RENAL PHYSIOLOGY

(a) ***To describe the functional anatomy of the kidneys and to explain the physiology of renal blood flow.***

(b) ***To describe glomerular filtration and tubular function.***

(c) ***To explain the counter-current mechanisms in the kidney.***

(d) ***To explain the mechanisms involved in the regulation of renal function.***

(e) ***To outline the endocrine functions of the kidney.***

(f) ***To describe the role of the kidneys in the maintenance of acid/base balance.***

(g) ***To describe the role of the kidneys in the maintenance of fluid and electrolyte balance.***

(h) ***To describe the role of the kidney in the handling of glucose, nitrogenous products and drugs.***

(i) ***To describe the principles of measurement of glomerular filtration rate and renal blood flow.***

(j) ***To describe the physiological effects and clinical assessment of renal dysfunction.***

(k) ***To explain the renal responses to hypovolaemia.***

(l) ***To explain the effects of general anaesthesia on renal function.***

Functions of the Kidneys:

Kidneys have the following functions:

- (1) Regulatory role (MOST vital)
  - Regulates water and electrolyte balance
  - Regulates plasma volume, plasma osmolality and systemic blood pressure
- (2) Excretion role – Eliminates toxic metabolic waste from the body (Eg. urea, creatinine, uric acid, bilirubin) and bioactive substances (Eg. drugs, hormones)
- (3) Endocrine role (Eg. production of EPO, renin, 1,25-dihydroxy-vitamin D)
- (4) Acid-base balance – Regulates urinary excretion of H^+ and HCO_3^-
- (5) Gluconeogenesis – With starvation, kidneys produce glucose from a.a's

Renal handling of any substance involves the following 4 processes → these permit the kidney to perform its "regulatory" and "excretory" functions:

- (1) Glomerular filtration – High renal blood flow (20% of CO) produces a very large volume ultrafiltrate of plasma (180 L/day) in the renal corpuscle → this permits excretion of metabolic/exogenous waste products (which are in low [ ] in plasma) in urine
- (2) This ultrafiltrate then passes through the tubular systems where it undergoes:
  - (a) Reabsorption – Most filtered fluid and its contents are reabsorbed into peritubular capillaries (especially at PCT) → thus, normal urine volume ~ 1-1.5 L/day
  - (b) Secretion – Substances (Eg. drugs) are removed from peritubular capillaries into tubular lumen for excretion
  - (c) Metabolism – Some substances within the tubular system are metabolised

Functional Anatomy of the Kidneys:

General structure of the kidney:

- Each kidney ~ 150 g → contains:
  - Outer "cortex" and an inner "medulla"
  - Medulla is arranged into "pyramids" with the inner zones forming the apex ("papillae") → this projects into the "Calyces" → then into the "Renal pelvis"
- "Nephron" is the basic cell unit of the kidney → each kidney contains 1-1.5 million nephrons (Nb. number is fixed throughout life)

Structure of the nephron:

(1) *Renal corpuscle*:

- Present in renal cortex as a compact tuft of capillary loops ("Glomerulus") that invaginates into "Bowman's capsule" → the glomerular capillaries are supplied by an "afferent arteriole" and drained by an "efferent arteriole"
- Acts as the "Filtering component" of the nephron → produces an ultrafiltrate of plasma from glomerular capillaries into Bowman's space under action of opposing $P_{HYDROSTATIC}$ and $P_{ONCOTIC}$ (Ie. bulk flow)

> Note: "Ultrafiltrate" produced by glomerulus contains electrolytes, glucose and amino acids in same [ ] as plasma BUT without cells and large -vely charged molecules (Eg. proteins)

- Filtration barrier between glomerular capillary blood and Bowman's capsule consists of three layers:
  - (1) Fenestrated glomerular capillary endothelium (with 100 nm pores)
  - (2) Basement membrane (with –vely charged glycoproteins)
  - (3) Bowman capsule epithelial cells ("Podocytes") that form "foot processes" → they interdigitate to form "Slit diaphragm" (~ 25 nm wide)

> Note: This barrier is highly permeable BUT very selective according to:
> - (i) Size – Particles < 7 kDa (Eg. glucose, ions, urea, H_2O) are filtered freely, between 7-70 kDa are ↓ freely filtered, and > 70 kDa (Eg. albumin) are not filtered; neutral particles < 4 mm diameter filtered freely, between 4-8 mm are partly filtered, and > 8 mm are excluded
> - (ii) Charge – Cationic substances filtered more readily cf. anionic substances (Ie. -vely charged plasma proteins, such as albumin) → due to –vely charged glycoproteins in filtration barrier

- "Mesangial cells" between BM and capillary endothelium → contractile role to regulate GFR

(2) *Tubules*:

- Consists of a single layer of epithelial cells separated from peritubular capillaries by a basement membrane. Apical tight junctions and interstitial space exist between these epithelial cells throughout the length of tubules
- Act to influence composition of plasma ultrafiltrate to be excreted in urine → via tubular reabsorption, secretion and metabolism in the order of the following tubular segments:
  - (a) Proximal convoluted tubules (PCT)
    - Convoluted (Pars convoluta) → then straightens out (Pars recta)
    - Tubular cell lining is noted to contain (i) "brush border" luminal edge, and (ii) "lateral intercellular spaces" between the cells' bases
    - Function → It collects a large volume of glomerular ultrafiltrate (180 L/day) whereby it:
      - (i) Reabsorbs 60-70% of the filtrate back into blood

        - 2° active reabsorption of Na^+ (65%)
        - Reabsorption of H_2O (65%) via "iso-osmotic reabsorption" (See below in renal H_2O handling) → allows tubular fluid to be isotonic to plasma
        - Reabsorption of K^+ [55%], Cl^- and urea [40%], HCO_3^- [85%], PO_4^{3-}, Ca^{2+} by passive diffusion → due to ↑ in their tubular [ ] 2° to H_2O reabsorption
        - 2° active reabsorption of nutrients (Eg. glucose, proteins) via co-transport with Na^+
    - (ii) Secretes a number of solutes
        - 2° active secretion of H^+ (via a Na^+/H^+ antiport)
        - Secretion of NH_4^+ (produced from glutamine)
        - Secretion of urate, and organic anions and cations
- (b) Loop of Henle (LOH)
    - Traverses from the cortex down into the medulla via the "thin descending limb" → hairpin bend then reascends back into the cortex via the "ascending limb" (initially "thin" → later "thick")

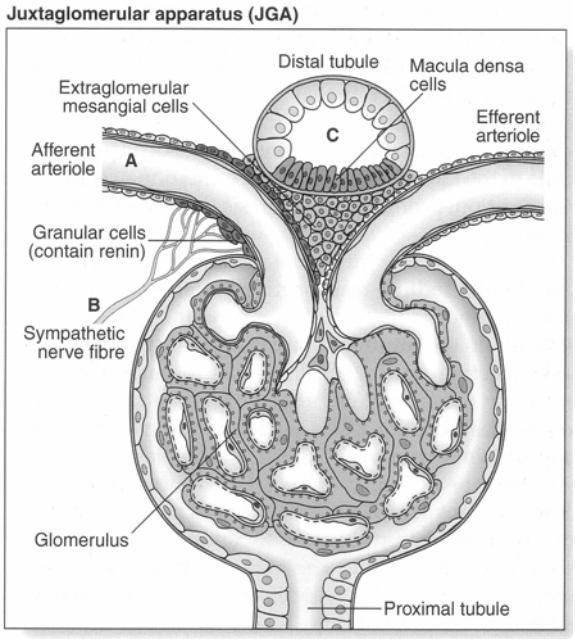
> Note – Length of "thin" ascending limbs varies with regards to type of nephron (cortical vs juxtamedullary – See below)

    - Functions:
        - (i) Generate ↑ interstitial osmotic gradient within the medulla by CCM in order to form concentrated (and diluted) urine
        - (ii) Thin descending limb → H_2O reabsorption (10%) and urea secretion (as part of "urea trapping")
        - (iii) TAL → Reabsorbs NaCl (25%), K^+ (30%), HCO_3^- (10%), and secretes H^+ → BUT impermeable to H_2O
        - (iv) Regulation of GFR by tubulo-glomerular feedback – End of the TAL possess modified tubular epithelium (Macula densa) → sense ionic composition of tubular fluid → regulate GFR via a paracrine process within the JGA
    - Nb. since more solute is reabsorbed than H_2O (25% NaCl vs 10% H_2O) → tubular fluid at end of LoH is hypotonic (~ 100 mosom/kg)
- (c) Distal convoluted tubule (DCT)
    - Early DCT is convoluted (like the PCT) before it straightens out
    - Functions:
        - (i) Reabsorbs NaCl (6-10%) by 2° active means, as well as HCO_3^- (5%), and Ca^{2+}
        - (ii) MAJOR site of K^+ and H^+ regulation → K^+ and H^+ secretion is varied according to Aldosterone levels
    - Nb. No H_2O reabsorption here → ongoing solute reabsorption causes tubular fluid to become ↑ hypotonic (< 100 mosm/kg)
- (d) Collecting ducts (CD)
    - Courses through cortex (as CCD) → medulla (as MCD) → several MCDs combine to form a large papillary CD at the apices of medullary pyramids → empty into a calyx of the renal pelvis
    - Two types of epithelial cells present:
        - (i) Principal cells (MAIN) → fine-tunes urine composition
            - MAJOR site of renal transport regulation of Na^+ and K^+ → via Aldosterone (↑ Na^+ reabsorption and K^+ secretion)
            - MAJOR site of controlling H_2O reabsorption → via ADH (↑ H_2O absorption) → 7% to 19.7% H_2O reabsorbed

- (ii) Intercalated cells (type A and B) → involved in acid-base regulation (H^+ secretion and HCO_3^- reabsorption)

Note – There are two types of nephrons (based upon position in cortex):
- (1) Cortical nephron (85%)
  - Glomeruli in the outer portion of renal cortex → Possess a "short" LoH with a shortened thin ascending limb
- (2) Juxtamedullary nephrons (15%)
  - Glomeruli in the juxta-glomerular regions of cortex → Possess a "long" LoH that extends far into the medullary pyramids and then ascends via a very long thin ascending limb
  - Associated with the "Vasa Recta" (originates from efferent arterioles of JM nephrons) → role in CCE → reabsorbs H_2O/solute from interstitium while maintaining ↑ medullary interstitial gradient

Juxta-Glomerular Apparatus (JGA):
- Formed where the TAL of LoH and DCT passes between the afferent and efferent arterioles at the vascular pole of the glomerulus
- Comprises of:
  - (1) Macula Densa
    - Modified tubular epithelial wall of the DCT (located at the angle between afferent and efferent arterioles)
    - Contains specialised cells that sense changes in the filtrate NaCl levels → involved in intrinsic regulation of GFR and RBF by "Tubuloglomerular feedback" and control of renin secretion
  - (2) Juxtaglomerular (Granular) cells
    - Specialised smooth muscle cells found in the afferent arteriolar wall (esp "media") that possess:
      - (a) Renin-containing granules → secreted to invoke RAAS
      - (b) Intrarenal-baroreceptors → involved in intrinsic autoregulation of RBF/GFR by a myogenic mechanism
  - (3) Extraglomerular mesangial (Lacis) cells
    - Participate in a signalling pathway of the Tubuloglomerular feedback
    - Possess contractile elements (actin and myosin) → contract in response to SNS stimulation → thereby ↓ glomerular surface area and GFR



Blood supply of the kidney and nephrons:

- Blood is delivered to and from the kidney via the following course:

  Renal artery → Interlobar art. → Arcuate art. → Interlobular art. → Afferent art. → Glomerular capillary loops → Efferent art. → Peritubular capillaries (+/- vasa recta) → Interlobular vein → Renal vein

- Each nephron has 2x arterioles (afferent and efferent) and 2x capillary beds (glomerular and peritubular)
- Note that in juxtamedullary nephrons → efferent arteriole divides into:
  - (i) Peritubular capillaries
  - (ii) Vasa recta → extend into inner medulla and forms hairpin loops → vital for (a) oxygenation of renal medulla and (b) counter-current exchange mechanism (Ie. maintains ↑ medullary interstitium gradient)

Nerve supply:

- Rich sympathetic innervation by NAd-ergic neurons (T10-L1) → to afferent/efferent art., JGA and tubular system (PCT, TAL of LoH, DCT)
- Some vagal innervation and DA neurons → unknown function

Renal Clearance:

Definition of renal clearance:

- Renal clearance is the volume of plasma completely cleared of a particular substance by the kidneys per unit time → unit is expressed as mL/min (volume of plasma over time)

Calculating renal clearance:

- The amount of substance excreted in urine per unit time (U_X x V) must EQUAL the amount of substance brought to the kidney in a relevant plasma volume over an equal period of time (C_X x P_X) → thus:

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

C_X = Renal clearance
U = urine [ ]
P = plasma [ ]
V = urine flow rate

Uses of renal clearance:

- Renal clearance of a substance is determined by the handling of the substance by the kidney → This involves – (i) Filtration (via the glomerulus), and (ii) Secretion and/or reabsorption (via the tubules)
- As a result, there is a range of renal clearance values of physiological significance:

Clearance (C_x)	Implication	Example of substance
0	Substance is either (i) completely reabsorbed by tubules, OR (ii) not filtered by glomerulus	Glucose → freely filtered at glomerulus BUT virtually 100% reabsorbed in PCT
$0 < C_x <$ GFR	Substance is partially reabsorbed by tubules	- Urea → freely filtered by glomerulus but 40-60% reabsorbed in tubules - Glucose in setting of DM → T_{MAX} is exceeded due to ↑ BGL → not all filtered glucose is reabsorbed
GFR	Substance is freely filtered by glomerulus and neither secreted nor reabsorbed by tubules	Inulin, ^{51}Cr-EDTA, ^{98}Tc-DTPA, and ^{125}I
GFR < C_X < RPF	Substance is freely filtered by glomerulus and partially secreted by tubules	Creatinine
RPF	Substance is completely filtered by glomerulus and completely secreted by tubules	Para-aminohippuric acid

Fractional excretion:

- Fraction excretion is the fraction of filtered mass of a substance that the excreted mass represents

$$FE_X = C_x/GFR$$

Thus,

- FE < 1 → net reabsorption
- FE = 1 → free filtration only
- FE > 1 → net secretion

Glomerular Filtration and Renal Blood Flow:

(I) Glomerular Filtration:

Glomerular ultrafiltration:

- It is the fluid filtered within the renal corpuscle from glomerular capillary blood across a filtration barrier into Bowman's space under action of opposing $P_{HYDROSTATIC}$ and $P_{ONCOTIC}$ (Ie. bulk flow)
- Its composition is influenced by the highly permeable but highly selective properties of the filtration barrier (See above) → contains electrolytes, glucose and amino acids in same [ ] as plasma BUT without cells and large -vely charged molecules (Eg. proteins)

Glomerular filtration rate:

- Defined as the amount of glomerular ultrafiltrate formed divided by the time of filtration (normally GFR is ~ 125 mL/min or 180 L/day)
- Note: The excessively ↑ GFR implies that plasma volume (3L) is filtered 60x per day → allows excretion of metabolic/exogenous wastes (which are in small quantities in plasma)

Determinants of glomerular filtration:

$GFR = K_F \bullet NFP$

Where:
- NFP = $[(P_{GC} - P_T) - \sigma(\pi_{GC} - \pi_T)]$
- K_F = (Filtration surface area) • (membrane permeability)

BUT π_T is negligible, thus → **$GFR = K_F \bullet [P_{GC} - P_T - \sigma \bullet \pi_{GC}]$**

Nb. GFR is heavily influenced by the $P_{HYDROSTATIC}$ of the glomerular capillary (P_{GC})

- (1) Net filtration pressure (NFP):
  - NFP is the balance of the hydrostatic and osmotic pressure across the glomerular filtration barrier between the glomerular capillary blood and Bowman's space

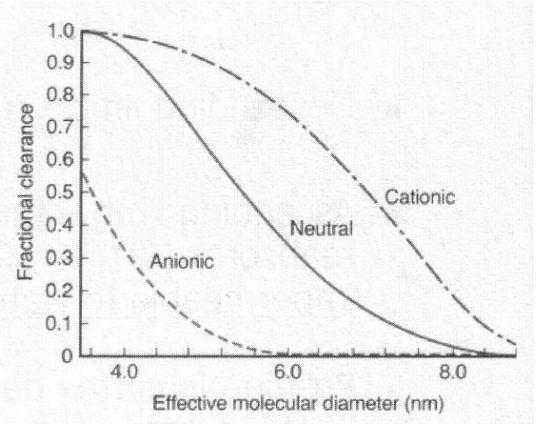
$$NFP = [(P_{GC} - P_T) - \sigma(\pi_{GC} - \pi_T)]$$

  - NFP ↓ along the length of the glomerular capillary due to:
    - (i) ↓ $P_{HYDROSTATIC}$ along the glomerular capillary → Note that the ↓ is minute (cf. systemic capillaries) → this is because of the large SA collectively providing a small resistance to flow
    - (ii) ↑ $P_{ONCOTIC}$ along the glomerular capillary → Note that the ↑ is substantial (cf. systemic capillaries) → this is because H_2O is filtered into Bowman's capsule, thus ↑ [protein] in vasculature

	Afferent end of glomerular capillary (mmHg)	Efferent end of glomerular capillary (mmHg)
P_{GC}	60	58
P_T	15	15
π_{GC}	21	33
NFP	24	10

$NFP_{AVERAGE}$ = 17 mmHg

- Determinants of NFP:
  - (i) Glomerular capillary hydrostatic pressure (P_{GC}) → influenced heavily by RBF which is determined by:
    - (a) Renal arterial pressure – Proportional to systemic BP (MAP) → BUT this effect is minimised by mechanisms that autoregulate RBF between MAP 75-170 mmHg
    - (b) Relative resistance of afferent and efferent arterioles
      - Afferent arteriole – Constriction → ↓ P_{GC} (and GFR); Dilation → ↑ P_{GC} (and GFR)
      - Efferent arteriole – Constriction → ↑ P_{GC} (and GFR); Dilation → ↓ P_{GC} (and GFR)
  - (ii) Bowman's capsule hydrostatic pressure (P_T):
    - Obstruction to flow of fluid along the tubules or extra-renal parts of urinary system (Eg. ureteral obstruction, oedema of kidney) → causes ↑ P_T → ↓ GFR
  - (iii) Glomerular capillary oncotic pressure (π_{GC}):
    - ↑ [plasma protein] (Eg. dehydration, ↓ RBF) → ↑ π_{GC} → ↓ GFR
    - ↓ [plasma protein] (Eg. cirrhosis, fluid overload) → ↓ π_{GC} → ↑ GFR
  - (iv) Tubular oncotic pressure (π_T) is negligible
  - (v) Reflection co-efficient (σ) → represents "leak" of plasma proteins across capillary membrane
    - Glomerulus normally has $\sigma = 1$ (no leak)
    - σ can ↓ with disease (Eg. nephritis → proteinuria) → ↑ GFR
- (2) Filtration coefficient of the glomerular filtration barrier (K_F):
  - (i) Filtration surface area (SA) → altered by contraction of mesangial cells in renal corpuscle:
    - AII, ADH, NAd → mesangial cells contraction → ↓ SA and GFR
    - ANP, DA, PGE2, PGI2 → relax mesangial cells → ↑ SA and GFR
  - (ii) Membrane permeability → Nb. 10-100x ↑ cf other capillary beds
    - (a) Size – Particles < 7 kDa (Eg. glucose, ions, urea, H_2O) are filtered freely, between 7-70 kDa are ↓ freely filtered, and > 70 kDa (Eg. albumin) are not filtered; neutral particles < 4 mm diameter filtered freely, between 4-8 mm are partly filtered, and > 8 mm are excluded
    - (b) Charge – Cationic substances filtered more readily cf. anionic substances (Ie. -vely charged plasma proteins, such as albumin) → due to –vely charged glycoproteins in filtration barrier



(II) Renal blood flow:

Overview of renal blood flow (and renal plasma flow):

- Kidneys receive 20% of CO at rest → thus "Renal blood flow" is 1200 mL blood/min
    - Highest blood flow per gram of tissue at 420 mL/100g/min (Nb. kidneys are 150 g each → 1% body weight) → thus very high O_2 delivery (10x more than required for metabolic rate)
    - Highest flow to cortex (95% of RBF) vs. medulla (5% of RBF)
    - Importance of ↑ RBF → produce large amounts of glomerular ultrafiltrate for urinary excretion of waste products

> Aside: Renal plasma flow (RPF) = [1 – HCrt] x RBF = 600 mL/min (assuming HCrt 45%)
> - 20% of plasma delivered → Forms GFR (125 mL/min) → of which 99% reabsorbed (so 1.5 L of urine made per day)
> - 80% of plasma delivered → enters peritubular capillaries → partakes in tubular function (reabsorption and secretion of substances)

- Control of RBF (and RPF) are dictated by the main resistance vessels (afferent and efferent arterioles):
    - Afferent arteriole – Constriction → ↓ RBF; Dilation → ↑ RBF
    - Efferent arteriole – Constriction → ↓ RBF; Dilation → ↑ RBF

Metabolic and O_2 demands of the kidney:

- Kidneys have a very ↑ metabolic rate at 6 mL O_2/100g/min (18 mL O_2/min) → related to (i) GFR, (ii) degree of active Na^+ reabsorption, and (ii) activity of H^+ ATPase pumps
- HOWEVER, renal O_2 delivery (and RBF) EXCEEDS renal O_2 metabolic demand by 10X → this means kidney O_2 extraction rate ↓ at 14 mL O_2/L blood (cf. 46 mL O_2/L blood for whole body) → but this varies on the part of the nephron:
    - Cortex → Low O_2 extraction ratio because (i) Not very metabolic (PO_2 50 mmHg) and (ii) very ↑ blood flow (10x more than medulla)
    - Medullary → High O_2 extraction ratio because (i) Very metabolic (need to maintain osmotic gradient in medullary interstitium with Na^+ reabsorption in TAL; PO_2 15 mmHg) and (ii) very ↓ blood flow

> Note: Despite O_2 delivery (and RBF) in excess of O_2 demand, the kidneys are susceptible to ischaemic damage (esp the medulla) if there is any large ↓ in total O_2 delivery (and RBF) → this is because:
> - (1) RBF is autoregulated to maintain GFR and Na^+ balance, rather than to meet renal metabolic demand
> - (2) Renal metabolic rate has little autoregulatory influence over peritubular capillary blood flow
> - (3) Counter-current exchange of O_2 in vasa recta ↓ peritubular capillary PO_2 as they descend into the medulla
> - (4) ↓ blood flow in medulla (by 10X cf. cortex)
> - (5) Medulla has the highest metabolic demand (cf. cortex) due to Na^+ reabsorption to maintain the ↑ medullary interstitial osmotic gradient

(III) Filtration fraction:

- "Filtration Fraction" is the ratio of GFR to the renal plasma flow (normally 20%)

$$\text{Filtration fraction} = \frac{\text{GFR}}{\text{RPF}} \times 100\% = \frac{125 \text{ mL/min}}{600 \text{ mL/min}} = 20\%$$

> Recall → RBF = RPF/(1-Hcrt)

Afferent arteriole				
Constriction	↓ P_{GC}	↓ GFR	↓ RBF (or RPF)	FF constant
Dilation	↑ P_{GC}	↑ GFR	↑ RBF (or RPF)	FF constant
Efferent arteriole				
Constriction	↑ P_{GC}	↑ GFR	↓ RBF (or RPF)	↑ FF
Dilation	↓ P_{GC}	↓ GFR	↑ RBF (or RPF)	↓ FF

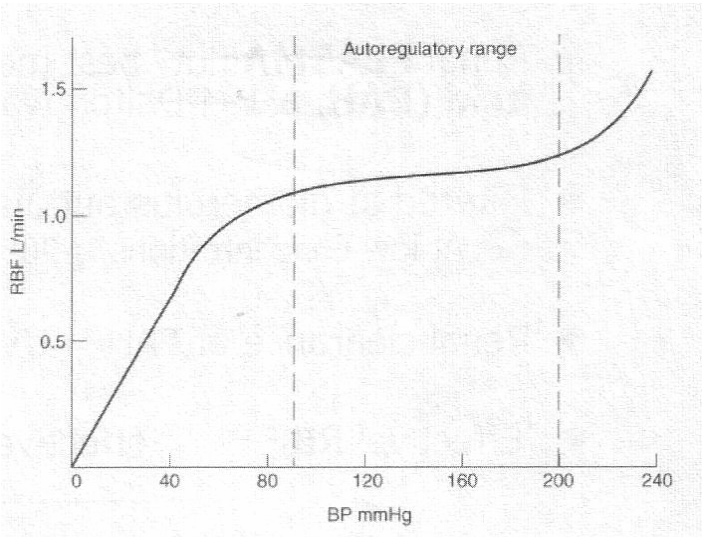
Note – It can be seen that:
- FF is INDEPENDENT on the AFFERENT arteriolar calibre (Ie. changes to the afferent arterioles produce a congruent Δ in GFR and RBF (or RPF) → thus, no Δ in FF)
- FF is DEPENDENT on the EFFERENT arteriolar calibre constriction or dilation, such that (Ie. produces incongruent Δ in GFR and RBF (or RPF) → thus, causes Δ in FF)

(IV) Regulation of GFR and RBF:

Remember:
- GFR is heavily influenced by the $P_{HYDROSTATIC}$ of the glomerular capillary → which is determined by RBF
- RBF is mainly influenced by the main resistance vessels of the kidneys → which is determined by the calibre of the Afferent and Efferent arterioles

- (1) Intrinsic (autoregulation) of GFR and RBF:
  - RBF (and thus GFR) are autoregulated and kept constant within a MAP range of 75-170 mmHg via changes in calibre (and resistance) of the AFFERENT arteriole of the kidney

Note:
- Efferent arteriole is NOT involved in autoregulation!
- Autoregulation of GFR and RBF can be overridden by external influences (Eg. hormones and SNS neurons), even when renal perfusion pressure is between MAP 75-170 mmHg!



  - (a) Myogenic regulation
    - VSMC of the afferent arterioles contract in response to vessel wall stretching caused by ↑ renal perfusion pressure → causes afferent arteriolar vasoconstriction → normalise GFR and RBF
  - (b) Tubuloglomerular feedback

- Intrinsic mechanism that regulates 1°ly GFR (and RBF 2°ly) → inherent to all nephrons within the kidney → it ensures that each nephron has a relatively constant rate of ultrafiltrate delivered to its DCT (whatever the GFR may be) by controlling its glomerular blood flow via a –ve feedback reflex effect on the afferent arteriolar tone
- It has a rapid effect (within seconds) → RAAS has a long-term role by adjusting the mechanism's set-point

Mechanism:
- Macula densa (within EDCT in JGA) indirectly measures GFR (and RBF) by gauging the rate of ultrafiltrate delivery to it → achieves this by measuring the ionic composition of tubular fluid (Na^+ and Cl^-) using a NKCCT co-transporter such that:
  - (i) ↑ [NaCl] → means ↑ UF delivery to MD → ↑ GFR (and RBF)
  - (ii) ↓ [NaCl] → means ↓ UF delivery to MD → ↓ GFR (and RBF)
- Through this "sensor mechanism", the macula densa then sends a feedback signal through the JGA to the glomerulus to regulate GFR (and RBF) by altering the tone of the renal afferent arteriole as follows:
  - (i) ↑ [NaCl] or GFR causes the macula densa to:
    - (a) Release Adenosine → binds A1 receptors on Extraglomerular mesangial cells → causes ↑ in IC [Ca^{2+}] → signal transmission across gap junctions to induce afferent arteriolar vasoconstriction → ↓ GFR
    - (b) ↓ PGE2, PGI2 and NO levels → inhibits afferent arteriolar vasodilation → ↓ GFR
    - (c) Long term → ↓ renin release → ↓ AII-induced efferent arteriolar vasoconstriction → ↓ GFR
  - (ii) ↓ [NaCl] or GFR causes the macula densa to:
    - (a) Initially release Nitrous Oxide → causes immediate afferent arteriolar vasodilation → ↑ GFR
    - (b) Delayed release of PGE2/PGI2 → causes (i) afferent arteriolar dilation, and (ii) renin release from JG cells → AII-induced efferent arteriolar constriction → ↑ GFR

- (2) Extrinsic control of GFR and RBF:
  - (a) Circulating hormones:
    - Afferent arterioles:
      - Dilation → PGE-2, PGI-2, DA, ANP, NO, kinins
      - Constriction → High dose AII, NAd, ET-1, Adenosine, ADH
    - Efferent arterioles:
      - Dilation → Inhibition of AII
      - Constriction → Low dose AII
    - Mesangial cell → Contracts due to AII, ADH and NAd (contraction response inhibited by ANP, DA, PGE2, PGI2)

Note: AII plays a vital role in regulating GFR:
- At physiological (low) doses → it maintains GFR by efferent arteriolar vasoconstriction (at expense of RBF)
- With ↑ AII levels → causes:
  - (i) BOTH afferent and efferent arterioles constriction→ ↓ GFR and RBF
  - (ii) Mesangial cell contraction in renal corpuscle → ↓ K_F → ↓ GFR

  - (b) Renal SNS (noradrenergic) nerves have a minor role:
    - (i) Constricts BOTH afferent and efferent arterioles → ↓ RBF >>> GFR

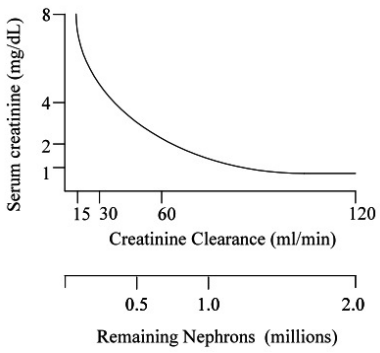
Note: GFR is only mildly affected cf. RBF because ↓ GFR caused by afferent arteriolar vasoconstriction, mesangial contraction, and ↑ π_{GC} (2° ↓ RBF) is offset by a ↑ P_{GC} and GFR caused by efferent arteriolar vasoconstriction

        - (ii) Stimulates renin secretion (via β1 receptors on JG cells) → ↑ AII production → afferent and efferent arteriolar vasoconstriction → ↓ RBF and GFR

(V) Measurement of GFR, RBF and RPF:

Measurement of GFR:

- GFR is determined by measuring the renal clearance of a substance that:
    - (1) Freely filtered from the glomerulus
    - (2) Neither secreted nor reabsorbed in the tubules
    - (3) Must not metabolised nor stored by the kidneys
    - (4) Must not affect renal function
    - (5) Must not be excreted extrarenally
    - (6) Non-toxic and easy to measure in plasma and urine
- Such substances include:
    - (i) Exogenous substances
        - Can use clearance of Inulin (5.2 kDa polymer of fructose → gold standard), or radiolabelled molecules (Eg. ^{51}Cr- EDTA, ^{98}-Tc-DPTA, ^{125}I)
        - Inconvenient measure of GFR as this requires → substance to be infused continuously IV until steady state plasma levels reached → then an accurately timed urine specimen is collected and plasma sample obtained → C_X then calculated using U_X, V_X and P_X
    - (ii) Endogenous substances
        - Creatinine clearance
            - Creatinine is a breakdown product of creatine phosphate (found in skeletal muscle) that is freely filtered by the glomerulus
            - ↑ convenient (cf. exogenous substances) because (i) it is steadily infused into plasma from muscle to produce a constant plasma [ ] (Ie. no infusions needed), and (ii) requires only an accurately timed urine specimen and single plasma sample to determine C_X
            - BUT it is ↓ accurate measure of GFR because creatinine is (i) partly secreted by tubules (overestimates GFR by 10-20%), and (ii) difficult to measure at low [ ] (overestimates GFR also)
        - Urea clearance
            - Urea is a nitrogenous waste product made in the liver whose clearance is ~ 50% of GFR
            - BUT it is not an accurate means of determining GFR because:
                - (a) Variable tubular reabsorption at the inner MCDs depending on the state of hydration (Ie. level of ADH) → varies between 40-60%
                - (b) Plasma [urea] depends on protein intake and metabolism
- Alternatively, GFR can also be "estimated' (eGFR) using plasma creatinine levels:
    - P_{Cr} is INVERSELY proportional to C_{Cr} (and GFR) in a hyperbolic fashion



- Using this principle, the following equations have been derived to estimate GFR:
  - (1) Cockcroft-Gault → GFR = (140-age) x weight x (1.23/P_{Cr}) x (0.85 if ♀)
  - (2) MDRD and Abberviated MDRD → latter requires only 4 variables (Age, gender, African-American ethnicity, and P_{Cr}) → more accurate and precise than the Cockcroft-Gault equation
- Although it is extremely convenient to measure (Ie. no need for infusions or urine and plasma collections) → it has limitations:
  - (1) Grossly inaccurate in situations that influence P_{Cr} (Ie. muscle wasting, malnutrition, Etc)
  - (2) Hyperbolic relationship of P_{Cr} and C_{Cr} implies:
    - P_{Cr} is a very insensitive measure of GFR (unless at very low GFRs) → 50% ↓ in GFR is needed to produce a detectable ↑ P_{Cr}
    - At very low GFRs, any further ↓ GFR produces a massive ↑ in P_{Cr}

Measurement of renal blood flow and renal plasma flow:

- Renal plasma flow (RPF)
  - Determined by calculating the renal clearance of a substance that undergoes complete glomerular filtration and tubular secretion such that NONE remains in the renal venous blood (Ie. 100% renal excretion)
  - The substance must also – (i) not metabolised nor stored by the kidneys, (ii) not affect renal function, (iii) not be excreted extrarenally, (iv) be non-toxic, and (v) be easy to measure in plasma and urine
  - Para-aminohippuric acid (PAH) is thus used to calculate RPF:
    - At low plasma [PAH], all plasma-perfusing, filtering and secreting parts of the kidney virtually clear it completely → 90% PAH excreted in urine
    - As a result, the renal clearance of PAH (C_{PAH}) is actually an underestimate of "Total" RBF → C_{PAH} is thus the "Effective" RPF

$$\text{"Total" RPF} = \frac{\text{ERPF}}{\text{Extraction ratio}} = \frac{625 \text{ mL/min}}{0.9} = 700 \text{ mL/min}$$

> Note: RPF is not clinically measured with PAH as it often underestimates it:
> - (i) Disease states and other organic acids interferes with PAH tubular secretion
> - (ii) When the PAH secretory mechanism is saturated (such as in high plasma [PAH]), PAH is no longer 100% excreted (Ie. ↓ secretion of PAH) → thus, C_{PAH} does not equal RPF (in fact, it approaches GFR)

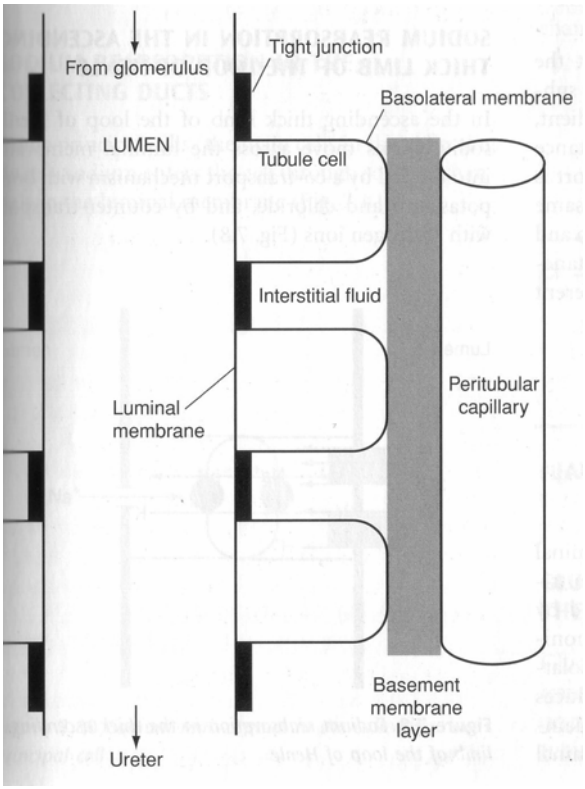
- Renal Blood Flow (RBF):

$$\text{"Renal blood flow"} = \frac{\text{Effective renal plasma flow}}{1 - \text{blood haematocrit}} = 1200 \text{ mL/min}$$

Process of Tubular Reabsorption and Secretion:

Tubular reabsorption and secretion:

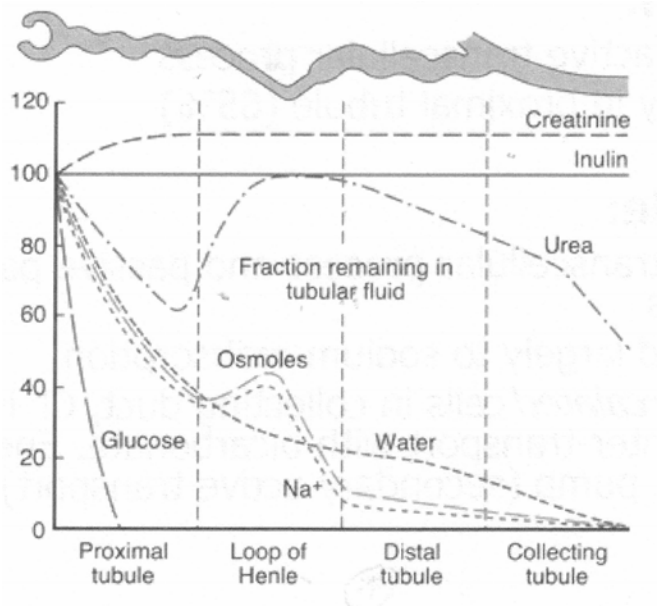
- Tubules consist of a single layer of epithelial cells separated from peritubular capillaries by a basement membrane. Apical tight junctions and interstitial space exist between these epithelial cells throughout the length of tubules



- They influence the composition of plasma ultrafiltrate to be excreted in urine via:
  - (1) Tubular reabsorption
    - MOST H_2O and substances (Eg. Na^+, K^+, Cl^-, HCO_3^-, glucose, urea, Etc.) filtered by the glomerulus is reabsorbed from the tubular lumen → into tubular interstitium → then into peritubular capillary system
    - This occurs via two routes:
      - (a) Transcellular route → across luminal and basolateral membranes of tubular cell
      - (b) Paracellular route → across tight junctions and interstitial space
    - Reabsorption of substances from interstitial space into peritubular capillaries is ALWAYS favoured → because $P_{HYDROSTATIC}$ of peritubular capillaries is LESS than its $\pi_{ONCOTIC}$

      Note – This is because:
      - $P_{HYDROSTATIC}$ ↓↓↓ after blood flows past the glomerular capillary beds
      - $\pi_{ONCOTIC}$ ↑↑↑ after protein-free fluid is filtered at the glomerulus
  - (2) Tubular secretion
    - Some substances are secreted into the tubular lumen for excretion (Eg. H^+, NH_4^+, drugs, Etc.)

Summary of substances handled by tubular reabsorption/secretion:



Mechanism of tubular reabsorption and secretion:

- (1) Passive transport
  - Simple diffusion → substance diffuses ↓ its electrochemical gradient via paracellular or transcellular routes
  - Facilitated diffusion → substance binds a specific membrane carrier protein that facilitates its movement ↓ its electrochemical gradient
  - Solvent drag → substance is carried by movement of H_2O via paracellular route
- (2) Primary active transport:
  - ATPase transporters (Eg. Na^+/K^+ ATPase, H^+ ATPase, H^+/K^+ ATPase, Etc.)
  - "Endocytosis" → reabsorption of small proteins and peptide hormones
- (3) Secondarily active transport
  - Na^+/K^+ ATPase on basolateral membrane of tubular cell actively extrudes Na^+ into interstitium → creates intracellular environment with (i) ↓ [Na^+] and (ii) –ve potential
  - 2° active transport occurs when a membrane protein carrier utilises the energy released from Na^+ movement down its electrochemical gradient (as generated by Na^+/K^+ ATPase) to power the movement of another substance against its electrochemical gradient → the movement of these substances can occur:
    - (i) In same direction across membrane → "Co-transporter"
    - (ii) In opposite directions across membrane → "Exchanger"
  - Important role in tubular transport of electrolytes (Na^+, Cl^-, K^+, P_i, H^+), organic substances (amino acids, glucose, lactate) and H_2O

Transport maximum (T_{MAX}) and Renal threshold:

- "T_{MAX}" is defined as the maximum amount of a particular substance that a membrane transport protein can transport per unit time due to its saturation kinetics → this implies:
  - (i) The amount of substance transported across a membrane transporter is proportional to its plasma [ ] until T_{MAX} is reached
  - (ii) When T_{MAX} is reached, the transporter is "saturated" and any further ↑ in plasma [ ] does not result in any further transport across the membrane

Note – Most T_{MAX} in kidney are very ↑ and difficult to meet → EXCEPT for glucose:

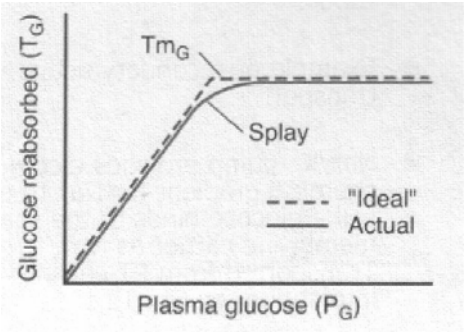
- T_{MAX} of glucose = 375 mg/min (2 mmol/min)
- Normally, all glucose is reabsorbed by PCT via GLUT transporter as GFR of glucose is < its T_{MAX} → with DM and ↑ BGL, GFR of glucose > its T_{MAX} thus glucosuria occurs

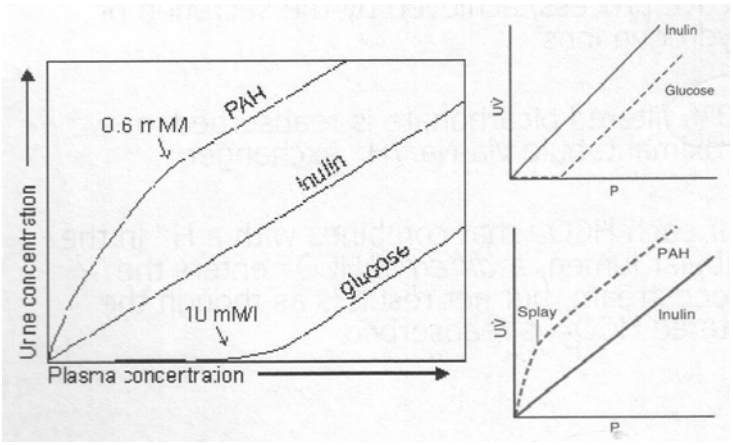
- "Renal threshold" of a substance is the plasma [ ] of the substance at which the outcome of transport saturation is apparent (Ie. plasma [glucose] when glucosuria occurs)

It important to note → the value for "Renal threshold" of a substance is invariably LESS than its "T_{MAX}" (Ie. plasma [glucose] at which glucosuria occurs is actually less then its T_{MAX}) → the difference between these two values is called the "Splay" → this is due to:

- (i) Tubules possessing slight variations in T_{MAX}'s
- (ii) Carrier kinetics → Maximal activity of transport system is substrate-dependent (Ie. 100% transport activity achieved only when luminal [ ] of substance is > T_{MAX})

Eg. For glucose – Renal threshold for glucosuria is a BGL of 10 mmol/L → BUT T_{MAX} occurs only at BGL of 16 mmol/L





Renal Handling of Na^+ and Cl^-:

(I) Renal handling of Na^+:

Overview of renal handling of Na^+:

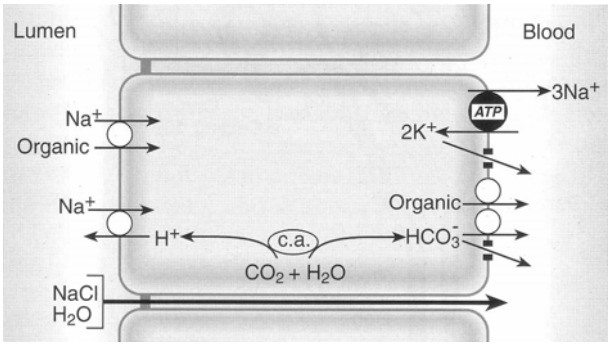
- Glomerulus freely filters a substantial amount of Na^+ → 140 mmol/L x 180 L/day → 25000 mmol Na^+ per day
- BUT only 140 mmol Na^+/day is excreted in urine → tubular system reabsorbed the vast majority (99.5%) of filtered Na^+ → thus, "fractional excretion" of Na^+ is 0.5%

Mechanism of renal Na^+ handling:

- Tubular reabsorption occurs in all tubular segments EXCEPT for the thin descending limb of LoH → PCT (65%), TAL of LoH (25%), EDCT (5%), LDCT and CD (4-5%)
- Majority of reabsorption involves 2°ly active transport via transcellular route:
    - Na^+/K^+ ATPase on basolateral membrane of tubular cell actively extrudes Na^+ into interstitium → depletes IC Na^+ and create –ve intracellular potential
    - This creates an electrochemical gradient within the tubular cell that favours intracellular Na^+ movement from the tubules → via (i) Na^+ channel (absorb Na^+ alone), (ii) Co-transporters (absorb Na^+ with other substances; Eg. glucose, a.a. Etc.), and (iii) Exchangers (exchange Na^+ for H^+)
- Some Na^+ is reabsorbed passively via paracellular route → via solvent drag or due to +ve luminal potential (via paracellular route)
- Note – Na^+ reabsorption plays a vital role in the tubular transport of other substances:
    - (i) Reabsorption of H_2O, electrolytes (K^+, Cl^-, HCO_3^-, PO_4^{3-}) and substances (a.a., glucose, lactate)
    - (ii) Secretion of organic substances and H^+

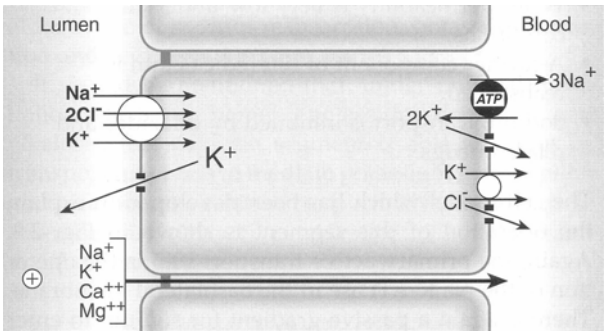
Na^+ reabsorption within the tubular system:

- (1) PCT (65% filtered Na^+ reabsorbed):
    - Na^+ is reabsorbed via 2° active transport transcellularly via:
        - (i) Luminal Na^+/organic co-transporters → Na^+ reabsorbed with glucose, amino acids, inorganic phosphates and lactate
        - (ii) Luminal Na^+/H^+ (NHE-3) exchanger → H^+ produced from IC H_2CO_3 (made from H_2O + CO_2 using CA) is exchanged for tubular Na^+
    - Interecellular junctions of PCT cells are not "tight" → thus, Na^+ can be reabsorbed passively via paracellular route due to:
        - (i) Solvent drag (along with H_2O and Cl^-)
        - (ii) +vely charged lumen (created by Cl^- reabsorption

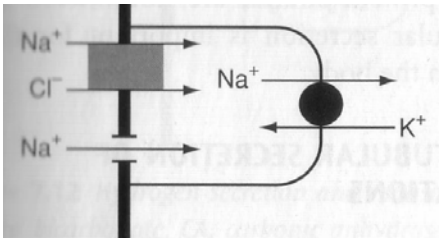


- (2) TAL of LOH (25% filtered Na^+ reabsorbed):
    - Na^+ is reabsorbed via 2° active transport transcellularly via:
        - (i) Luminal Na^+-K^+-$2Cl^-$ (NKCCT) co-transporter → requires luminal K^+ channel for extrusion (recycling) of K^+ to allow NKCCT to function
        - (ii) Luminal Na^+/H^+ (NHE-3) exchanger (similar to in PCT)

- Interecellular junctions also not "tight" → thus, Na^+ (along with other cations) can be reabsorbed passively via paracellular route due to +vely charged lumen

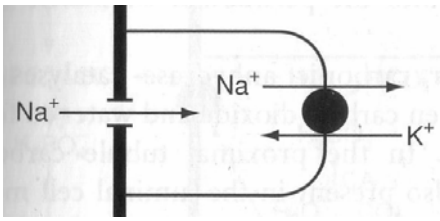


- (3) Early DCT (5% filtered Na^+ reabsorbed):
  - Na^+ is reabsorbed via 2° active transport transcellularly via → (i) Luminal Na^+-Cl^- co-transporter, and (ii) Luminal Na^+ channel



- (4) Late DCT and Collecting duct (4-5% filtered Na^+ reabsorbed):
  - Na^+ is reabsorbed via 2° active transport transcellularly via luminal ENaC channels → found on Principle cells (main cells of the CD)

Note – Main site of Na^+ regulation → affected by aldosterone



(II) <u>Renal handling of Cl^-</u>:

Overview of renal handling of Cl^-:

- Glomerulus freely filters a substantial amount of Cl^- → 18000 mmol Cl^- per day
- BUT only 140 mmol Cl^-/day is excreted in urine → tubular system reabsorbed the vast majority (99.2%) of filtered Cl^- → thus, "fractional excretion" of Cl^- is 0.8%

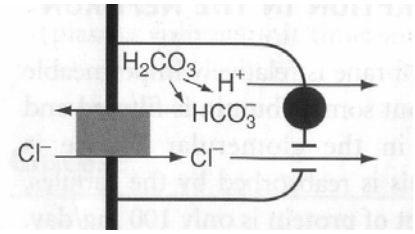
Note – Daily Cl^- intake requirement → 1.3-1.9 mmol/kg

Mechanism of renal Cl^- handling:

- Tubular reabsorption occurs in all tubular segments EXCEPT for the thin descending limb of LoH → PCT (65%), TAL of LoH (25%), DCT and CCD (~ 10%)
- Majority of reabsorption involves 2°ly active transport coupled to Na^+ reabsorption (via transcellular route)
- Minority of reabsorption is passively due to solvent drag or –vely charged lumen (via paracellular route)

Cl^- reabsorption within the tubular system:

- (1) PCT (65% of filtered Cl^- reabsorbed) → Cl^- reabsorbed passively (along with Na^+) via paracellular route due to solvent drag
- (2) TAL of LoH (25% of filtered Cl^- reabsorbed) → Cl^- reabsorbed by 2° active transport transcellularly via NKCCT (along with Na^+)
- (3) DCT and CCD (~10% of filtered Cl^- reabsorbed):
    - DCT → Cl^- reabsorbed by:
        - 2° active transport transcellularly (along with Na^+) via Na^+/Cl^- co-transporter
        - Passively via paracellular route due to –vely charged lumen
    - CCD (type B intercalated cells) → Cl^- reabsorbed by:
        - 2° active transport transcellularly via HCO_3^-/Cl^- exchanger → IC CA hydrates CO_2 into H_2CO_3 which dissociates into H^+ and HCO_3^- → HCO_3^- is the exchanged for tubular Cl^-, while H^+ is reabsorbed using H^+ ATPase
        - Passively via paracellular route due to –vely charged lumen



(III) <u>Control of Renal Na^+ Handling</u>:

Importance of Na^+ regulation in body:

- ECF content of Na^+ is the main determinant of ECFV → reasons:
    - ECFV is determined by the total amount of osmotically active solute present in this fluid compartment → 1°ly Na^+ and Cl^- (90%)
    - BUT Δ in ECF Cl^- content occurs 2° to Δ in ECF Na^+ content → thus, ECF Na^+ content is the true determinant of ECFV
- ECFV (as determined by ECF Na^+ content) → influences PV → which determines CVS $P_{HYDROSTATIC}$ → thus:
    - ↓ ECFV (a/w ↓ body Na^+ content) → ↓ PV → ↓ CVS $P_{HYDROSTATIC}$ (or MAP)
    - ↑ ECFV (a/w ↑ body Na^+ content) → ↑ PV → ↑ CVS $P_{HYDROSTATIC}$ (or MAP)
- -ve feedback reflex system regulates MAP → involves sensing Δ in CVS $P_{HYDROSTATIC}$ via:
    - (i) Low pressure baroreceptors (atria and great vessels) → sense 5-10% Δ PV
    - (ii) High pressure baroreceptors (carotid sinus and aortic arch) → sense > 10% Δ PV
    - (iii) Intra-renal baroreceptors (JG cells in JGA) → sense Δ renal arterial BP
- Signals from these baroreceptors → sent to integration centres → effector responses involve modulating body Na^+ content (esp by kidneys) → restores ECFV (and PV) and Δ in MAP

> Note – ECF content of Na^+ → influenced by body Na^+ balance:
> - Daily Na^+ intake → 1-1.4 mmol/kg/day (~ 100-300 mmol/day in 70 kg adult) orally
> - Na^+ loss → Kidney (most), then sweat and faeces (least)

Overview of renal Na^+ regulation:

$$Na^+ \text{ excretion} = Na^+ \text{ filtered} - Na^+ \text{ reabsorbed}$$

Thus, renal Na^+ regulation depends on:

- (1) Degree of glomerular filtration of Na^+ → GFR
- (2) Degree of tubular reabsorption of Na^+

> Note – Control of tubular reabsorption of Na^+ is a MORE important than GFR in terms of long-term Na^+ excretion because:
> - (i) GFR is heavily autoregulated
> - (ii) Glomerulotubular balance blunts any major changes in Na^+ excretion that would have resulted from minor changes in GFR changes that actually occurs

> Note – Given that the kidney filters 25,000 mmol Na^+/day and reabsorbs 99.5% of it → a 1% change in Na^+ renal handling contributes to a significant flux in Na^+ content → major impact on ECFV and MAP!

Renal Na^+ regulation: Control of GFR

- (1) Intrinsic autoregulatory factors (tubuloglomerular feedback and myogenic mechanism)
  - MAP has minor effect on GFR over MAP range 70-175 mmHg → BUT changes in BP that invoke baroreceptor reflexes (BRR) can override these autoregulatory mechanisms → alter GFR and amount of Na^+ filtered
- (2) Extrinsic factors: Body Na^+ content (via ECFV)
  - (a) Direct renal effects – ↓ $[Na^+]$ (or ↓ ECFV) → results in ↓ GFR due to a ↓ glomerular capillary $P_{HYDROSTATIC}$ and ↑ glomerular capillary $P_{ONCOTIC}$ → ↓ GFR and Na^+ filtered
  - (b) Indirect renal effects – ↓ $[Na^+]$ (or ↓ ECFV) → stimulates arterial, venous and cardiac BRR → neurohormonal response → to ↓ GFR and Na^+ filtered via:
    - (i) ↑ SNS and RAAS activity → cause afferent and efferent arteriolar constriction and mesangial cell contraction
    - (ii) ↑ ADH → cause afferent arteriolar constriction and mesangial cell contraction
    - (iii) ↓ ANP → inhibit afferent arteriolar dilation and mesangial cell relaxation

Renal Na^+ regulation: Control of Tubular Reabsorption

- (1) Glomerulotubular balance:
  - Intrinsic autoregulatory mechanism that minimises the effect of changes in GFR on Na^+ and H_2O excretion
  - It functions on the basis that the PCT reabsorbs a constant proportion of glomerular filtrate (65% of filtered Na^+/H_2O), rather than a constant amount

    > In effect – ↑ GFR = ↑ filtration of Na^+/H_2O = ↑ Na^+/H_2O reabsorption

  - Mechanism:
    - With ↑ GFR → large amount of plasma is filtered at the glomerulus → leads to ↑ $\pi_{ONCOTIC}$ of plasma in peritubular capillaries
    - This results in an ↑ gradient that – (i) Favours tubular reabsorption, and (ii) Counteracts the effect of ↑ GFR on fluid leaving the PCT
- (2) Renal interstitial hydrostatic pressure:
  - ↓ ECFV (and ↓ Na^+) results in ↓ MAP → leads to (i) ↓ $P_{HYDROSTATIC}$ and (ii) ↑ $\pi_{ONCOTIC}$ of peritubular capillaries → thus, ↑ Na^+ (and ↑ H_2O) reabsorption from tubular interstitium into peritubular capillaries
- (3) Hormonal influences:
  - (a) Aldosterone

        - Steroid hormone made in zona glomerulosa of adrenal cortex → secreted in response to AII (via RAAS), ACTH, and ↑ plasma [K^+]
        - Alters protein translation (inducing production of tubular basolateral Na^+/K^+ ATPase and luminal ENaC and K^+ channels) → causes ↑ Na^+ reabsorption by DCT and Principal cells of CCD

        > Nb – MOST important regulator of Na^+ reabsorption → as ~2% of filtered Na^+ can be reabsorbed (= total plasma Na^+ content)!

    - (b) AII
        - Peptide hormone produced as part of RAAS in response to → activation of intrarenal baroreceptor reflex, SNS stimulation of JG cells, ↓ NaCl content in macula densa (due to ↓ GFR)
        - Causes → (i) Direct stimulation of Na^+ reabsorption at PCT, and (ii) Indirect stimulation of Na^+ reabsorption via SNS, AII, and aldosterone
    - (c) Renal SNS stimulation (NAd nerves and circulating NAd/Adr)
        - ↑ SNS outflow triggered by a baroreceptor reflex in response to ↓ MAP
        - Causes → (i) Direct stimulation of Na^+ reabsorption at the PCT (α1 and β1 receptors), and (ii) Indirect stimulation of Na^+ reabsorption via RAAS
    - (d) ADH
        - Peptide hormone produced in hypothalamus → released from posterior pituitary by BRR triggered in response to ↓ MAP
        - Causes → ↑ Na^+ reabsorption at the CCD (principal cells) → acts synergistically with aldosterone here!
    - (e) ANP
        - Peptide hormone produced by RA → released in response to ↑ RA pressures (caused by ↑ MAP)
        - Causes natriuresis via → (i) Inhibition of Na^+ reabsorption in the CDs, and (ii) ↓ RAAS and ↓ ADH activity

"Pressure diuresis and natriuresis":

- A renal compensatory mechanism that maintains long-term regulation of arterial BP by controlling the kidney's excretory ability of Na^+ and H_2O
- Mechanism → Ability of kidney to excrete Na^+ and H_2O is directly proportional to the arterial BP (and ECFV/PV) → via:
    - (a) Renal mechanism
        - In the presence of ↑ arterial BP (or ↑ ECFB or PV) → there is ↑ urinary Na^+ and H_2O excretion due to:
            - (i) ↓ tubular reabsorption 2° to ↑ peritubular capillary $P_{HYDROSTATIC}$ (major)
            - (ii) ↑ glomerular filtration of Na^+ and H_2O (minor)
        - This results in ↓ plasma volume (and ECFV) due to loss of Na^+ and H_2O → normalises arterial BP by ↓ it
    - (b) Extra-renal mechanisms:
        - In the presence of ↑ arterial BP (or ↑ ECFB or PV) → there is ↓ SNS outflow, ↓ RAAS response, ↓ ADH release but ↑ ANP secretion
        - This results in ↓ plasma volume (and ECFV) due to loss of Na^+ and H_2O → normalises arterial BP by ↓ it

Renal Handling of H_2O:

(I) Body H_2O Balance:

- Total body water (TBW) is $2/3^{rd}$ of body weight (42 L in 70 kg adult) → subdivided into:
  - (1) Intracellular fluid (ICF) compartment → $2/3^{rd}$ of TBW (28 L in 70 kg adult)
  - (2) Extracellular fluid (ECF) compartment → $1/3^{rd}$ of TBW (14 L in 70 kg adult) → subdivided further into (i) Intravascular (3 L) and (ii) Interstitial (11 L)
- TBW state is determined by the body's H_2O balance (daily H_2O intake vs loss) → normally, it is balanced (as per table below):

Daily H_2O intake	
Drinking	1200 mL
Food	1000 mL
Metabolism (Eg. ETC)	350 mL
Total intake	*2550 mL/day (in 70 kg adult) → 25-35 mL/kg/day*
Daily H_2O loss	
Urine	1500 mL (includes obligatory loss ~ 430 mL)
Insensible losses (skin, lungs)	900 mL
Faecal	100 mL
Sweat	50 mL
Total loss	*2550 mL/day*

- However, abnormal TBW states arise when an imbalance in body H_2O exists::
  - (i) ↓ TBW ("H_2O deficit" → due to H_2O loss > intake) → results in ↑ plasma osmolality due to a relative ↑ plasma $[Na^+]$ → associated with ↓ ECFV (and PV)
  - (ii) ↑ TBW ("H_2O excess" → due to H_2O intake > loss) → results in ↓ plasma osmolality due to a relative ↓ plasma $[Na^+]$ → associated with ↑ ECFV (and PV)

(II) Daily Urine Output:

- Kidneys filter 180 L H_2O/day at the glomerulus → normally 99.4% is reabsorbed → thus average daily urine output is 1-1.5 L/day
- Daily urine output is determined by two key factors:
  - (1) Need to excrete a constant "solute load" each day (600-700 mosm/day → contains urea 400 mmol/day, NaCl 200 mmmol/day, K^+ 70-100 mmol/day, sulphate, phosphate and other waste products)
  - (2) Need to maintain body H_2O balance → body must excrete H_2O in proportion to the amount in which H_2O intake EXCEEDS "obligatory H_2O losses" in urine, insensible losses, faeces and sweat (= 430 mL + 900 mL + 100 mL + 50 mL = 1480 mL) → since H_2O intake normally exceeds obligatory losses, large volume and more dilute urine is produced cf. "obligatory urine loss"
- As a result, the constant "solute load" is excreted in a daily urine output that varies depending on the body's TBW state (or hydration state):
  - (i) Normal hydration state → 99.4% of filtered H_2O is reabsorbed within the tubular system (in the presence of some ADH) → thus, ~ 1-1.5 L urine is produced each day (at 500-800 mosom/kg) to excrete the "solute load"
  - (ii) Overhydrated state (↑ TBW) → as little as 87% of filtered H_2O is reabsorbed within the tubular system (in the absence of ADH) → thus, as much as 25 L urine is produced each day (min 30-60 mosm/kg) to excrete the "solute load"
  - (iii) Dehydrated state (↓ TBW) → up to 99.7% of filtered H_2O is reabsorbed within the tubular system (in presence of max ADH secretion) → thus, as little as 430 mL urine is produced each day (max 1200-1400 mosm/kg) to excrete the "solute load" → See "Obligatory loss in urine" below

Important to note → "Obligatory loss in urine":

- Minimal urine volume needed to excrete the daily "solute load" → 430 mL/day
- This is because:
  - (i) Body must excrete a daily "solute load" ~ 600 mosmol/day in urine
  - (ii) In presence of ADH, the kidneys can achieve a maximum concentration effect of 1200-1400 mOsm/kg H_2O

- Content of urine (as compared to plasma):
    - (1) Nitrogen containing compounds (Eg. urea [400 mmol/day], creatinine [7 mmol/day], ammonia, uric acid) in high levels
    - (2) Variable amounts of electrolytes (NaCl [100-300 mmol/day], K^+ [50-300 mmol/day], Ca^{2+} [3-8 mmol/day] → depending on homeostasis of plasma levels of electrolytes
    - (3) Virtual absence of glucose, HCO_3^-, amino acids, proteins
    - (4) Acidic pH (5-6) due to net acid excretion of fixed/metabolic acids

(III) Counter-Current Mechanism and Exchanger:

Counter-Current Multiplier (CCM):

- Overview of CCM:
    - CCM is generated by the counter-current flow of tubular fluid within the two limbs of LoH (↓ descending limb, then ↑ the ascending limb) → produces a graded ↑ in medullary interstitial osmolality along its length (max. 1400 mosm/kg H_2O at LoH tip in inner medulla)
    - Its function is vital in transforming glomerular filtration (isotonic to plasma at 300 mosm/kg H_2O) → into either (i) concentrated urine (1200-1400 mosm/kg H_2O), or (ii) dilute urine (30-60 mosm/kg H_2O)

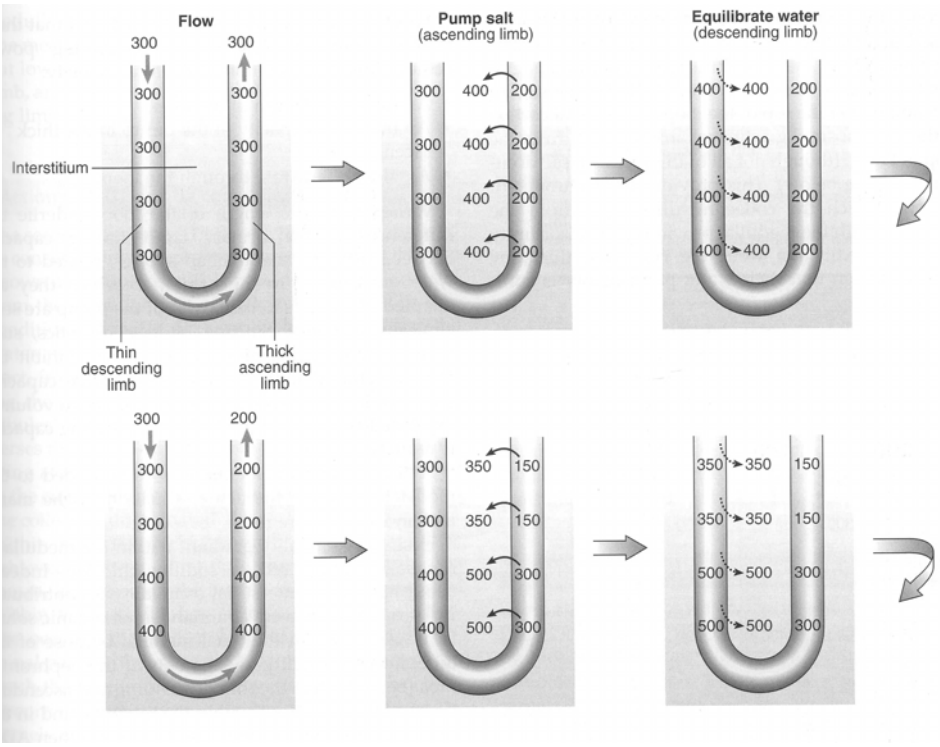
Note:
- (1) Production of concentrated urine (Ie. water deprivation):
    - CCM generates a very hypertonic medullary interstitium (1400 mosm/kg H_2O at tip → urea 650 mosm/kg; NaCl 750 mosm/kg) → this produces hypotonic fluid (100 mosm/kg H_2O) that enters the DCT and CD
    - Within DCT → ongoing NaCl reabsorption BUT no H_2O reabsorption (as it is impermeable to H_2O) → ↓ tubular fluid osmolality < 100 mosm/kg H_2O
    - Within CD → ADH ↑↑↑ its H_2O permeability → H_2O reabsorbed into cortical and medullary interstitium → thus small amounts of concentrated urine is excreted (obligatory urine loss ~ 430 mL at 1200-1400 mosm/kg H_2O → urea 700 mosm/kg; other solutes 700 mosm/kg)
- (2) Production of diluted urine (Ie. water excess):
    - CCM generates a less hypertonic medullary interstitium (650 mosm/kg H_2O at tip → urea 300 mosm/kg; NaCl 350 mosm/kg) → this produces a hypotonic fluid (100 mosm/kg H_2O) that enters the DCT and CD
    - Within DCT and → ongoing NaCl reabsorption BUT no H_2O reabsorption (due to lack of ADH) → thus large volumes of dilute urine is excreted (30-60 mosm/kg H_2O → urea 30 mosm/kg; other solutes 10 mosm/kg)

- Properties of the LOH required to generate CCM:
    - (1) Descending limb of LOH → permeable ONLY to H_2O (and NOT to NaCl)
    - (2) Ascending limb of LOH → comprises of two parts, both of which are ONLY permeable to NaCl (and NOT to H_2O):
        - (i) "Thin" limb → passive NaCl reabsorption (unknown mechanism)
        - (ii) "Thick" limb → NaCl is reabsorbed via (a) NKCCT co-transporter (via 2° active transport; MAIN), and (b) paracellular diffusion due to +ve luminal potential created by NKCCT co-transporter
- Generation of the CCM:
    - (1) Tubular fluid (300 mosm/kg) enters LOH → equilibrates with entire loop and medullary interstitium → thus osmolality of the entire system is 300 mosm/kg
    - (2) TAL of LoH then actively pumps 100 mosm/kg of NaCl into the interstitium → this causes:
        - (i) ↓ TAL osmolality to 200 mosm/kg (due to the efflux of NaCl)
        - (ii) ↑ interstitial osmolality to 400 mosm/kg (due to influx of NaCl)

- (iii) H_2O in descending limb of LOH to then flows into the interstitium → results in osmolality of descending limb of LOH to ↑ to 400 mosm/kg

> Note – TAL of LoH ALWAYS reabsorbs 100 mosm/kg of solute to produce 200 mosm/kg gradient between the TAL and the interstitium

- (3) But fluid continues to enter the LOH at 300 mosm/kg → and above processes keep repeating itself (Ie. fluid flows down the descending loop with H_2O being reabsorbed, followed by NaCl being actively reabsorbed in the ascending loop)
- (4) Net effect is that:
  - (i) Interstitium gets more hypertonic, especially towards the tip of the LOH (max ~ 1400 mosm/kg)
  - (ii) Fluid entering the LOH is 300 mosm/kg but gets more hypertonic as well, especially towards the tip of the LOH (max 1400 mosm/kg)
  - (iii) Fluid leaving the LOH gets more hypotonic towards the DCT (approaches 100 mosm/kg)
  - (iv) LOH reabsorbs 25% of filtered NaCl and 10% of filtered H_2O

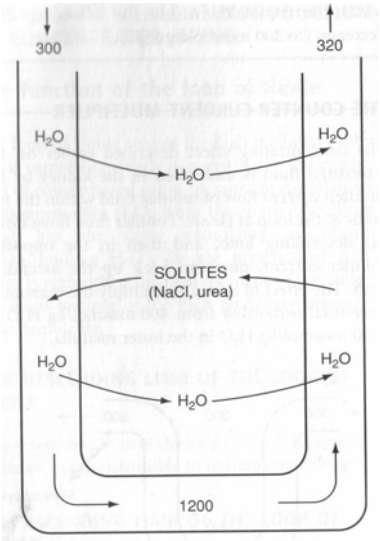


- Basis of the ↑ interstitial osmolality:
  - (1) Na^+, Cl^-, K^+ (and other solutes)
    - Accounts for 50% of osmolality (350 mosm/kg up to 750 mosm/kg)
    - Generated by the 2° active reabsorption of Na^+, Cl^- and K^+ from tubular fluid lumen in TAL of LoH via NKCCT co-transporter
  - (2) Urea
    - Accounts for 50% of osmolality (300 mosm/kg up to 650 mosm/kg)
    - Generated by urea reabsorption in inner MCD as follows:
      - [Urea] in the inner MCDs is ↑↑↑ because – (i) Urea is impermeable in the most of the tubular system, and (ii) it is secreted in LOH due to the very high interstitial [urea] (part of "Urea Trapping")
      - In the presence of ADH → "urea transporters" are upregulated in inner MCD → ↑ facilitate transport of urea into interstitium → ↑

concentrating ability of CCM and allows ↑ H_2O to be reabsorbed concurrently → produce ↑ concentrated urine

Counter-current exchanger (CCE):

- CCE comprises of the Vasa Recta → arranged in hairpin loops closely applied to the limbs of LoH of juxta-medullary nephrons only (NOT cortical nephrons)
- It provides a circulatory means to remove reabsorbed H_2O and solutes from the LoH and CD, WITHOUT compromising the hypertonic interstitial gradient created by the CCM!
- Mechanism of CCE:
    - Passive diffusion of solutes and osmosis of H_2O between the descending and ascending vessels of the vasa recta within the medullary interstitium:
        - (i) With descent into medulla → H_2O is lost into interstitium and NaCl absorbed into vessels → thus plasma osmolality of vasa recta ↑ from 300 mosm/kg to 400 mosm/kg at tip of the loop
        - (ii) With ascent back into cortex → this process is reversed (Ie. H_2O reabsorbed into vasa recta and NaCl leaves into interstitium) → plasma osmolality ↓ to 320 mosm/kg (slightly ↑ than plasma entering it)
    - Note → this process is facilitated by the SLOW flow of blood within the low pressure vessels of the vasa recta



Important to note → CCM's (and CCE's) ability to concentrate urine depends on 4 factors:

- (1) Loop length → JM nephrons have long LoH (due to presence of a "thin" ascending limb) → produce a very concentrated inner medulla
- (2) Strength of ion pump in ascending limb of LOH
- (3) Degree of H_2O impermeability of ascending limb of LOH
- (4) Optimal flow rate in loop → ↑ flow rates (Ie. osmotic diuresis) can wash out medullary gradient, while ↓ flow rates (Ie. ↓ GFR) fails to deliver NaCl to LoH
- (5) Optimal medullary blood flow rate (esp in vasa recta) → ↑ flow rates or lack of blood flow can disrupt the medullary gradient
- (6) Structurally intact kidney (esp medulla) and nephron (esp LoH)

Note → If any of these factors are pertubed (Ie. due to renal disease) → medullary concentration gradient is destroyed → lose urinary concentrating ability (Ie. polyuria)

(III) <u>Renal Handling of H_2O</u>:

Overview of renal H_2O balance:

H_2O excretion in urine = (H_2O filtered by glomerulus) – (H_2O reabsorbed by renal tubular system)

- Glomerulus filters 180 L of fluid per day
- Variable amount of H_2O is reabsorbed within the tubular system (87% to 99.7% or 155 L to 179.5 L) → PCT 65%, TAL of LoH 10%, DCT 5%, CD (7-19.7%)

Mechanism of tubular H2O reabsorption:
- Tubular H_2O reabsorption relies on "Iso-osmotic reabsorption" → this involves:
    - Luminal osmotic pressure ↓ (and tonicity of reabsorbed fluid ↑) due to tubular reabsorption of solutes (esp Na^+)
    - In order to maintain an iso-osmotic environment (and tonicity) → H_2O is consequently reabsorbed via "osmosis" → via trans- and/or paracellular routes in H_2O permeable parts of the tubular system

Renal tubular handling of H_2O:
- (1) PCT:
    - 2° active reabsorption of Na^+ (and other solutes) causes iso-osmotic reabsorption of H_2O via transcellular routes (AQ1 transporters) and paracellular routes (TJs)
    - Thus → 65% of filtered H_2O is reabsorbed along with 60-70% of filtered solute (Na^+ 65%, K^+ 55%) → isotonicity of tubular fluid is maintained (300 mosm/kg)
- (2) LoH:
    - Descending limb (H_2O permeable only) → iso-osmotic reabsorption of H_2O into hypertonic medullary interstitium (as generated by CCM) → produces hypertonic tubular fluid (max 1400 mosm/kg)
    - Ascending limb (NaCl permeable only) → no reabsorption of H_2O, but Na^+, K^+ and Cl^- as reabsorbed by 2° active means (via NKCCT transporter)
    - Thus → 10% of filtered H_2O is reabsorbed, along with 25% of Na^+ and 30% of K^+ → fluid entering DCT is hypotonic (100 mosm/kg)
- (3) DCT:
    - Relatively impermeable to H_2O (only 5% H_2O reabsorbed), but there is ongoing reabsorption of Na^+ (~6-10%) and variable K^+ secretion → thus, fluid entering the CD is even more hypotonic (< 100 mosm/kg)
- (4) CD:
    - Reabsorption of H_2O here varies between 7% and 19.7%, depending on the level of plasma ADH (as determined by body hydration state or H_2O content):
        - In the absence of ADH (Ie. H_2O excess state) → CD remain relatively impermeable to H_2O → only 7% of filtered H_2O is reabsorbed here via iso-osmotic reabsorption into peritubular capillaries → thus, a hypotonic and voluminous urine is produced (25 L at 30-60 mosm/kg)
        - In the presence of ADH (Ie. H_2O deprived state) → CD is permeable to H_2O → up to 19.7% of filtered H_2O is reabsorbed here via iso-osmotic reabsorption into peritubular capillaries → thus, a concentrated and small volume urine is produced (430 mL at 1200-1400 mosm/kg)

Control of renal H_2O handling:
- TBW content (or hydration state) is detected by sensors:
    - (1) Osmoreceptors (anterior hypothalamus)
        - Responds to ↑ plasma osmolality (normal level ~ 280-295 mosm/kg)
        - Very sensitive (detects 1% change) → threshold for stimulation is 280 mosm/kg (near lower normal limit) → steep linear rise > 290 mosm/kg
    - (2) Low-pressure baroreceptors (right atrium and great vessels)
        - Responds to ↓ plasma volume indirectly by ↓ CVS $P_{HYDROSTATIC}$ (↓ MAP)
        - ↓ sensitive cf. osmoreceptors (detects 5-10% change in PV)

    - (3) High-pressure baroreceptors (carotid sinus and aortic arch)
        - Responds to ↓ plasma volume indirectly by ↓ CVS $P_{HYDROSTATIC}$ (↓ MAP)
        - Even ↓ sensitive cf. osmoreceptors (detects > 10% change in PV → large H_2O deficits) → BUT its response overrides that of the osmoreceptors!
- Afferent signals from these sensors are integrated by the hypothalamus → controls effector response via the kidneys (main effector organ) in regulating TBW content
- Renal H_2O handling is influenced by the control of:
    - (1) Tubular H_2O reabsorption (MAJOR) → determined by plasma [ADH] (Ie. affects H_2O permeability at CD) → influenced 1°ly by TBW state (plasma osmolality → hypothalamic osmoreceptors)
    - (2) Glomerular filtration of H_2O (minor) → determined by factors controlling GFR (and RBF) (Ie. intrinsic autoregulatory mechanisms (myogenic and TG feedback mechanisms) and neurohormones (SNS, RAAS, ADH, ANP)) → influenced 1°ly by ECFV (Ie. MAP → BRR)

(IV) <u>Free Water Clearance and Osmolar Clearance</u>:

Overview of free H_2O and osmolar clearance:

- Kidneys produce urine composed of → (i) Solute-containing (or osmotically obligated) H_2O, and (ii) Solute-free (or non-osmotically obligated) H_2O (aka. "Free H_2O")

> Note – "Free H_2O" is defined as → non-osmotically obligated H_2O → it is present in excess of that required to produce a solution with the same osmolality as plasma (Ie. if the solution has an osmolality less than plasma, there is +ve "Free H_2O")

- As a result, urine flow (V) is the sum of:
    - (1) "Free H_2O Clearance" (C_{H2O}) → volume of plasma that is cleared of free H_2O (or non-osmotically obligated or solute-free H_2O) per unit time by the kidneys
    - (2) "Osmolar Clearance" (C_{OSM}) → volume of plasma that is cleared of osmotically active solute-containing H_2O by the kidneys per unit of time

$$V = C_{osm} + C_{H_2O}$$

Free H_2O clearance:

- C_{H2O} can also be defined as the difference between actual urine flow rate (V) and C_{OSM}:

$$C_{H_2O} = V - C_{osm}$$

- It is used to determine individual's hydration state → quantifies relative loss or gain of "free H_2O" (or solute-free H_2O) in urine:
    - $C_{H2O} = 0$ → kidney is producing urine iso-osmotic to plasma (Ie. $C_{OSM} = V$)
    - $C_{H2O} > 0$ → kidney is producing dilute urine by excretion of "free H_2O" (Ie. $V > C_{OSM}$) → note that C_{H2O} can be +14.5 mL/min in absence of ADH
    - $C_{H2O} < 0$ → kidney is producing concentrated urine by conserving "free H_2O" (Ie. $V < C_{OSM}$) → note that C_{H2O} can be -1.3 mL/min with max antidiuresis

Osmolar clearance:

- C_{OSM} can be determined by the product of urine flow (V) and the ratio of urine to plasma osmolality:

$$C_{OSM} = (V \bullet U_{OSM}) / P_{OSM}$$

- As a result, it can also be defined as the urine flow rate (V) necessary to secrete an osmotic load if urine is isotonic to plasma → Ie. $U_{OSM}/P_{OSM} = 1$, and thus $C_{OSM} = V$

Renal Handling of K^+:

Importance of K^+ regulation in body:

- K^+ is the main intracellular cation → ratio of IC:EC of K^+ is vital in determining RMP and excitability of excitable cells (Eg. muscle, nerve, heart cells)
- Daily K^+ intake requirement is 0.7-0.9 mmol/kg/day (~ 60 mmol/day in 70 kg adult)
- Normally, patients remain in K^+ balance by renally excreting any excess K^+ ingested that is not lost in sweat or faeces:

Renal K^+ excretion = (Dietary K^+ intake) – (K^+ eliminated in sweat and faeces)

Overview of renal K^+ excretion:

Urinary K^+ excretion = [K^+ filtered by glomerulus] + [K^+ secreted by CCD/LDCT] – [K^+ reabsorbed by renal tubules]

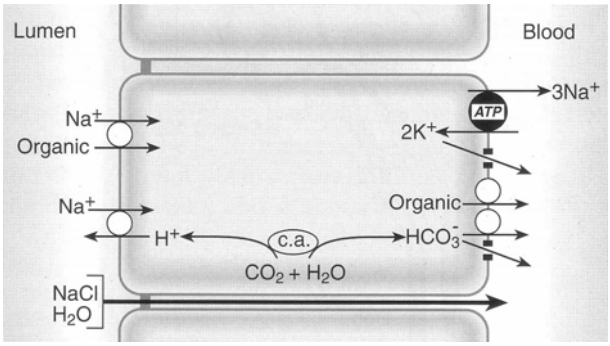
- At the glomerulus → K^+ is freely filtered → 5 mmol/L x 180 L/day → 900 mmol/day
- Within tubular system:
  - (i) Most filtered K^+ is reabsorbed → at PCT (55%), TAL of LoH (30%), Type A intercalated cell of CCD (10%), MCD (5%)
  - (ii) Secretion of K^+ occurs at → Principal cell of CCD and LDCT (varies between 0% to > 15%) → Nb. more K^+ is secreted at CCD than LDCT

Important to note → Renal K^+ regulation occurs MAINLY via control of K^+ secretion at the distal nephron (CCD and LDCT) → and NOT via control of filtration or reabsorption

Important to note → Renal K^+ regulation depends on dietary intake of K^+ such that:
- With normal intake → net effect of LDCT and CCD is to secrete K^+ → thus, fractional excretion of K^+ is 10-20% of filtered load (~ 100-200 mmol K^+/day)
- With high intake → further K^+ secretion by DCT/CCD → thus, fractional excretion of K^+ ↑ to 30% (~ 300 mmol K^+/day)
- With reduced intake → K^+ secretion by DCT/CCD is reduced → thus, net reabsorption so fractional excretion of K^+ ↓ to 5% (~ 50 mmol K^+/day)

K^+ handling within the tubular system:

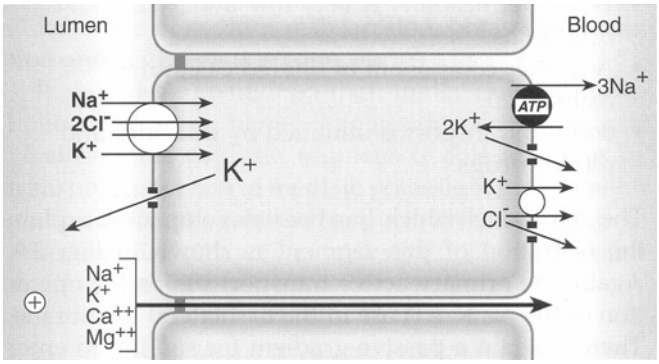
- (1) PCT (55% of filtered K^+ is reabsorbed):
  - K^+ is reabsorbed passively via the paracellular route due to:
    - Solvent drag → coupled to flow of Na^+ and H_2O
    - [ ] gradient → created by reabsorption of H_2O
    - Electrical gradient → due to +ve luminal charge in late PCT



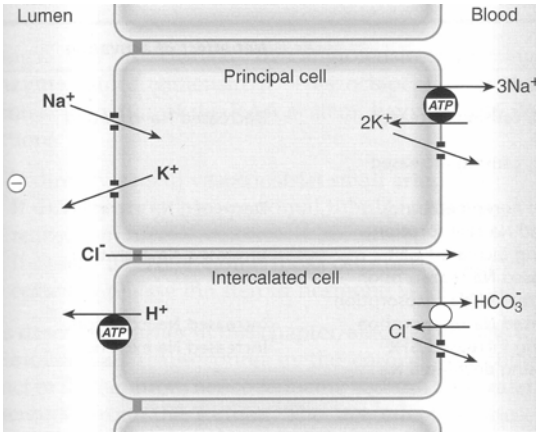
- (2) TAL of LoH (30% of filtered K^+ is reabsorbed):

- K^+ is reabsorbed by 2° active transport via paracellular route → +ve charged lumen generated by ion flux created by NKCCT transporter causes K^+ to be reabsorbed by diffusion down its electrical gradient

> Note:
> - Luminal NKCCT co-transporter causes K^+ to be reabsorbed into the tubular cell, but it is promptly recycled back into the lumen via a luminal K^+ channel → this is needed to allow the NKCCT to function
> - K^+ brought intracellularly by the basolateral Na^+/K^+ ATPase is promptly recycled into the peritubular capillary via a basolateral K^+ channel and K^+/Cl^- co-transporter



- (3) LDCT and CCD (0 to > 15% K^+ is secreted):
  - Principal cells of CCD and LDCT → 2° active secretion K^+ → via:
    - Basolateral Na^+/K^+ ATPase → (i) generates an electrochemical gradient that draws Na^+ intracellularly from the tubular lumen (via the ENaC channel), and (ii) pumps K^+ from peritubular capillaries into tubular cell
    - The –vely charged lumen generated by influx of Na^+ across ENaC channel → favours tubular secretion of K^+
  - Type A intercalated cells of CCD and LDCT → 2° active reabsorption of K^+:
    - H^+ is produced within tubular cell by hydration of CO_2 (using CA) → H^+ is then exchanged for tubular K^+



> Note – CCD and LDCT are the major sites of renal K^+ regulation (mainly influenced by Aldosterone) → they 1°ly secrete K^+ via principal cells (rather than absorb K^+ via type A intercalated cells)

- (4) MCD (5% of filtered K^+ is reabsorbed)

Renal K^+ regulation:

- Renal K^+ regulation occurs MAINLY via control of K^+ secretion at the distal nephron (CCD and LDCT) via the following factors:
    - (1) Circulating factors:
        - (a) Plasma $[K^+]$
            - ↑ $[K^+]$ causes ↑ K^+ secretion by → (i) Directly stimulating Na^+/K^+ ATPase in principal cells, and (ii) Directly stimulating Aldosterone release from adrenal cortex
        - (b) Aldosterone
            - ↑ aldosterone causes ↑ K^+ secretion by → ↑ production of Na^+/K^+ ATPase, K^+ channels and ENaC Na^+ channels in the principal cells
        - (c) Plasma pH
            - Alkalosis via ↓ plasma $[H^+]$ causes ↑ K^+ secretion by → stimulating Na^+/K^+ ATPase in principal cells
    - (2) Luminal factors:
        - (a) Flow of tubular fluid in DCT and CCD
            - ↑ tubular fluid flow causes ↑ K^+ secretion by → maintaining ↓ luminal $[K^+]$ (Ie. continuously washing it away) → permits passive diffusion of K^+ ↓ its [ ] gradient into tubular lumen
        - (b) ↑ Na^+ delivery rate to DCT and CCD → ↑ K^+ secretion
        - (c) -ve lumen potential difference → ↑ K^+ secretion

Note – K^+ balance is NOT affected by:

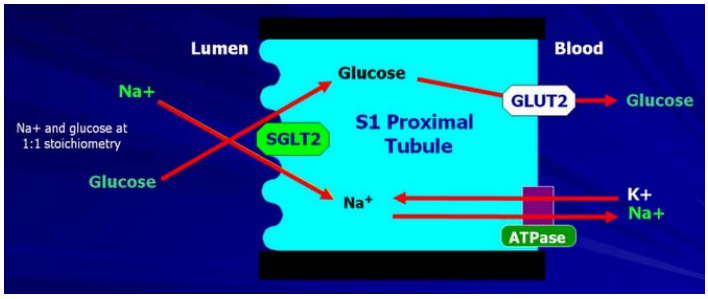
- (i) Na^+ balance
    - ↑ body Na^+ content (and ECFV) inhibits aldosterone secretion → ↓ Na^+ reabsorption AND K^+ secretion also!
    - BUT high tubular flow rates due to ↑ glomerular $P_{HYDROSTATIC}$ → ↑ K^+ secretion in distal nephron → effectively restores K^+ balance in kidneys!
    - Opposite is true with ↓ body Na^+ content (and ECFV)
- (ii) Water balance
    - ↑ TBW inhibits ADH secretion → this causes ↓ K^+ secretion
    - BUT high tubular flow rates due to ↑ urine production → ↑ K^+ secretion in distal nephron → effectively restores K^+ balance in kidneys!
    - Opposite is true with ↓ TBW

Renal Handling of Glucose, Nitrogenous Waste Products, and Drugs:

Renal Handling of Glucose:

Overview of renal glucose handling:

- Glucose is filtered freely at the glomerulus → plasma [glucose] x GFR (or 4.5 mmol/L x 125 mL/min) → rate of 0.56 mmol/min (= 180 g/day)
- Reabsorption of glucose at the PCT via a 2° active transport mechanism:
    - Glucose is cotransported with Na^+ into tubular cell via luminal SGLT-2 → Na^+ moves ↓ electrochemical gradient created by a basolateral Na^+/K^+ ATPase and releases energy to co-transport glucose against its [ ] gradient
    - Glucose is then transported from tubular cell into peritubular capillaries via basolateral GLUT 2 transporters



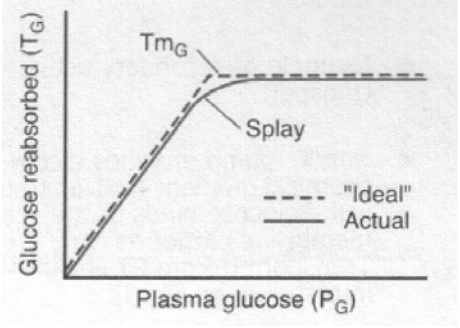
T_{MAX} and renal threshold of glucose:

- T_{MAX} of glucose transport mechanism is ~ 2 mmol/min (or 375 mg/min) → this means:
    - At normal BGL (assuming a normal GFR) → rate of glucose filtration at glomerulus (0.56 mmol/min) is LESS than T_{MAX} → thus, ALL glucose is reabsorbed at the PCT and none is excreted in urine ($C_{GLUCOSE}$ = 0 mL/min)
    - At ↑ BGL (Eg. DM) → rate of glomerular filtration of glucose EXCEEDS T_{MAX} → saturated glucose transporters cannot reabsorb all filtered glucose → glucosuria occurs as ($C_{GLUCOSE}$ > 0 mL/min)

> Note – Assuming a normal GFR → T_{MAX} of glucose transporters is reached at ~ 15-16 mmol/L!

- Renal threshold for glucose is the plasma [glucose] at which it first appears in the urine → ~ 10-12 mmol/L

> It important to note → the renal threshold of glucose is LESS than its "T_{MAX}" (Ie. plasma [glucose] at which glucosuria occurs is actually less then its T_{MAX}) → the difference between these two values is called the "Splay" → this is due to:
> - (i) Tubules possessing slight variations in T_{MAX}'s
> - (ii) Carrier kinetics → Maximal activity of transport system is substrate-dependent (Ie. 100% transport activity achieved only when luminal [ ] of substance is > T_{MAX})



Renal Handling of Protein:

- Glomerular membrane is impermeable to large molecules (esp proteins) → BUT some albumin is filtered (~ 10 mg/L in glomerular ultrafiltrate)
- Tubular system reabsorbs MOST filtered proteins → only 100 mg/day excreted in urine
    - Large proteins are reabsorbed by tubular cells via endocytosis → broken down by lysosomes to a.a. that are taken up by peritubular capillaries
    - T_{MAX} of protein transport mechanism can be easily exceeded (similar to glucose) → results in proteinuria

Renal Handling of Urea:

Overview of urea:

Urea is a nitrogenous waste product made in the liver from the breakdown of protein → excreted in kidneys

$$\text{Glutamate} \xrightarrow{\text{Deaminated in liver}} NH_4^+ \xrightarrow{\text{Urea cycle in liver}} \text{Urea (main) + Glutamine (minor)}$$

Renal handling of urea:

- Urea is filtered freely by glomerulus → plasma [urea] (5 mmol/L) x GFR → 900 mmol/day (Nb. depends on protein intake/metabolism)
- Within the tubular system → Urea is secreted and a variable amount is reabsorbed (40-60% of filtered urea; 350-550 mmol/day) → depending on the state of hydration:
    - (a) PCT → 50% of filtered urea is reabsorbed by passive diffusion → caused by Na^+/H_2O reabsorption which effectively ↑ tubular [urea]
    - (b) LOH, DCT and CCD → tubular [urea] gradually ↑ within the tubular system such that fluid entering the MCD has a very ↑↑↑ [urea] → this is because:
        - (i) These tubular segments are impermeable to urea → but there is ongoing H_2O reabsorption → effectively ↑ tubular [urea]
        - (ii) "Urea trapping" – ↑ medullary interstitial [urea] causes it to be secreted back in the thin descending limb of LOH → allows urea to be recycled back into medullary interstitium at the CDs in the presence of ADH
    - (c) MCD → urea reabsorption is variable → additional 10% filtered urea can be reabsorbed by passive diffusion (depending on the state of hydration):
        - (i) Dehydrated state → ↑ ADH upregulates "Urea transporters" in MCD → ↑ facilitated transport of urea into medullary interstitium → additional 10% filtered urea reabsorbed
        - (ii) Overhydrated state → No ADH → No urea reabsorbed
- Thus daily urea excretion → 400-700 mmol/day (20-50% of filtered load)

> Important points to note:
> - Urea reabsorbed into medullary interstitium (in presence of ADH) → accounts for the ↑ medullary interstitial osmolality (650 mosm/kg of 1400 mosm/kg) → vital for the Counter-current mechanism to function in concentrating and diluting urine
> - Variable degree of tubular urea reabsorption (~ 50% of GFR) → means that it is NOT a substance that can be used to accurately determine GFR

Renal Handling of Creatinine:

- Creatinine is a breakdown product of creatine phosphate found in skeletal muscle

- It is freely filtered by glomerulus → within tubular system it is NOT reabsorbed, BUT it is partly secreted

> Note – $C_{CREATININE}$ overestimates GFR by 10-20% because it is partly secreted by the tubules

Renal Handling of Inulin:

- Inulin is a 5.2 kDa polymer of fructose whose renal clearance approximates GFR → this is because it is freely filtered by the glomerulus and NOT reabsorbed nor secreted by the tubular system

Renal Handling of Para-aminohippuric acid:

- PAH is an organic anion whose renal clearance approximates "effective" RPF → this is because at low plasma [PAH], all plasma-perfusing nephrons freely filters PAH and completely secretes it from the tubular system
- At high plasma [PAH], T_{MAX} of PAH transport system is saturated → not all PAH is secreted → thus, C_{PAH} underestimates RPF

Renal Handling of Drugs and Exogenous Wastes:

- Drugs (Eg. penicillin, aspirin, probenecid, Etc.) and exogenous wastes (Eg. urate, bile salts, PGs, FAs, Etc.) exist as organic cations and anions
- Some are freely filtered by the glomerulus, while others are not and depend on tubular secretion (via active transport carrier) for their excretion

Renal Handling of Acid-Base:

H^+ Balance in the Body:

H^+ production in the body:

- (1) "Volatile acids" (aka. "Respiratory acids")
  - CO_2 is produced by [O] metabolism of carbohydrates and triglycerides (Ie. decarboxylation in TCA cycle) → Majority (75%) is hydrated in plasma to form Carbonic acid (H_2CO_3)
  - H_2CO_3 produces 15000 mmol H^+/day
  - "Volatile acids" do NOT contribute to net acid balance in the body → because all H_2CO_3 in plasma is reformed as CO_2 in the lungs and eliminated
- (2) "Non-volatile acids" (aka. "Metabolic acids" or "Fixed acids")
  - (a) Lactate production
    - Produced from anaerobic metabolism of glucose and glycogen in RBC, skin and skeletal muscle → Produces 1500 mmol H^+day
    - Does not contribute to net acid balance in the body → because lactate is oxidised in the liver to regenerate HCO_3^- (via Cori cycle) → Exception is with excessive production (Ie. lactic acidosis with tissue hypoxia)
  - (b) Sulphuric acid
    - Produced from metabolism of S-containing a.a (esp Cys and Met) → Produces of 45 mmol H^+/day
  - (c) Phosphoric acid
    - Produced from hydrolysis of phosphoproteins → Produces 13 mmol H^+/day
  - (d) Other acids (Eg. HCl produced from a.a. metabolism, ketoacids produced from fat metabolism, Etc.) → Produces 12 mmol H^+/day

> Note:
> - These acids are termed "Fixed acids" because they cannot be excreted by the lungs → Must be excreted by the kidneys (sulphuric acid, phosphoric acid and other acids) or metabolised by the liver (lactate)
> - They contribute to net acid balance in the body → Normally produced at 1-1.5 mmol H^+/kg/day (or ~ 70 mmol H^+/day) → Thus, to maintain acid-base balance, these "fixed acids" must be completely excreted!

H^+ excretion from the body:

- (1) Lungs
  - Eliminate all "volatile acids" (H_2CO_3) → 15000 mmol H^+/day
- (2) Liver
  - Eliminates "fixed acids" (mainly lactate) → 1500 mmol H^+/day
- (3) Kidney
  - Eliminates "fixed acids" (mainly phosphoric acid, sulphuric acid, other acids) as NH_4^+ and "titratable acids"
  - Accounts for at least 70 mmol H^+/day → 30 mmol/day as "titratable acids" and 40 mmol/day as NH_4^+

Renal Regulation of Acid-Base:

Renal regulation of acid-base balance involves tubular H^+ secretion:

- At least 4390 mmol H^+ is actively secreted by kidneys each day:

    - (i) Majority is used to reabsorb filtered HCO_3^- → 4320 mmol H^+ is actively secreted by the tubular system each day to facilitate reabsorption of all 4320 mmol HCO_3^- filtered by the glomerulus (24 mmol HCO_3^-/L x 180 L/day = 4320 mmol) → this does not lead to net excretion of H^+ in urine or addition of new HCO_3^- to blood
    - (ii) Additional 70 mmol H^+ is actively secreted to excrete "fixed/metabolic" acids to achieve a "net acid balance" of zero → 30 mmol/day as filtered buffers (titratable acids) and 40 mmol/day as manufactured buffers (NH_4^+) → this leads to net excretion of H^+ in urine (and addition of new HCO_3^- to blood)
- During acidosis:
    - (a) Tubular H^+ secretion leads to all filtered HCO_3^- being reabsorbed
    - (b) Excess H^+ is secreted and lost in urine bound to non-absorbable buffers (filtered buffers and manufactured buffers) → results in an additional 300 mmol H^+ excreted in urine per day (mainly bound to NH_4^+) and an additional HCO_3^- added to blood
- During alkalosis, excess plasma HCO_3^- (> 28 mmol/L) is excreted in urine by:
    - (a) Loss of filtered HCO_3^- (Ie. not all is reabsorbed in tubular system)
    - (b) Secretion of HCO_3^- from "type B intercalated cells" in CCD

Summary of "net acid excretion":

$$NAE = NH_4^+ + \text{"Titratable acids"} - HCO_3^-$$

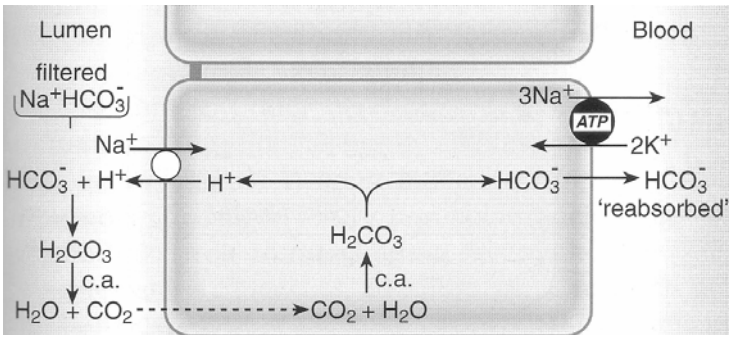
	Normal	Acidosis	Alkalosis
Titratable acids (mmol/day)	30	40	0
NH_4^+ (mmol/day)	40	160	0
HCO_3^- (mmol/day)	0	0	80
NAE / "New" HCO_3^- added (mmol/day)	+70	200	-80
Urine pH	6.0	4.6	8.0

Note: Filtered H^+ at the glomerulus accounts for a very small % of excreted H^+ → 36 nM x 180 L/day = 6.48 umol of excreted H^+ per day

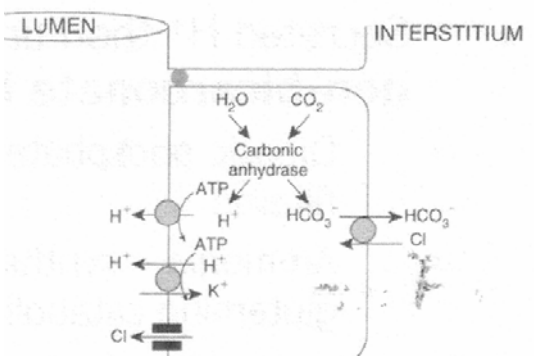
Renal regulation of acid-base balance involves the following processes:

- (1) Secretion of H^+ associated with HCO_3^- reabsorption by renal tubules:
    - Majority of HCO_3^- is reabsorbed by proximal tubular system → PCT (85%) and TAL (10%):
        - High capacity and low gradient system → Because 3900 mmol H^+ secreted/day (and 95% filtered HCO_3^- reabsorbed) BUT lowest urine pH achieved is only 7
        - In the brush border membrane of PCT tubular luminal cells:
            - H^+ is secreted into the tubular lumen via apical Na^+/H^+ antiport → Secondarily active transport dependent on Na^+ gradient generated by basolateral Na^+/K^+ ATPase
            - This H^+ then binds tubular luminal HCO_3^- filtered freely at the glomerulus to form H_2CO_3 → brush border CA then catalyses conversion of H_2CO_3 into CO_2 and H_2O, which are reabsorbed into tubular cells
        - Within tubular cells throughout the tubular system:
            - H_2O and CO_2 reabsorbed from the tubular lumen is catalysed by intracellular CA into H_2CO_3, which dissociates into H^+ and HCO_3^-
            - HCO_3^- produced is reabsorbed into the peritubular capillary

- H^+ produced is then actively secreted back into tubular lumen where it can – (a) Aid in reabsorbing more HCO_3^-, or (b) Participate in net acid excretion once all HCO_3^- has been bound

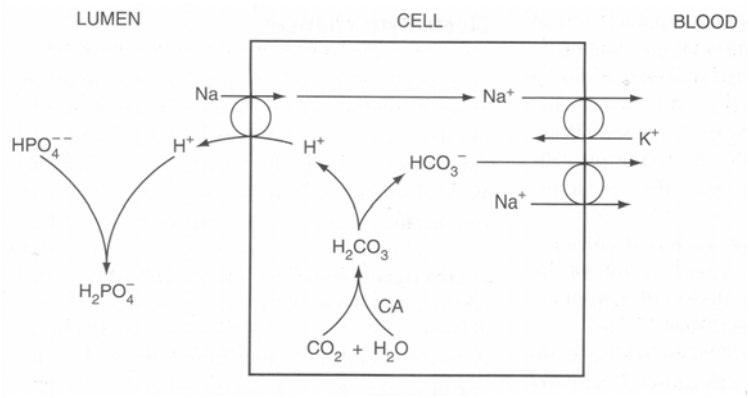


- Minority is reabsorbed by distal tubular system → DCT/CCD (5%):
  - Low capacity and high gradient system → Because 420 mmol H^+ secreted/day (and 5% filtered HCO_3^- reabsorbed) BUT urine pH created is as low as 4.5
  - Within "type A intercalated cells", CO_2 is hydrated to form H_2CO_3 (via intracellular CA) → dissociates into H^+ and HCO_3^-
  - H^+ derived from this reaction is secrete H^+ into the tubular lumen via apical H^+ ATPase and H^+/K^+ ATPase (primary active transports) → controlled by aldosterone
  - HCO_3^- produced via this reaction is absorbed into peritubular capillary



Note – Type B intercalated cells in CCD → secrete HCO_3^- produced from IC dissociation of H_2CO_3 via a luminal Cl^-/HCO_3^- exchanger → little importance because:
- (i) Very few type B intercalated cells (more type A cells in CD)
- (ii) Net tubular HCO_3^- handling is ALWAYS reabsorption (never secretion)

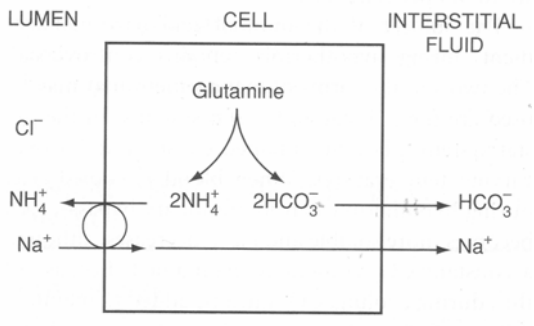
- (2) Formation of titratable acidity:
  - Once all HCO_3^- in tubular fluid are reabsorbed by H^+ secretion, excess H^+ secreted in tubular fluid are bound to "filtered buffers" in the distal tubular system (DCT and collecting ducts) and excreted in urine:
    - (i) Mainly phosphate buffer system → H^+ combines 1°ly with HPO_4^{2-} to form $H_2PO_4^-$ (as urine acidity ~ 4.5 → close to HPO_4^{2-} buffer pKa of 6.8)
    - (ii) Organic buffer systems (Eg. creatinine, sulphate, β-hydroxybutyrate)
  - Within distal tubular cells, CO_2 is hydrated to form H_2CO_3 (via intracellular CA) → dissociates into H^+ (which is secreted into tubular lumen via a secondarily active Na^+/H^+ anti-port → H^+ bind "filtered buffers") and a newly formed HCO_3^- (which is absorbed into peritubular capillary)



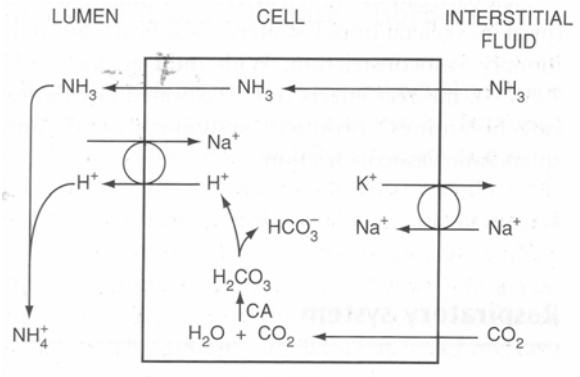
- "Filtered buffers" are vital for excretion of "fixed acids" under normal conditions → Leads to excretion of 30 mmol of "fixed acids" per day
- Note – They cannot be ↑ to excrete additional acid load during acidosis because:
  - (i) Availability of filtered buffers cannot be easily ↑ cf. "manufactured buffers" (Ie. phosphate buffer amount is dependent on diet and PTH levels)
  - (ii) Buffering capacity of filtered buffers maxed out at ↓ urine pHs

> Aside: "Titratable acidity"
> - Measured by the amount of alkali (as NaOH) that must be added to urine to return its pH to 7.4 (which is the pH of glomerular filtrate)
> - Accounts for only a FRACTION of the H^+ secreted by the kidneys:
>   - Accounts mainly for the H^+ that are bound to "filtered buffers" (phosphate and organic buffer systems) and excreted in urine
>   - Does not account for HCO_3^- buffer system → because H_2CO_3 is converted and reabsorbed as CO_2 and H_2O → thus alters urine pH minimally
>   - Ammonia buffer system contributes little to "titratable acidity" → because of high pKa of buffer system (9.1) (Ie. urine only titrated up to a pH of 7.4 and not any further)

- (3) Ammonia secretion:
  - Once all HCO_3^- in tubular fluid are reabsorbed by H^+ secretion, excess H^+ secreted in tubular fluid are also bound to "manufactured buffers" in the distal tubular system (medullary collecting ducts) and excreted in urine
  - Ammoniagenesis → PCT tubular cells produce NH_3:
    - Filtered glutamine is taken up by PCT tubular cells and deaminated via Glutaminase (which is upregulated with chronic acidosis) into NH_4^+ and HCO_3^-
    - NH_4^+ is secreted into the tubular lumen via a Na^+/ NH_4^+ antiport, while "newly formed" HCO_3^- is reabsorbed into peritubular capillary blood



- Ammonium cycling → In the TAL of LOH, 80% of tubular NH_4^+ is reabsorbed → this generates ↑ [ ] gradient of NH_4^+ in the medullary interstitium
- Ammonium excretion → Later in the medullary CD:
  - NH_3 diffuses from medullary interstitium into tubular cells of the CD, then into tubular fluid
  - Within the tubular cells of the CD, H^+ and HCO_3^- is formed by hydration of CO_2 using intracellular CA
  - H^+ secreted into tubular fluid by Na^+/H^+ antiport → combines with tubular NH_3 to form NH_4^+ → "ion trapped" in tubular fluid



- These "manufactured buffers" are vital for excretion of "fixed acids" under both:
  - (a) Normal conditions → Accounts for 40 meq of H^+ excretion per day
  - (b) Acidotic conditions → Can excrete an additional 300 mmol H^+ per day due to:
    - (i) ↑ transfer at low urine pH due to high pKa 9.2 of buffer system (Ie. allows additional H+ to be excreted even when tubular fluid has reached maximal acidity)
    - (ii) ↑ production of glutamine (very slow process)

Control of renal acid-base balance:

- H^+ secretion and excretion is ↑ by the following factors:
  - (1) Raised $PaCO_2$
  - (2) High EC $[H^+]$ → Stimulates glutamine production
  - (3) K^+ and Cl^- depletion → ↑ renal HCO_3^- reabsorption
  - (4) Inhibition of CA → H^+ secretion is inhibited
  - (5) Aldosterone (and cortisol) → Upregulates H^+ and H^+/K^+ ATPases
  - (6) ECF volume depletion → ↑ renal HCO_3^- reabsorption
  - (7) ↓ GFR → ↓ filtration of HCO_3^-

Minimum urinary pH:

- Only a small amount of H^+ can be excreted in its free form → because active transport of H^+ secretion is inhibited at high urinary $[H^+]$ → thus lowest urinary pH achieved is 4.4
- As a result, H^+ secretion and excretion in urine is dependent on binding to "urinary buffers" → (i) HCO_3^- buffer (pKa 6.1), (ii) HPO_4^{2-} buffer (pKa 6.8), and (iii) NH_3 buffer (pKa 9.1)
- In the absence these urinary buffer systems → urine pH of 4.4 would be reached very rapidly and any further H^+ secretion would cease

Endocrine Functions of the Kidneys:

(I) Hormones acting on the kidneys:

Hormone	Synthesis/release and mechanism of action	Effect on kidney
Angiotensin II	- Renin (glycoprotein) made in JG cells (in afferent arteriole) → secreted by: - (1) Renal SNS nerves and circulating catecholamines → via β1 effect - (2) ↓ renal arteriolar BP (as sensed by intrarenal baroreceptors) - (3) ↓ tubular [NaCl] in TAL/EDCT (as sensed by Macula densa as part of tubulo-glomerular feedback) - (4) Prostacyclin - Nb. ADH and AII both inhibit renin secretion - Renin causes a cascade of events → it cleaves Angiotensinogen (liver) to AI → AI converted by ACE (lungs) to AII → AII - AII acts via GPCR via Gq → activates PLC to ↑ IP3 → ↑ IC Ca^{2+}	- (1) ↑ tubular reabsorption of Na^+ and Cl^- (and ↑ ECVF) and ↑ tubular K^+ secretion via → (i) Direct effects on the PCT, and (ii) Release of aldosterone from the adrenal cortex - (2) ↑ body H_2O content via → (i) ↑ thirst/H_2O intake (via hypothalamus), and (ii) ADH secretion (↑ H_2O reabsorption in renal CD) - (3) ↓ RBF and GFR (esp at ↑↑↑ levels of AII) via: - (i) Renal afferent and efferent arteriolar vasoconstriction (↓ GFR and RBF) - (ii) Mesangial contraction (↓ GFR) - Nb. at low levels of AII → it maintains GFR via renal afferent arteriolar constriction alone! - (4) Net tubular fluid/electrolyte reabsorption → due to ↓ peritublar capillary $P_{HYDROSTATIC}$ 2° to renal arteriolar vasoconstriction - (5) Potent vasoconstrictor → ↑ BP - (6) ↑ SNS activity → facilitates NAd release from post-ganglionic SNS neurons - (7) Inhibits renin release at JG cells (in JGA) → -ve feedback
Aldosterone	- Steroid hormone secreted from adrenal cortex (zona glomerulosa) in response to: - (1) Angiotensin-II (most important) - (2) High plasma $[K^+]$ - (3) ACTH - Binds to IC mineralocorticoid receptors → cause DNA-binding domain of receptor to be exposed → binds to DNA and promotes mRNA formation → alter protein translation	- (1) ↑ renal tubular Na^+ reabsorption and K^+ secretion at the Principal cells of DCT and CCD → MAIN determinant of Na^+ tubular reabsorption! - (2) ↑ H^+ secretion (and HCO_3^- reabsorption) by type A intercalated cells of CCD
ADH	- Peptide hormone synthesized in the hypothalamus (SON/PVN) → transported to posterior pituitary where it is secreted by: - (1) ↑ plasma osmolality → detected by osmoreceptors (in anterior hypothalamus) → very sensitive (detects 1% change) - (2) ↓ PV (↓ MAP sensed by baroreceptors (mainly low-pressure BR in atrium and great vessels; also high pressure BR in carotic sinus and aortic body) → ↓ sensitivity (detects 5-10% change) - Others: ↑ AII, pain, N/V,	- V1 receptor (GPCR via Gq → activates PLC to ↑ IP3 → ↑ IC $[Ca^{2+}]$ → smooth muscle contraction) → this causes: - (1) Renal afferent arteriolar constriction → ↓ GFR/RBF - (2) Contraction of renal mesangial cells → ↓ GFR - V2 receptor (GPCR via Gs → activates AC to ↑ cAMP → activates PKA) → this causes: - (1) Upregulates insertion of luminal AQP2 (stored in vesicles) in all parts of CD → ↑ H_2O permeability → ↑ H_2O reabsorption into hypertonic medullary interstitium (across BLM AQP3 and 4) - (2) Upregulates "urea transporters" in inner MCD → ↑ permeability to urea → ↑ urea absorption to maintain ↑ medullary osmolality (strengthens

	exercise, drugs	CCM) - (3) ↑ Na^+ reabsorption and K^+ secretion by principal cells of CCD
ANP	- Peptide hormone produced by right atrial cardiac cells in response to ↑ plasma volume → causes ↑ CVP and RAP → causes RA cardiac cells to stretch → release ANP - ANP receptor binding → ↑ GC activity → ↑ cGMP → activates MLC and MLCK phosphatase → ↓ IC Ca^{2+} → VSMC relaxation	- (1) Directly inhibiting Na^+ reabsorption in the CDs - (2) Inhibiting RAAS → attenuates Aldosterone secretion and renin release - (3) Inhibiting ADH release - (4) ↑ GFR (and FF) by: - ↑ glomerular $P_{HYDROSTATIC}$ 2° to direct afferent arteriolar dilation and efferent arteriolar constriction (and inhibiting constrictor effects of AII) - ↑ K_F → relaxes mesangial cells
1,25-dihydroxy-cholecalciferol	- Steroid hormone → Vit D3 (cholecalciferol) is made in the skin by UV light → hydroxylated in liver to 25-HO-cholecalciferol (inactive) - In the proximal nephron, 1α-hydroxylase (which is stimulated by PTH) hydroxylates it to 1,25-dihydroxy-cholecalciferol (active)	- ↑ plasma [Ca^{2+}] and [PO_4^{3-}] by directly ↑ distal renal tubular reabsorption of Ca^{2+} and PO_4^{3-}
PTH	- Peptide hormone produced by the parathyroid gland in response to – (i) ↓ [Ca^{2+}], (ii) ↑ [PO_4^{3-}], and (iii) ↓ vitamin D levels	- (i) ↑ Ca^{2+} reabsorption at the TAL and DCT - (ii) Inhibit PO_4^{3-} reabsorption at the PCT - (iii) Stimulate renal 1α-hydroxylase (to produce more activated vitamin D)
Catecholamines	- Renal noradrenergic neurons (NAd SNS nerves) and circulating catecholamines (NAd, Adr) have a minor role - Tonic discharge at rest, but ↑ with (i) BRR (due to ↓ MAP), (ii) central SNS stimulation, and (iii) exercise	- (1) ↓ RBF due to afferent and efferent arteriolar vasoconstriction - (2) Causes a milder ↓ in GFR – ↑ π_{GC} (caused by ↓ RBF) and mesangial contraction both cause ↓ GFR → but these effects are minimised by the ↑ filtration caused by ↑ P_{GC} - (3) ↑ renin release at JGA (β1 effect) → RAAS - (4) ↑ Tubular Na^+ reabsorption (PCT, DCT and TAL of LoH via α1 and β1 effect)
Prostaglandin (PGE-2/PGI-2)	- Products of arachidonic acid produced in kidney (medullary interstitial cells, CD cells, MD cells, arterioles) in response to: - (i) Clinical states a/w ↑ [ ] of circulating vasoconstrictors → ↑ SNS activity, ↑ AII and ↑ ADH - (ii) ↓ NaCl delivery to macula densa (TG feedback)	- Dampen vasoconstrictor effects on kidney (Ie. maintain RBF) → prevent hypoxic renal damage → via: - (i) Afferent arteriolar vasodilation - (ii) Stimulate renin secretion from JG cells → low AII levels that maintain GFR - (iii) Inhibit mesangial contraction by AII, ADH, NAd

(II) Hormones produced by the kidneys:

Hormone	**Synthesis/release**	**Effect**
Hormones directly produced by the kidneys		
Calcitriol (1,25-dihydroxy-cholecalciferol)	- Steroid hormone → Vit D3 (cholecalciferol) is made in the skin by UV light → hydroxylated in liver to 25-HO-cholecalciferol (inactive) - In the proximal nephron, 1α-hydroxylase (which is stimulated by PTH) hydroxylates it to 1,25-dihydroxy-cholecalciferol (active)	- ↑ plasma [Ca^{2+}] and [PO_4^{3-}] by: - (i) ↑ bone resorption - (ii) ↑ distal renal tubular reabsorption of Ca^{2+} and PO_4^{3-} - (iii) ↑ small bowel Ca^{2+} reabsorption
Erythropoeitin (EPO)	EPO is a glycoprotein that is mainly produced in the kidney (90%; 10% in	- Main regulator of RBC production in the bone marrow:

	liver) → by peritubular capillary endothelium in response to ↓ renal PO_2 (caused by ↓ PaO_2, anaemia, ↓ RBF)	- (i) Stimulates RBC precursor production - (ii) Enhances the rate of RBC maturation
Prostaglandin	- Products of arachidonic acid produced in kidney (medullary interstitial cells, CD cells, MD cells, arterioles) in response to: - (i) Clinical states a/w ↑ [ ] of circulating vasoconstrictors → ↑ SNS activity, ↑ AII and ↑ ADH (ii) ↓ NaCl delivery to macula densa (TG feedback)	- Dampen vasoconstrictor effects on kidney (Ie. maintain RBF) → prevent hypoxic renal damage → via: - (i) Afferent arteriolar vasodilation - (ii) Stimulate renin secretion from JG cells → low AII levels that maintain GFR - (iii) Inhibit mesangial contraction by AII, ADH, NAd
Hormones indirectly formed by the kidneys as a result of enzymes released by the kidneys		
Renin → forming AII and Aldosterone	- Glycoprotein made in JG cells (in afferent arteriole) → secreted by: - (1) Renal SNS nerves and circulating catecholamines → via β1 effect - (2) ↓ renal arteriolar BP (as sensed by intrarenal baroreceptors) - (3) ↓ tubular [NaCl] in TAL/EDCT (as sensed by Macula densa as part of tubulo-glomerular feedback) - (4) Prostacyclin - Nb. ADH and AII both inhibit renin secretion	- Renin causes a cascade of events → it cleaves Angiotensinogen (liver) to AI → AI converted by ACE (lungs) to AII → AII releases Aldosterone (adrenal cortex) - AII and Aldosterone cause: - (i) ↑ Na^+ reabsorption in GIT, kidneys, saliva, sweat → ↑ ECFV - (ii) ↑ body H_2O content → induce thirst and ADH release (H_2O reabsorption) - (iii) ↑ renal K^+ and H^+ secretion - (iv) Vasoconstriction of systemic vessels (↑ SVR) and renal arterioles (↓GFR and RBF) - (v) ↑ SNS activity
Kallikrein	- Kallikrein converts kinonogens into active kinins (Eg. bradykinin)	- Active kinins have vasoactive properties – especially, VSMC relaxation leading to vasodilation and increased blood flow

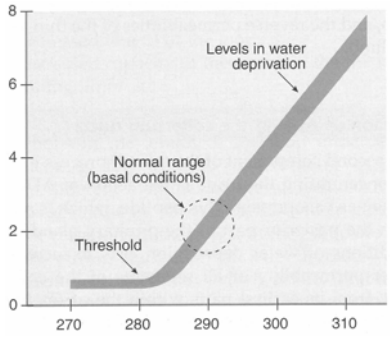
(III) <u>Renin-Angiotensin-Aldosterone System: Overview</u>

- Renin:
  - Glycoprotein produced as pre-pro-hormone and stored in secretory granules within Juxtaglomerular cells in walls of afferent arterioles (part of JGA)
  - It is secreted in response to:
    - (1) Renal SNS NAd nerves and circulating catecholamines → via β1 effect
    - (2) ↓ renal arteriolar BP (as sensed by intrarenal baroreceptors)
    - (3) ↓ tubular [NaCl] in TAL/EDCT (as sensed by Macula densa as part of tubulo-glomerular feedback)
    - (4) Prostacyclin
    - Nb. ADH and AII both INHIBIT renin secretion
- Angiotensin I (AI):
  - Renin cleaves Angiotensinogen (α2 globulin produced in liver) to an inactive 10 a.a. peptide AI
  - This is the RATE LIMITING STEP of the RAAS pathway!
- Angiotensin II (AII):
  - Angiotensin-Converting Enzyme in the capillary endothelium (esp in lungs) converts AI to an active 8 a.a peptide AII
  - AII acts via GPCR via Gq → activates PLC to ↑ IP3 → ↑ IC Ca^{2+} to cause:
    - (1) ↑ tubular reabsorption of Na^+ and Cl^- (and ↑ ECVF) and ↑ tubular K^+ secretion via → (i) Direct effects on the PCT, and (ii) Release of Aldosterone from the adrenal cortex
    - (2) ↑ body H_2O content via → (i) ↑ thirst/H_2O intake (via hypothalamus), and (ii) ADH secretion (↑ H_2O reabsorption in renal CD)

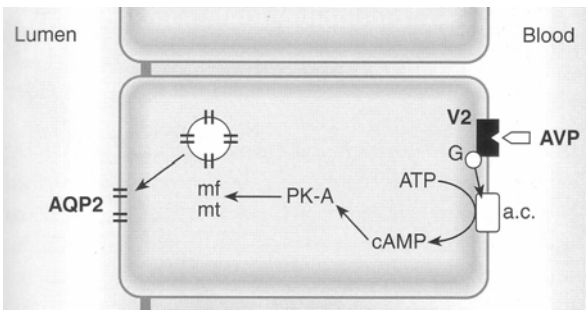
        - (3) ↓ RBF and GFR (esp at ↑↑↑ levels of AII) via:
            - (i) Renal afferent and efferent arteriolar vasoconstriction (↓ GFR and RBF)
            - (ii) Mesangial contraction (↓ GFR)
            - Nb. at low levels of AII → it maintains GFR via renal afferent arteriolar constriction alone!
        - (4) Net tubular fluid/electrolyte reabsorption → due to ↓ peritublar capillary $P_{HYDROSTATIC}$ 2° to renal arteriolar vasoconstriction
        - (5) Potent vasoconstrictor → ↑ BP
        - (6) ↑ SNS activity → facilitates NAd release from post-ganglionic SNS neurons
        - (7) Inhibits renin release at JG cells (in JGA) → -ve feedback
- Angiotensin III (AIII):
    - Additional a.a. moieties are split from AII to produce AIII in some tissues → AIII has similar actions to AII, but with much less potency
- Aldosterone:
    - Steroid hormone secreted from the zona glomerulosa of the adrenal cortex in response to:
        - (1) Angiotensin-II (most important) → via Gq effect → stimulates (i) synthesis (converts cholesterol to pregnenolone) and (ii) secretion
        - (2) ↑ plasma $[K^+]$ → secreted with 1% ↑ $[K^+]$ → due to depolarisation of cell membrane
        - (3) ACTH (mild effect) → via Gs effect → stimulates synthesis (converts cholesterol to pregnenolone)
        - (4) ↓ plasma $[Na^+]$ → secreted with 10% ↓ $[Na^+]$ (Nb. this is overridden by Δ ECFV → so it is not released with hypervolaemic hyponatraemia)
    - Aldosterone binds to IC mineralocorticoid receptors → cause DNA-binding domain of receptor to be exposed → binds to DNA and promotes mRNA formation → alter protein translation
    - Effects:
        - (1) ↑ plasma Na^+ (and ↑ ECVF)
            - (i) ↑ renal tubular Na^+ reabsorption at Principal cells of DCT/CCD → upregulates basolateral Na^+/K^+ ATPase and luminal ENaC → MAIN determinant of Na^+ tubular handling (reabsorbs 1-2% of filtered Na^+)
            - (ii) ↑ Na^+ reabsorption throughout the GI tract, sweat and salivary glands → upregulates Na^+/K^+ ATPase
            - (iii) ↑ renal tubular H_2O reabsorption (a/w Na^+ reabsorption) → ↑ ECFV 5-15% only → limited by "Escape phenomenon", where initial ↑ ECFV is limited by diuretic/natriuretic effects of ANP secretion
        - (2) ↓ plasma K^+
            - (i) ↑ renal tubular K^+ secretion at Principal cells of DCT/CCD → upregulates basolateral Na^+/K^+ ATPase and luminal K^+ channels → MAIN determinant of K^+ tubular secretion
            - (ii) ↑ K^+ secretion throughout the GI tract, sweat and salivary glands → upregulates Na^+/K^+ ATPase
            - (iii) ↑ IC uptake of K^+
        - (3) Alkalosis by renal H^+ loss → ↑ H^+ secretion (and HCO_3^- reabsorption) by type A intercalated cells of CCD → upregulates H^+ and H^+/K^+ ATPase
        - (4) ↑ plasma Cl^- → ↑ Cl^- reabsorption in distal tubule (Nb. This can lead to hyperchloraemic acidosis with aldosterone excess)

(IV) Anti-Diuretic Hormone: Overview

- Nonapeptide (9 a.a.) synthesized 1°ly in cell body of SON (some also in PVN) of the hypothalamus → transported to posterior pituitary via infundibulum where it is stored
- It is secreted in response to:
  - (1) ↑ plasma osmolality (Major determinant)
    - Detected by osmoreceptors (in anterior hypothalamus near SON/PVN)
    - Very sensitive (detects 1% change in osmolality) → threshold for ADH release is 280 mosm/kg (slightly less than normal plasma osmolality) → steep linear rise > 290 mosm/kg



  - (2) Non-osmotic stimuli:
    - (i) Haemodynamic changes
      - ↓ PV → ↓ MAP that is sensed by baroreceptors (mainly low-pressure BR in atrium) → cause ↑ ADH release
      - ↓ sensitivity cf. osmotic stimuli (detects 5-10% change in PV)
      - BUT very potent → overrides osmoreceptors (in terms of control of ADH secretion) when there are LARGE changes in PV!!!
    - (ii) ↑ AII
    - (iii) Pain
    - (iv) Nausea/vomiting (powerful stimuli)
    - (v) Exercise
    - (vi) Drugs (↑ release – morphine, nicotine, barbiturate; ↓ release – EtOH)
- ADH exerts its effects via two receptors:
  - (1) V1 receptor (GPCR via Gq → activates PLC to ↑ IP3 → ↑ IC [Ca^{2+}] → smooth muscle contraction) → this causes:
    - (a) Contraction of vascular SM cells (potent vasoconstrictor effect) → ↑ TPR and MAP
    - (b) Renal afferent arteriolar constriction and contraction of renal mesangial cells → ↓ GFR/RBF
    - (c) Platelet aggregation and degranulation
  - (2) V2 receptor (GPCR via Gs → activates AC to ↑ cAMP → activates PKA) → this causes:
    - (a) Upregulates insertion of apical membrane AQP2 (stored in vesicles) in principal cells of CCD and MCD → ↑ H_2O permeability → ↑ H_2O reabsorption into hypertonic medullary interstitium (across BLM AQP3 and 4) → causes ↓ plasma osmolality (and ↑ urine concentration)



        - (b) Upregulates "urea transporters" in principal cells of inner MCD → ↑ permeability to urea → ↑ urea absorption to maintain ↑ medullary osmolality (strengthens CCM)
        - (c) ↑ Na^+ reabsorption and K^+ secretion by principal cells of CCD
        - (d) ↑ CF VIII release by vascular endothelium
    - (3) Other effects:
        - CNS → promotes memory, learning, attention and concentration
        - ACTH release form anterior pituitary gland
- ADH's effect is very short-lived → short t ½ ~ 20 mins (rapidly inactivated by tissue peptidases → excreted by liver and kidney)

(V) Atrial Natriuretic Peptide: Overview
- ANP is a peptide hormone produced and secreted by RA cardiac myocytes in response to RA wall stress/stretching 2° to ↑ RA pressure (correlating with ↑ plasma volume) → linear relationship between RAP and plasma ANP levels exists
- It is produced as a stored prohormone (126 a.a.) → when secreted, it is cleaved into "active" ANP (28 a.a.) and "inactive" N-ANP (98 a.a.)
- ANP receptor binding → ↑ GC activity → ↑ cGMP → activates MLC and MLCK phosphatase → ↓ IC Ca^{2+} → VSMC relaxation
- Functions of ANP → short-lived (t ½ 2.5 min):
    - (1) Renal effects → ↑ natriuresis and water excretion by:
        - (i) Directly inhibiting Na^+ reabsorption in the CDs
        - (ii) Inhibiting renin release → ↓ AII and aldosterone production
        - (iii) Inhibiting ADH release
        - (iv) ↑ GFR (and FF) by:
            - ↑ glomerular $P_{HYDROSTATIC}$ 2° to direct afferent arteriolar dilation and efferent arteriolar constriction (and inhibiting high level AII efferent arteriolar constrictor activity)
            - ↑ K_F by relaxing mesangial cells
    - (2) Adrenal effects → inhibits aldosterone secretion stimulated by AII, ACTH and cAMP
    - (3) Peripheral vascular effects → ↓ MAP 2° to vasodilation of resistance and capacitance vessels via a direct effect (and also indirect effect via ↓ AII/ADH)

(VI) SNS (NAd) Nerves and Circulating Catecholamines (NAd/Adr): Overview
- Renal noradrenergic neurons (NAd SNS nerves) and circulating catecholamines (NAd, Adr) have a minor role
- Tonic discharge at rest, but ↑ with (i) BRR (due to ↓ MAP), (ii) central SNS stimulation, and (iii) exercise → Causes:
    - (1) ↓ RBF due to afferent and efferent arteriolar vasoconstriction
    - (2) Causes a milder ↓ in GFR – ↑ π_{GC} (caused by ↓ RBF) and mesangial contraction both cause ↓ GFR → but these effects are minimised by the ↑ filtration caused by ↑ P_{GC} (caused by efferent arteriolar constriction)
    - (3) ↑ renin release at JGA (β1 effect) → RAAS
    - (4) ↑ Tubular Na^+ reabsorption (PCT, DCT and TAL of LoH via α1 and β1 effect)

(VII) Prostaglandins (PGE-2/PGI-2): Overview
- Products of arachidonic acid produced in kidney (medullary interstitial cells, CD cells, MD cells, arterioles) in response to:

    - (i) Clinical states a/w ↑ [ ] of circulating vasoconstrictors → ↑ SNS activity, ↑ AII and ↑ ADH
    - (ii) ↓ NaCl delivery to macula densa (TG feedback)
- Role → dampen vasoconstrictor effects on kidney (Ie. maintain RBF) → prevent hypoxic renal damage → via:
    - (i) Afferent arteriolar vasodilation
    - (ii) Stimulate renin secretion from JG cells → low AII levels that maintain GFR
    - (iii) Inhibit mesangial contraction by AII, ADH, NAd
- Nb. NSAIDs inhibit PG formation → ↓ RBF and renal hypoxia!

Physiological Effects and Clinical Assessment of Renal Dysfunction:

(I) Physiological Effects of Renal Dysfunction:

- (1) Uraemia:
  - Breakdown products of protein metabolism leads to production of nitrogenous waste (esp creatinine and urea) → accumulate in renal dysfunction due to – (i) impairment in renal excretion, and (ii) –ve N-balance a/w renal dysfunction
- (2) Loss of concentrating and diluting ability of the kidney:
  - Due to loss of – (i) CCM, and (ii) functional nephrons
  - Produces polyuria → "osmotic diuresis" occurs as few remaining functional nephrons need to excrete the "solute load" → produces voluminous urine with osmolality similar to that of plasma ("isotonic urine")
  - Oliguria and anuria occur when all functioning nephrons are lost
- (3) Fluid and electrolyte imbalance:
  - (a) ↑ $[K^+]$ – Due to kidney's failure to secrete K^+ → Further exacerbated by acidosis
  - (b) ↑ $[PO_4^{3-}]$ and ↓ $[Ca^{2+}]$ – Due to impaired renal excretion of PO_4^{3-} and ↓ 1,25-dihydroxy-cholecalciferol levels
  - (c) Na^+ and H_2O retention:
    - Due to (i) ↓ filtered Na^+/H_2O due to glomerular damage (↓ Na^+/H_2O excreted) and (ii) ↑ RAAS and SNS activity 2° to ↓ PV a/w ↓ π_{PLASMA} with hypoproteinaemia (Ie. nephrotic syndrome)
    - BUT causes no Δ in plasma $[Na^+]$ (EXCEPT when large volumes of dilute urine are produced) → b/c Na^+/H_2O retained in equal proportions
    - ALSO ↑ osmolality → Mostly due to ↑ urea
- (4) Metabolic acidosis:
  - Due to kidney's failure to excrete metabolic acids or inability to reabsorb HCO_3^- → often ↑ anion-gap type due to accumulation of unmeasured anion (but can have normal AG with renal tubular acidosis)
- (5) Metabolic bone disease:
  - 2° hyperparathyroidism → later 3° (autonomous) hyperparathyroidism – Due to ↑ PO_4^{3-} , ↓ Ca^{2+} and ↓ vitamin D
  - ↓ 1,25-dihydroxy-cholecalciferol (vitamin D levels) – Due to loss of 1α-hydroxylase in proximal nephron → cannot hydroxylate and activate 25-hydroxy-cholecalciferol
- (6) Haematological disturbances:
  - (a) Anaemia – ↓ EPO production from renal peritubular capillary endothelium
  - (b) Bleeding tendency – Uraemia disturbs platelet function, and coagulopathy occurs with loss of clotting factors (esp in nephrotic syndrome)
  - (c) Immunodeficiency – Uraemia disturbs white cell function, and there is loss of immunoglobulins (esp in nephrotic syndrome)
- (7) Metabolic disturbances:
  - Dyslipidaemia (especially with nephrotic syndrome)
  - Impaired glucose tolerance due to insulin resistance
  - Raised inflammatory markers (CRP and homocysteine)
  - Sex hormone derangements

(II) Clinical Assessment of Renal Dysfunction:

Overview of renal function tests:

- "Renal function tests" indirectly measure renal function by assessing the kidney's:
  - (1) Glomerular function → assess ability to filter fluid/solutes
  - (2) Renal tubular function → assess ability to reabsorb and/or secrete substances
  - (3) Cortico-medullary interstitial function → assess ability to concentrate and dilute urine

Measurement of glomerular function:

- (1) Plasma creatinine and urea:
  - (a) P_{Cr} → convenient measure of renal function (Ie. no infusions/collections) → estimate GFR using Cockcroft-Gault/MDRD → BUT it has limitations:
    - (i) It is inversely related to GFR (via hyperbolic relationship) → thus:
      - Insensitive measure of GFR (unless at very ↓ GFRs) → 50% ↓ in GFR is needed to produce a detectable ↑ P_{Cr}
      - At very ↓ GFRs, any further ↓ GFR produces a massive ↑ in P_{Cr}
    - (ii) Grossly inaccurate in situations that influence P_{Cr} (Ie. muscle wasting)
  - (b) P_{UREA} → worse marker of renal function because → (i) tubular absorption is variable and (ii) its levels are not raised above normal range until at least 50% of total renal function is lost
- (2) Creatinine clearance
  - ↑ convenient (cf. inulin) because (i) it is steadily infused into plasma from muscle to produce a constant plasma [ ] (Ie. no infusions needed), and (ii) requires only an accurately timed urine specimen and single plasma sample to determine C_X
  - BUT it is ↓ accurate measure of GFR because it is (i) partly secreted by tubules (overestimates GFR by 10-20%), and (ii) difficult to measure at low [ ] (overestimates GFR also)
- (3) Clearance of exogenous substances (Eg. ^{98}-Tc-DPTA, ^{125}I, inulin)
  - Inconvenient measure of GFR as this requires → substance to be infused continuously IV until steady state plasma levels reached → then an accurately timed urine specimen is collected and plasma sample obtained to determine C_X

Measurement of renal tubular and cortico-medullary interstitial function:

- (1) Plasma and urine electrolytes (Na^+, Cl^-, K^+, Ca^{2+}, PO_4^{3-}, Mg^{2+}) → assess tubular reabsorption and/or secretion of electrolytes
- (2) Plasma and urine osmolality → assess concentrating and diluting ability of kidney
- (3) Urine volume → assess concentrating and diluting ability of kidney
- (3) ABG (plasma pH and HCO_3^-) → assess renal handling of acid-base
- (4) Plasma hormone levels (Eg. PTH, Vitamin D) → assess endocrine function of kidney
- (5) Urine collection for casts, protein, glucose → determine nature of renal pathology

(III) <u>Principles of Dialysis</u>:

Overview of dialysis:

- "Dialysis" is a process whereby the composition of a solution (patient's blood) is altered by exposure to a second solution (dialysate) through a semi-permeable membrane → involves transfer of H_2O and LMWT solutes across the membrane between the solutions via diffusion, osmosis and solvent drag
- Dialysis is used in patients with renal failure as a form of renal replacement therapy:
  - (1) Removes waste products (such as urea, creatinine, Etc.)
  - (2) Achieves H_2O balance → usually remove free H_2O from blood
  - (3) Achieves electrolyte balance
  - (4) Achieves acid-base balance
- There are two major types of dialysis:
  - (1) Peritoneal dialysis – Peritoneum acts as the semi-permeable membrane
  - (2) Haemodialysis – Involves a "Dialyser" containing a semi-permeable membrane filter whereby counter-current flow of blood and dialysate occurs on opposing sides of it → this ↑ dialysis efficiency as it maintains a maximal [ ] gradient across the membrane for diffusion

Factors that influence movement of solvent during dialysis:

- (1) Osmosis:
  - A passive process by which H_2O diffuses across a semi-permeable membrane down its concentration gradient (Nb. $[H_2O]$ is determined by concentration of solute in it, in particular proteins)

> Note – "Tonicity" is a measure of the osmotic pressure gradient of two solutions separated by a semi-permeable membrane → osmosis involves H_2O flows from a hypotonic solution (↓ $P_{OSMOTIC}$) across a semi-permeable membrane to a hypertonic one (↑ $P_{OSMOTIC}$)

  - Dialysate is "hypertonic" (↑ $P_{OSMOTIC}$ gradient cf. blood) due to addition of glucose → causes osmosis of H_2O out of plasma into dialysate → removes excess free H_2O to achieve H_2O balance (Nb. inadvertently causes glucose uptake → hyperglycaemia)
  - Note – Osmosis is a relatively fast process compared to diffusion
- (2) Ultrafiltration:

$$H_2O \text{ flux into dialysate} = K_F \bullet [(P_B - P_D) - \sigma(\pi_B - \pi_D)]$$

  - Starling's forces → balance of $P_{HYDROSTATIC}$ and $P_{ONCOTIC}$ across semi-permeable membrane → determine bulk flow of H_2O between plasma and dialysate
  - During dialysis → there is net H_2O movement from plasma into dialysate (haemodialysis > peritoneal dialysis)

> - Peritoneal dialysis → P_B = 35 mmHg, π_B = 28 mmHg, P_D and π_D = 0, σ = 1 (assuming no peritoneal pathology) → NFP = 7 mmHg into dialysate
> - Haemodialysis → P_B = 90 mmHg, π_B = 20 mmHg, P_D and π_D = 0 → NFP = 70 mmHg into dialysate

Factors that influence movement of solute during dialysis:

- (1) Diffusion → a passive process by which movement of a solute in solution moves down its concentration gradient → influenced by:
  - (a) Fick's law of diffusion
    - Rate of diffusion is proportional to:
      - (i) [ ] gradient of the substance across the cell membrane
      - (ii) SA of the cell membrane
      - (iii) Diffusion coefficient (which is the solubility of the substance in the lipid bilayer, divided by the square-root of the substance's molecular weight)
      - (iv) Inverse of the cell membrane's thickness

$$J = -\frac{D \times A \times [\ ]_{GRADIENT}}{t} \quad ; \; D = \frac{\text{Solubility in membrane}}{\sqrt{MWT}}$$

    - With dialysis:
      - (i) Dialysate is produced with a desired [ ] of solutes to achieve an intended dialysis outcome:
        - If solutes need to be removed from plasma (Eg. K^+, Ca^{2+}) → ↓ [ ] in dialysate
        - If solutes need to be transferred into plasma (Eg. lactate for acid-base balance) → ↑ [ ] in dialysate
        - If no solute transfer is required (Eg. Na^+) → [ ] in dialysate same as in plasma

        - (ii) Solutes with ↑ molecular size (Eg. proteins) cannot diffuse across semi-permeable membrane
        - (iii) Peritoneum can be used because it has a large SA → permits diffusion; but infection/inflammation causes ↓ membrane permeability and ↑ thickness → hinders diffusion
    - (b) Gibbs-Donnan effect
        - When a semipermeable membrane separates two solutions, one of which contains a non-diffusible ion (Eg. protein), the distribution of diffusions ions across the membrane is altered such that at equilibrium → (i) the concentration ratios of the diffusible ions between the two solutions will be equal and (ii) the osmolality of the solution containing the non-diffusion ion will be greater
        - In dialysis → -ve charged plasma proteins affect transfer of charged particles across the peritoneum
- (2) Solvent drag (see ultrafiltration above)
    - Solute movement across a membrane is determined purely by the bulk flow of H_2O across it → determined by (i) driving pressure of ultrafiltration and (ii) membrane porosity
    - Permits removal of LMWT solutes from plasma into dialysate via bulk-flow

Effects of General Anaesthesia on Renal Function:

ALL GA agents have INDIRECT effects on renal function → ↓ RBF (and GFR) via ↓ MAP

ONLY volatile agents have a direct effect on renal function → ↓ responsiveness of RAAS and ADH via a central effect