the plasma
• only a very small quantity of H^+ is in free solution in equilibrium with buffer
• the source of essentially all excreted H^+ is ***tubular secretion***, glomerular filtration makes no significant contribution (~ 0.1 mmol/d)
• the two most important urinary buffers are ***phosphate*** and ***ammonia***
• the quantity of urinary buffer limits the rate at which the kidneys can excrete acid
• in the DT the ***minimum* pH ~ 4.4**, limited by inhibition of the luminal H^+-pump at low pH
• therefore, the quantity of buffer determines the ***mass*** of H^+ which may be secreted before the limiting pH is reached

■ *Urinary Phosphate and Organic Buffers*

• the relationship between monobasic and dibasic phosphate is as follows,

$$HPO_4^{=} + H^+ \longleftrightarrow H_2PO_4^-$$

$$pH = 6.8 + \log \frac{[HPO_4^{=}]}{[H_2PO_4^-]}$$

• therefore, at pH = 7.4 the ratio of ***dibasic:monobasic*** **~ 4:1**
• by the time the limiting pH of 4.4 is reached, the ratio ~ 1:250
• effectiveness as a buffer is limited by,

a. protein binding slightly reduces the amount filtered
b. only 80% of the filtered mass is in the ***dibasic form***
c. tubular reabsorption → ~ 75% of the filtered mass
→ end result is only **~ 35-40 mmol/d** is available for buffering secreted H^+

• normally, phosphate and ammonia are the only important buffers
• however, under abnormal conditions the urine may contain large quantities of anions of keto-acids → acetoacetate & β-hydroxybutyrate
• these appear as their tubular T_{Max}'s are exceeded
• however, they have only limited usefulness as buffers due to their low pKa's ~ 4.5
• therefore, only 1/2 of the excreted keto-acid anions are available to accept H^+

▪ *Urinary Ammonia Buffer*

• the ammonia/ammonium reaction is as follows,

$$NH_3 + H^+ \longleftrightarrow NH_4^+$$

$$pH = 9.2 + \log \frac{[NH_3]}{[NH_4^+]}$$

• at pH = 7.4, the ratio will be **~ 1:63**
• therefore, virtually all synthesised NH_3 entering the lumen will immediately pick-up a H^+ ion

NB: accordingly, as long as NH_3 is available from the tubular cells, urinary acid excretion and addition of bicarbonate to the plasma can continue

▪ *Ammonia Synthesis & Diffusion Trapping*

• glomerular filtration is ***not*** a significant source of NH_3, as its combined $[NH_3/NH_4^+]$ is very low, and only ~ 1.5% of this is in the NH_3 form
• the source of ammonia is ***synthesis*** in renal tubules from glutamine,

glutamine → glutamate + NH_4^+ *glutaminase*

glutamate → α-ketoglutarate + NH_4^+ *glutamic acid dehydrogenase*

glycine } + α-ketoglutarate → ***glutamate*** + α-keto-acids
alanine }

• thus, the generation of a-***ketoglutarate*** also generates **$2H^+$**, which has 3 possible fates,

1. complete oxidation to CO_2 and water
2. gluconeogenesis
3. recycling to glutamate (above)

• therefore, the generation of ammonia itself does ***not*** add H^+ to the body
• prolonged ***acidosis*** → adaptation of ammonia synthesis, involving enhanced transport of glutamine into the mitochondrion ± increased glutaminase
• once synthesised, ammonia diffuses rapidly across the luminal membrane by ***passive diffusion***, or ***diffusion trapping***
• in both cell and lumen the base/conjugate-acid pair are in equilibrium, the relative quantities of each being pH dependent
• due to the low pH of luminal fluid, almost all NH_3 entering the tubule accepts a H^+ ion, thereby maintaining a concentration gradient for the diffusion of NH_3 from the cell
• ratio of NH_3:NH_4^+ at pH = 4.4 **~ 1:63,000**
• as the luminal membrane is virtually ***impermeable*** to ammonium, at low pH ammonia passively diffuses into the lumen and is trapped there by conversion to ammonium
• as long as ammonia synthesis can keep pace with acid secretion, tubular pH will not fall
• ammonium excretion can increase from the normal 20-30 mmol/d → > 500 mmol/d
• in contrast, ***phosphate*** may only increase by ~ 20-40 mmol/d

• conversely, if the urine pH is not low the luminal [NH_3] will rise opposing any further diffusion and ammonium excretion will be low
• ammonia is synthesised in both the PT and distal segments, however urine pH only falls significantly in the distal segments, therefore most ammonia synthesis and trapping occurs distally
• some of the ammonia in the DT actually short-circuits the loop by diffusing from the PT and enters the CT from the medullary interstitium

Integration of Bicarbonate Reabsorption and Acid Excretion

• the fate of secreted H^+ depends on whether it combines with HCO_3^- effecting its reabsorption, or with urinary buffer effecting acid secretion
• which of these two processes occurs is determined by,

1. the ***mass*** of each buffer present
2. the **pK_A's** of the conjugate pairs
3. the ***luminal pH***

• compared to HCO_3^-, relatively little other buffer is present, therefore little non-bicarbonate buffer is titrated until almost all of the HCO_3^- is reabsorbed
• conversely once the bicarbonate has been largely reabsorbed, most secreted H^+ combines with urinary buffer
• ergo, the PT secretes a far greater ***mass*** of H^+ than the distal segments, however this effects bicarbonate reabsorption and the luminal pH falls < 1 unit, only a small amount of H^+ being picked-up by phosphate etc.
• in contrast, the DT [HCO_3^-] is low and secreted H^+ is sufficient to effect its reabsorption plus lower the pH allowing titration of other buffers and trapping of ammonia
• however, should a large quantity of bicarbonate reach the distal segments, most secreted H^+ would be expended in bicarbonate reabsorption rather than in titration of urinary buffer

Measurement of Tubular Acid Secretion

- the total rate of tubular H^+ secretion is equal to the sum of,

a.	HCO_3^- reabsorption	~ 4300	mmol/d
b.	excretion of titratable acid	~ 20	mmol/d
c.	excretion of NH_4^+	~ 40	mmol/d

NB: values are for average "Western" diet

- HCO_3^- reabsorption is calculated from,

mass filtered ($GFR.[HCO_3^-]_{pl}$) - mass excreted

Def'n: ***titratable acid*** is the mmol of NaOH required to reach a urine pH = 7.4

- this will equal the number of H^+ ions that were added to tubular fluid and combined with phosphate and other organic buffers
- this ***will not*** include those H^+ ions that combine with NH_3 due to the high pK_A (~ 9.2) for this reaction, hence the quantity of urinary ammonium must be added

Measurement of New Bicarbonate Added to Blood

- total net gain or loss of HCO_3^- from the body is equal to,

excretion of titratable acid + excretion of NH_4^+ - excretion of HCO_3^-

(+)'ve value = net gain of bicarbonate
(-)'ve value = net loss of bicarbonate

Homeostatic Control of Tubular Acid excretion

▪ *Glomerulotubular Balance for Bicarbonate*

• as seen with Na^+ reabsorption, H^+ secretion & HCO_3^- reabsorption vary directly with GFR
• ie., if GFR increases 25%, bicarbonate reabsorption increases by a similar amount
• the underlying mechanism is likely a part of the same process that operates for sodium
• this is adaptive, in that it prevents large alterations of acid/base balance with changes in GFR

▪ *PCO_2 and Renal Intracellular pH*

• the single most important determinant renal H^+ secretion is the P_{aCO2}
• in the physiological range, P_{aCO2} lies on "shoulder" of curve (? virtually linear)
• renal tubular cells themselves respond directly to the P_{CO2} of blood perfusing them
• CO_2 raises the intracellular $[H^+]$ by ***mass action*** and this increases the rate of H^+ secretion and increases the number of luminal H-pumps
• intracellular pH is more dependent on P_{aCO2} than arterial pH due to the low membrane permeability of H^+ & HCO_3^-

Respiratory Acidosis and Alkalosis

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

$$K_A = \frac{[HCO_3^-].[H^+]}{[H_2CO_3]}$$ by the law of ***mass action***

• but as K_A only applies to infinitely dilute solutions with negligible interionic forces, the ***apparent dissociation constant***, $\mathbf{K_A'}$, is used
• CO_2 can be used instead of H_2CO_3 because their concentrations are always in direct proportion
• this may be rewritten for hydrogen ion, viz.

$$[H^+] = \frac{K_A' \times \alpha . P_{CO_2}}{[HCO_3^-]}$$

• in respiratory insufficiency the reaction is shifted to the right, with resulting acidosis
• bicarbonate increases but not to the same degree as CO_2, as $[H^+].[HCO_3^-] \propto K_A'.[CO_2]$

NB: increases in P_{aCO2} increase the ***product*** of $[H^+].[HCO_3^-]$

• pH is restored by elevating $[HCO_3^-]$ to the same degree as $[CO_2]$
• increased P_{aCO2} stimulates tubular H^+ secretion, which reabsorbs all the filtered bicarbonate and "new" bicarbonate is added to the blood by the formation of titratable acid and ammonium
• this continues to a new steady-state point where the elevated H^+ secretion can only serve to reabsorb the increased filtered load of HCO_3^-
• the sequence of events for alkalosis is the direct opposite

NB: compensation is not perfect, $[HCO_3^-]$ is not elevated to the same degree as $[CO_2]$

Metabolic Acidosis and Alkalosis

Metabolic Acidosis

• caused by the primary addition of acid to, or loss of alkali from the body
• the kidneys compensate by increasing H^+ ion secretion, thereby raising the plasma [HCO_3^-] and restoring the pH
• this occurs in the ***absence*** of any apparent stimulus to the kidney, in fact frequently occurs with a ***decreased stimulus***, due to reflexly increased ventilation and a lowered P_{aCO2}
• therefore, renal tubular cell pH is likely to be increased early in a metabolic acidosis
• in time the pH returns to normal, or actually decreases due to altered basolateral transport of H^+
• compensation is achieved as the ***mass*** of filtered bicarbonate is dramatically reduced and less H^+ ion secretion is required for HCO_3^- reabsorption and the formation of titratable acid and ammonium, eg.

	Normal		***Acidosis***	
Plasma HCO_3^-	24	mmol/l	12	mmol/l
Filtered HCO_3^-	4320	mmol/d	2160	mmol/d
Reabsorbed HCO_3^-	4315	mmol/d	2160	mmol/d
Total H^+ secreted	4375	mmol/d	2360	mmol/d
Titratable Acid & NH_4^+	60	mmol/d	**200**	**mmol/d**

• thus, even in the presence of greatly reduced total ***acid secretion***, the kidneys are able to compensate for metabolic acidosis
• the limiting factor for this compensation is the availability of buffer
• there is recent evidence that the rate of H^+ secretion in the collecting ducts may in fact be increased, despite the lowered CO_2, the mechanism is unknown but may involve ***aldosterone***

Metabolic Alkalosis

• the situation for alkalosis is exactly the opposite
• despite the reflexly elevated P_{aCO2} and increased H^+ secretion, the load of filtered HCO_3^- becomes so great that much escapes reabsorption and little or no titratable acid or ammonium is formed
• there is some evidence that there may be active secretion of bicarbonate into the collecting ducts
• this description may not apply to chronic alkalosis

Other Factors Influencing Hydrogen Ion Secretion

▪ *Extracellular Volume Depletion*

• presence of salt depletion and ECV contraction interferes with the ability of the kidney to compensate for a ***metabolic alkalosis***, as may occur in high GIT obstruction
• salt depletion not only stimulates Na^+ reabsorption but also H^+ secretion
• this occurs mainly in the proximal segments, the mechanism is unclear but probably involves Na^+/H^+ ***counter-transport*** across the luminal membrane
• therefore, all filtered HCO_3^- is reabsorbed and the metabolic alkalosis is uncompensated

> ***NB:*** salt depletion itself ***will not*** generate an alkalosis, (arguable point !)
> merely impair the kidneys ability to compensate for such

• the major reason for this is that salt depletion *per se* has little effect on the distal nephrons secretion of H^+
• isolated losses of ***chloride***, in addition to the above, maintain an alkalosis by stimulating hydrogen-ion secretion

▪ *Aldosterone Excess and Potassium Depletion*

• ***aldosterone*** and other mineralocorticoids stimulate H^+ secretion and ammonia production by a direct action on the DT and collecting ducts
• this is distinct from their effects on Na^+ & K^+
• this effect alone is relatively small but is physiologically significant as aldosterone,

a. tonically facilitates H^+ secretion (permissive effect)
b. increases during metabolic acidosis and facilitates H^+ secretion in the collecting ducts
c. may contribute to the increased H^+ secretion seen in salt depletion
   - although more proximal factors are more important

• ***potassium depletion*** also stimulates H^+ secretion and ammonia production, presumably by decreasing tubular cell pH
• only when K^+ depletion is extremely severe will it *de novo* alter the renal acid-base balance
• hypokalaemia decreases aldosterone secretion, tending to negate any increase in H^+ secretion

• the combination of ***hypokalaemia*** and ***hyperaldosteronism*** acts synergistically to markedly stimulate H^+ secretion and thereby ***generate*** a metabolic alkalosis
• this combination occurs in a number of clinical conditions,

a. primary hyperaldosteronism - may itself cause hypokalaemia
b. GIT fluid losses - upper GI or SI fistula losses
c. extensive use of diuretics - especially in CCF and cirrhosis

• the later may be worsened by concurrent ***salt depletion*** stimulating the reabsorption of HCO_3^-
• the reverse can occur in patients unable to secrete aldosterone, ie. ensuing hyperkalaemia and modest metabolic acidosis

- ***Cortisol and PTH***

• when present in high concentration, cortisol will exert ***mineralocorticoid*** effects, ie.

1. sodium retention
2. potassium depletion
3. metabolic alkalosis

• PTH exerts a direct effect on the PT ***inhibiting*** H^+ ion secretion with ensuing loss of bicarbonate and ***metabolic acidosis***

Influence of H^+ Secretion on NaCl Reabsorption

• H^+ ion secretion in the PT is directly coupled to ***countertransport*** of Na^+
• ergo, were H^+ ion secretion inhibited, Na^+ reabsorption would decrease
• moreover, even in the distal segments, Na^+ reabsorption is ***indirectly coupled*** to H^+ ion secretion by the δ[EC]
• this stems from the fact that bicarbonate ions are ~ 25% of the anions in glomerular filtrate, unless reabsorbed at the same rate as Na^+ a large charge separation occurs

NB: since bicarbonate is reabsorbed as a result of H^+ ion secretion, there is, effectively an "exchange" of Na^+ for H^+ even in the absence of direct coupling

• this also occurs with the formation of titratable acid and ammonium, both of which increase the (+)'ve charge of the lumen and facilitate the reabsorption of Na^+
• in effect, Na^+ is either reabsorbed with Cl^- or in exchange for H^+, therefore,

a. there is usually an inverse correlation between the excretion rates of ***bicarbonate*** and ***chloride***
b. whenever acid secretion is inadequate to effect bicarbonate reabsorption, there is usually an obligatory excretion of Na^+

• increased renal excretion of Cl^- (a) is, therefore, one of the reasons plasma [Cl^-] decreases during renal compensation for metabolic acidosis
• in (b), Na^+ excretion is usually not as great as the losses of bicarbonate due to the increased K^+ secretion induced by an alkalosis

• inhibition of ***carbonic anhydrase*** therefore reduces renal acid excretion, thereby causing an increased excretion of sodium, bicarbonate and water
• further, this alkalinises the tubular cells, increasing K^+ secretion so a large fraction of the excreted bicarbonate is accompanied by K^+

REGULATION OF CALCIUM HOMEOSTASIS

- total body content **~ 380 mmol/kg**, distributed as follows,

a.	bone	~ 99%
b.	ICF	~ 0.004%
c.	ECF	~ 0.01%
d.	exchangeable	~ 1%

- this equates to ~ 1100 g in an average adult → **~ 27.5 mol** of Ca^{++}
- the daily requirement in the adult ~ 0.11 mmol/kg
- concentration ranges vary between tissues,

a. ECF **~ 2.2-2.8 mmol/l**
   - i. 45% - ionised Ca^{++}
   - ii. 15% - complexed to low MW anions (citrate, HPO_4^-)
   - iii. 40% - reversibly bound to plasma proteins (alb, glob.)
     - non-filterable fraction

b. ICF ~ 1 mmol/l total
   ~ 10^{-4} mmol/l as free ionised Ca^{++}
   ~ 99% bound to enzymes in SR, cisternae, & tubules

- only plasma ***ionised*** Ca^{++} is biologically active
- the most important influence on protein binding is ***plasma pH***
- an increase of pH increasing the binding of Ca^{++} due to the exposure of more anionic sites

→ decreased ionised Ca^{++}

Effector Sites for Homeostasis

GIT

- the ***major variable*** under control for homeostasis is GIT absorption of Ca^{++}
- on typical daily intake of 1000 mg → absorption **~ 10%**
- the GIT actually secretes large quantities of Ca^{++}, up to 600 mg/d
- this is reabsorbed along with the above 10%
- thus, by altering GIT absorption, Ca^{++} balance can be achieved and this is ***quantitatively*** the most important variable

- ***Kidney***

• only 60% of plasma Ca^{++} is filterable at the glomerulus
• reabsorption occurs throughout the nephron with the ***exception*** of the ***DLH***, similar to Na^{+}
• 60% is reabsorbed in the PT, the remainder in the ALH and DT to ~ 98-99%
• the kidneys are involved in Ca^{++} balance, but has been estimated that only about 5% of an increment in dietary Ca^{++} appears in the urine
• the rate of Ca^{++} reabsorption is under control of ***PTH***, but is also affected by large number of other inputs, especially Na^{+} and acid-base changes
• there is some form of ***coupling*** of Na^{+}/Ca^{++} in the PT and ALH
• however, this coupling must be dissociated in more distal segments as,

1. alterations of ***aldosterone*** or ***PTH*** do not affect distal handling of both ions
2. ***thiazides*** inhibit distal Na^{+} reabsorption but ***enhance*** Ca^{++} reabsorption

NB: in contrast, proximal or loop diuretics which increase excretion of ***both*** ions

• chronic ***metabolic acidosis*** markedly increases Ca^{++} excretion with subsequent loss from bone
• alkalosis does the opposite

- ***Bone***

• exchange between ECF and bone affect the ***internal distribution*** not body mass of Ca^{++}
• ~ 99% of total body Ca^{++} held in bone as ***hydroxyapetite***
• this acts as an enormous sink for exchange of Ca^{++} with the ECF

Hormonal Control of Effector Sites

▪ *Parathyroid Hormone PTH*

• polypeptide hormone secreted by the parathyroid glands in direct response to a lowered serum Ca^{++} level
• exerts at least four distinct effects on Ca^{++} homeostasis,

1. increases movement of Ca^{++} and phosphate out of bone
2. ↑ tubular reabsorption of Ca^{++}
3. ↓ tubular reabsorption of phosphate
4. stimulates the production of ***Vitamin D*** → ***indirect effects***

• when PTH induces bone reabsorption both Ca^{++} and phosphate are released
• similarly, the vitamin D induced GIT absorption also increases plasma phosphate
• the action of PTH on the renal tubules, ***increasing*** phosphate excretion prevents elevation of the plasma phosphate
• maximal amounts of PTH can reduce phosphate reabsorption from 80% → **~ 15%**
• plasma phosphate may actually ***decrease*** in response to hypocalcaemic induced increases in PTH
• this aids in further reabsorption of bone due to local interactions between Ca^{++} and phosphate
• PTH also inhibits proximal tubular H^+ secretion and, therefore, bicarbonate reabsorption
• this results in a ***decrease*** in plasma ***pH*** which displaces Ca^{++} from plasma protein and bone

• ***hyperparathyroidism*** causes enhanced bone reabsorption with cysts, an elevated plasma calcium and lowered phosphate, ectopic calcification and renal stones
• renal calcium excretion ***increases***, despite the elevated PTH, as the filtered mass increases more than the reabsorptive increase

▪ *Vitamin D*

• actually a group of closely related sterols
• Vit.D_3, ***cholecalciferol***, is formed by the action of UV light on ***7-dehydrocholesterol*** in the skin
• Vit.D is also absorbed from the GIT and the plant form differs only slightly from the endogenous variety
• Vit.D_3 is hydroxylated in the liver at the 25-position and activated by the kidney by further hydroxylation at the ***1-position*** to 1,25-$(OH)_2D_3$, which by definition is a ***hormone*** not a vitamin
• final hydroxylation in the kidney is stimulated by,

1. elevated PTH
2. a low plasma phosphate
3. increased oestrogen and prolactin - as in pregnancy

• the major action of Vit.D is to enhance the absorption of calcium and phosphate from the ***GIT***
• Vit.D also enhances the reabsorption of calcium and phosphate from bone, ? by interaction with PTH, and can stimulate the renal tubular reabsorption of calcium, but the significance of this is unsettled

- ***Calcitonin***

• peptide hormone secreted by the ***parafollicular cells*** of the thyroid gland in response to a raised plasma [Ca^{++}]
• calcitonin lowers the plasma calcium, principally by inhibiting ***bone reabsorption***
• overall contribution to homeostasis is minor c.f. PTH & Vit.D

- ***Other Hormones***

• high levels of ***cortisol*** can induce a negative calcium balance by decreasing GIT absorption and increasing renal excretion
• ***growth hormone*** also increases urinary excretion of calcium but simultaneously increases GIT absorption, the net effect usually being (+)'ve

RENAL HANDLING OF PHOSPHATE

• ***urinary excretion*** is the major homeostatic regulator for total body phosphate balance
• ~ 5-10% is protein bound, therefore 90-95% filterable at glomerulus
• about 75% is actively reabsorbed, mostly in the PT in co-transport with Na^+

NB: there is ***no*** conclusive evidence for tubular secretion of phosphate

• reabsorptive T_{Max} for phosphate is very close to normal filtered load, therefore even small increases in the plasma level result in relatively large increases in renal excretion
• the reabsorptive rate, and T_{Max}, alters over time in response to alterations in plasma phosphate levels, ***not*** as a result of PTH or Vit.D
• the mechanism for such changes is still unclear

• factors affecting ***tubular reabsorption*** of phosphate are,

a. PTH ↓
b. glucagon ↓
c. dietary phosphate ↓
d. $1,25\text{-}(OH)_2D_3$ ↑
e. insulin ↑