Introduction to the kidneys + urinary system

Dr Vikram Khullar (v.khullar@imperial.ac.uk)

1. Draw a simple diagram of the urinary system including the following: kidney, renal pelvis, ureter, bladder, urethra, sphincter vesicae, sphincter urethrae

2. Outline the means of urine transport down the ureters into the bladder + explain the mechanism preventing reflux of urine from the bladder

3. Describe with anatomical + histological features allowing expansion of the bladder as it fills with urine

4. Distinguish between the sphincter urethrae + sphincter vesicae muscles and their nerve supplies

5. Describe the mechanisms involved in the reflex contraction of the bladder in response to distension. State the approximate volume of urine in the bladder that normally initiates a reflex contraction in the adult.

The urinary apparatus

Main functional components of the urinary apparatus:

- kidneys
- calyces, renal pelvis + ureters
- urinary bladder
- urethra + associated sphincters
- neurological control systems for the bladder muscle + the sphincters
- well-adapted blood supply

The Kidneys

Location

- the kidneys lie posteriorly between ribs 11 + 12, and are overlapped by both the diaphragm + pleural cavity. They lie embedded in retroperitoneal fat
  - clinical correlate = kidney problems often misdiagnosed as chest infection
- the kidneys are described as retro-peritoneal organs, NOT abdominal, therefore pain is referred via iliohypogastric + ilioinguinal nerve (pain posterior, and travels antero-inferiorly towards the groin)
- the liver is superior to the right kidney, and the spleen is superior to the left kidney
  - the liver is a large organ, thus pushes the right kidney inferiorly, so it is not on the identical plane to the left kidney
- the upper part of each kidney rests on the diaphragm, therefore on inhalation, the kidneys move inferiorly on flattening of the diaphragm
- superior to each kidney is an associated suprarenal gland (also known as adrenal gland)
Blood supply
- **Arterial** – from renal arteries which branch directly from the abdominal aorta (lies posterior to the left renal vein)
  - These arteries are short, fat therefore receive a good blood supply at HIGH pressure (this high pressure drives the ultrafiltration process by glomerular capillaries)
  - Renal arteries branch profusely into arcuate arteries which supply each glomerulus
- **Venous** – renal vein from each kidney drain directly into the IVC

Structure
- Each kidney is surrounded by a deep dense fibrous capsule, a middle adipose capsule and a superficial renal fascia
  - Clinical correlate: kidney infection/abscess – pressure builds up within the capsule which leads to a reduced blood supply and risk of necrosis. Treatment involves needle insertion + drainage from capsule
- Each kidney consists of a superior + inferior pole, and can be described as segmental/multilobular
- Frontal section through kidney reveals two distinct regions: a granular looking cortex + an inner striated medulla
- The renal medulla consists of several cone-shaped renal pyramids. The base of each pyramid faces the renal cortex, and its apex (called the renal papilla) points towards the renal hilum
- The granular-looking renal cortex extends from the renal capsule to the bases of each renal pyramid, forming renal columns between each pyramid
- A renal lobe consists of a renal pyramid, its overlying area of renal cortex, and ½ of each adjacent renal column
- Together, the renal cortex + renal pyramids constitute the parenchyma of the kidney (functional portion), with associated functional units called nephrons.
  - The cortex + medulla each contain distinct parts of the nephrons, with the cortex consisting of the glomeruli surrounded by the convoluted tubules, and the medulla containing loops of Henle (in parallel bundles – gives striated appearance)
  - Urine formed by the nephrons drain into large papillary ducts which extends through the renal papilla (involved in making urine concentrated)
  - The papilla then drain into cuplike structures called minor + major calyces
  - Each minor calyx receives urine from the papillary ducts of one renal papilla, and delivers it to a major calyx
  - From the major calyx, urine is drained into a single large cavity called the renal pelvis, then out through the ureter to the urinary bladder
• NB: the renal collecting system provides an effective barrier preventing waste from re-entering the bloodstream
• The renal cortex has a large blood supply (arcuate arteries supply glomerulus), which then go into capillaries into loop of Henle and finally to renal papilla
  o This means the renal papilla is most vulnerable to death following trauma
  o In acute renal failure, following blood transfusion with associated increase in bp, polyuria still occurs due to malfunctioning as papilla (as papilla involved in concentrating urine)

Ureters
• From the major calyx, urine is drained into a single large cavity called the renal pelvis, then out through the ureter to the urinary bladder
• The ureters run vertically down the posterior abdominal wall, lying across the transverse spinal processes on each side of the vertebral column
• Clinical correlate: sites of renal colic caused by kidney stones passing through the uterers at specific junctions:
  o The ureteropelvic junction (between renal pelvis + ureter)
  o The ureter segment near the sacroiliac joint
  o The ureterovesical junction (between ureter + bladder)
• Urine is transported along the ureter by peristalsis of smooth muscle (both circular + longitudinal muscles present)
• The ureters open obliquely through the bladder wall, thus acting as a valve ensuring unidirectional movement of urine
  o Clinical correlate: vesicoureteral reflux – the abnormal movement of urine FROM the bladder INTO the ureter/kidneys. The urine collects in the pelvis in saces, dripping back down into the bladder → incomplete micturition (emptying) + infection
• Cross section of ureter shows urothelium with tight gap junctions + plaques preventing leakage (acts as barrier/seal)
  o Folded walls with many cells enable stretch to accommodate urine during peristalsis
  o Urothelium = 3-layered epithelium with very slow cell turnover
  o The large luminal cells have a highly specialised low-permeability luminal membrane, which prevents the dissipation of urine-plasma gradients.

Bladder + Urethra
• Vessel with pyramidal shape when empty, more spherical shape when full
• Capacity of 450-550ml, which voids 120-250ml of urine at each go.
  o NB: the bladder should NOT contract between voids
• Can be subdivided into fundus (base), superior + inferolateral bladder
• Urine transported into bladder via ureters, acts as storage vessel which then empties urine into urethra via neck
• NB: a median umbilical ligament exists at the apex of the bladder, as a remnant of the umbilical cord – this may be patent
 occasionally

- Control of urine transport from bladder to urethra via smooth muscle sphincters; both internal + external
  (striated muscle which produce tonic contractions in order to prevent uncontrolled urination)
  - Clinical correlate: during childbirth, the pelvic floor may increase x30 → damage of urinary
    sphincters → slight loss of bladder control
- Internal sphincter (sphincter vesicae) is under involuntary parasympathetic control, as it is a continuation of
detrusor muscle (Ach synaptic control) – reflex opening in response to bladder wall tension involving Ach
  - Clinical correlate: loss of bladder control due to inappropriate opening of internal sphincter can be
    treated with anticholinergics
- External sphincter (sphincter urethrae) is in the perineum – opened by voluntary inhibition of somatic
  pudendal nerve (S2, 3 +4)
  - Sustained closure keeps the sphincter vesicae closed + reduces bladder tone
- In males, the sphincters lie on either side of the prostatic urethra
  - The urethra is longer (12-14cm), therefore hasa reduced pressure therefore requires more effort to
    void the bladder
  - The internal sphincter prevents retrograde ejaculation into the bladder → sperm in urine

Structural basis of kidney function
Dr Vikram Khullar (v.khullar@imperial.ac.uk)

1. Describe the structural organisation of the kidney, as seen at a macroscopic level.
2. Draw a diagram showing the main constituent parts of a nephron.
3. Draw a diagram of the structures separating glomerular capillary plasma from the fluid in Bowman's
capsule.
4. List the features of the cellular structure of the tubules in different parts of the nephron which make
   possible the concentration of urine.
5. Draw a diagram showing the pattern of blood vessels in the kidney, and state which features contribute to
   the filtration process, to the reabsorption process, and to the countercurrent mechanism.

NOTE: there are both passive + active mechanisms for maintaining homeostasis, including the regulation of
osmolality, nitrogen etc., and the control of body fluid volume. These mechanisms include exhalation, sweat + urine
(urine is especially key in regulating sodium and potassium levels)

Kidney function

The production of urine
This is a multi-step process involving...
  1) Ultrafiltration of blood through glomerulus
  2) Selective reabsorption in proximal convoluted tubule
  3) Creation of hyperosmotic extracellular fluid by counter-current mechanism in loop of Henle
  4) Adjustment of ion content in distal convoluted tubule
  5) Adjustment to concentration of urine in collecting duct as necessary

Endocrine signal
- To the rest of the body, e.g. renin, erythropoietin, 1,25-OH vitamin D

Structure
The functional unit of urine production is called a **nephron** (below) – there are millions within the parenchyma of the kidney. Glomerulus supplied by afferent arteriole from arcuate arteries (branched from renal artery from aorta). The afferent arteriole is of much higher pressure than the efferent arteriole, creating a pressure gradient which drives ultrafiltration. The glomerulus is surrounded by the glomerular capsule; also known as BOWMAN’S CAPSULE.

**Renal corpuscle**
- Structure composed of the glomerulus + Bowman’s capsule
- The glomerulus consists of capillaries with associated podocytes (visceral epithelial cells); these “wrap” around the capillaries, with long processes/feet that leave slits/gaps between them = fenestrations
- Blood supply: from afferent to efferent arteriole, the blood supply enters at the vascular pole of the corpuscle
- Filtration barrier: the fenestrae between the processes of the podocytes have a specialised basal lamina which allows the passage of ions and molecules \(< ~50,000\text{ m weight}\) to pass from the blood (i.e. first step in producing urine)
- The filtrate then drains into the proximal convoluted tubule at the urinary pole of the corpuscle

**Mechanism of urine production**

1) **Ultrafiltration**
- Blood passing through **glomerulus** is filtered
- Filtrate consists of all components of \(< ~50,000\text{ m weight}\), then drains into proximal convoluted tubule (look at notes above on renal corpuscle for more detail)

2) **Selective Reabsorption**
- Material that needs to be retained in the blood is then reabsorbed in the **proximal convoluted tubule** (70% reabsorbed)
- This includes ions, glucose, amino acids, small proteins, water etc
- Methods of uptake:
  - Na\(^+\) uptake by basolateral Na\(^+\) pump. Water and anions then follow the Na\(^+\) (along osmotic + electrochemical gradient)
  - Glucose uptake is via Na\(^+\)/glucose co-transporter
  - Amino acids by Na\(^+\)/amino acid co-transporter
  - Protein uptake by endocytosis
- Structural features:
  - large diameter lumen (larger than distal convoluted tubule)
  - cuboidal epithelium sealed with tight junctions (act as paracellular seals)
  - brush border at apical surface + basolateral interdigitations - increase the membrane surface area
  - aquaporins (membrane protein channel carriers) – mediate transcellular water diffusion
  - prominent mitochondria (reflect high energy requirements)

3) Creation of hyper-osmotic (concentrated) ECF
- Achieved by loop of Henle (both descending + ascending limb) + vasa recta (blood vessels supplying loop) by a countercurrent mechanism
- Descending limb:
  - Thin, simple squamous epithelium
  - Aquaporins present on apical membrane allow passive H2O reabsorption to continue until a passive osmotic equilibrium is established
- Ascending limb:
  - Thick, cuboidal epithelium with few microvilli + prominent mitochondria (required for the active pumping of ions)
  - Na+ + Cl- are actively pumped out of the tubular fluid into the ECF. However there are very water-impermeant tight junctions between the epithelial cells, which also lack aquaporins, therefore H2O not reabsorbed further
  - This results in a HYPER-osmotic ECF (with a corresponding HYPO-osmotic tubular fluid)
- Vasa recta
  - Loop structure stabilises the hyperosmotic ECF by forming a rapid equilibrium

4) Adjustment of ion content of urine
- Achieved by distal convoluted tubule (also known as cortical collecting duct)
- Cuboidal epithelium, with few microvilli + numerous large mitochondria, complex lateral membrane interdigitations with Na+ pumps
- Adjusts Na+, K+, H+ and NH4+ (urea) – under control by aldosterone
- Re-equilibirilates luminal fluid + ECF (under control by vasopressin)
- Also specialisation at macula densa, which forms part of the juxtaglomerular apparatus (this regulates the function of the nephron via renin-angiotensin system – found at vascular pole of renal corpuscle)

5) Final adjustment of urine concentration
- Occurs in medullary collecting duct – involves the movement of water down osmotic gradient into extracellular fluid; completes ion adjustment + controls urine osmolarity
Simple cuboidal epithium with single cilium per cell and no interdigitations on cell boundaries
- Little active pumping therefore fewer mitochondria required
- However contain organelles associated with secretory activity e.g. golgi

Rate due to aquaporin-2 in apical membrane
- Content is varied by exo/endocytosis mechanism
- Controlled by vasopressin (ADH; antidiuretic hormone)

Basolateral membrane has aquaporin-3 for transcellular water movement, but this is not under vasopressin control

Urine then drains into minor calyx at apex of medullary pyramid → major calyx → renal pelvis → ureter
- Both calyces + renal pelvis have specialised urinary epithelium
- Urinary epithelium – a specialised epithelium also known as urothelium; properties are resistance to urine, ability to stretch
  - Cells appear squamous or cuboidal depending on degree of stretch
  - The luminal cells are also highly specialised for low permeability

Mechanism of Renin release – the Juxtaglomerular apparatus

- Endocrine specialisation
- Cellular components:
  - Macular densa of distal convoluted tubule – detects [CL-]
  - Juxtaglomerular cells of afferent arteriole – senses stretch in arteriole wall
- Response to Cl⁻ + stretch: secretes Renin, which controls blood pressure (and hence nephron function) via renin-angiotensin system

Renal blood flow + glomerular filtration

1. Indicate what proportion of the cardiac output normally perfuses the kidney
2. Define the term freely filtered. State that the permeability barrier in the glomerulus discriminates mainly on the basis of size (although electrical charge also influences the filtration of charged proteins).
3. Compare the composition of glomerular filtrate + plasma
4. Define glomerular filtration rate (GFR) and filtration fraction + give typical values for each in a normal healthy young adult.
5. Write an equation for the net filtration pressure across the glomerular membrane in terms of the hydrostatic pressure + osmotic pressure involved.
6. Explain how net filtration pressure will be affected by:
   a. A large fall in arterial blood pressure
   b. A fall in plasma protein concentration
   c. Ureteral obstruction
7. Describe + explain the effect of change in renal blood flow on GFR
8. Define renal clearance + explain is use in assessing renal function

Basic Renal Process

Renal input = renal artery
Glomerular filtration
Renal output = renal vein and ureter
Final urine (Excretion)

Amount excreted = Amount filtered + Amount secreted + Amount absorbed
Not all substances undergo all processes
**Introduction**

**Basic functions of the kidney:**
- Excretion of metabolic products e.g. urea, uric acid + creatinine
- Excretion of foreign substances (e.g. drugs – link with pharmokinetics)
- Homeostasis of cell volume (through regulation of body fluids, electrolytes + acid-base balance)
- Regulation of blood pressure – link with cardiovascular system
- Secretion of hormones (e.g. renin, erythropoietin – link with endocrinology)

**Glomerular filtration**
- “The formation of an ultrafiltrate of plasma in the glomerulus of a kidney nephron”
- Renal failure – an abrupt fall in glomerular filtration
- Abnormalities in renal circulation + urine production lead to reduced glomerular filtration, e.g. cause kidney failure

**Glomerular filtration detail**
- Defined as a passive process (no active transport involved) whereby fluid is driven (by hydrostatic pressure of the afferent arteriole) through the semipermeable/fenestrated walls of the glomerular capillaries into the Bowmans capsule space (surrounding the glomerulus)
- The filtration barrier is highly permeable to fluids + small solutes (<50,000 m weight – are freely filtered therefore there is no change in concentration of these solutes)
- The filtration barrier is impermeable to cells, proteins + drugs etc which are bound to plasma proteins
- The ultrafiltrate forms is a clear fluid completely free from blood cells + proteins, and contains only electrolytes + small solutes = primary urine
- Glomerular filtration is then followed by subsequent processes which may change the concentration of the urine, including reabsorption + secretion
  - Not all substances undergo all processes, thus for each substance:
    - amount excreted = amount filtered – amount reabsorbed + amount secreted

**Glomerular filtration pressures**
- Driving force = hydrostatic pressure in the glomerular capillaries (caused by the high blood pressure in the afferent renal arteriole due to the short, wide renal arteries which branch directly from the aorta) – this is written as $P_{gc}$
- There are 2 opposing pressures:
  - Hydrostatic pressure IN the tubule – written as $P_t$
    - Clinical correlation: obstruction within the tubule may increase its hydrostatic pressure, thus reducing the amount of fluid filtered through
  - Oncotic pressure of the plasma proteins in the glomerular capillaries – written as $\pi_{gc}$
- Together, these forces determine the net ultrafiltration pressure, which determines the amount of fluid filtered – written as $P_{uf}$
  - This can be calculated from $P_{uf} = P_{gc} - P_t - \pi_{gc}$
  - From example opposite, $= 45-10-25 = 10\text{mmHg}$ (pressure driving urine formation)
- The normal range for net ultrafiltration pressure = 10-20mmHg
Glomerular filtration rate (GFR)

- Pressure is not the only thing that influences the glomerular filtration rate. Other factors include:
  - Permeability of tubule membrane
    - Clinical correlation: kidney disease may result in a change in membrane permeability
  - Surface area of membrane available for filtration
    - Clinical correlation: kidney disease may damage nephrons, thus reducing the number of functioning glomeruli → recused SA available for ultrafiltration
- These factors are considered in the ultrafiltration coefficient – written as $K_f$
- Glomerular filtration – written as $GFR = P_{uf} \times K_f$
- Definition of GFR: the amount of fluid fluid from the glomeruli capillaries into the Bowmans capsule per unit time (ml/min) – this considers ALL the functioning nephrons
- GFR is used as an index of kidney function. Normal is approx. 120ml/min

Renal blood flow (RBF)

- GFR is closely linked with renal blood flow, as the driving force for ultrafiltration pressure is the hydrostatic pressure of the heart
- Renal blood flow delivers oxygen, nutrients + substance for excretion to the kidneys, via renal arteries which branch directly from the aorta
- Kidneys receive 20% of the cardiac output (where 5L/m = total CO, therefore kidneys receive 1L/m)
  - As ultrafiltration is more to do with the plasma volume, as all the blood cells remain in the capillaries – we consider the renal plasma flow (RPF) as more important = approx. 0.6L/min
- Not all of the plasma is filtered into the glomerulus; some just passes through the glomerular capillaries into the efferent arteriole
  - The friction fraction (FF) is the ratio between RPF and the amount of filtrate filtered by the glomerulus, i.e. the fraction of the primary urine volume/renal plasma volume
  - This is normally 20%
- Glomerular filtration rate can also be calculated as $GRP = RPF \times FF$

Autoregulation of GFR

- Normal value for GFR is approx. 120ml/min, and this is kept within a narrow range
- The GFR depends on a number of factors:
  - Glomerular capillary pressure ($P_{gc}$)
  - Plasma oncotic pressure ($\pi_{gc}$)
  - Tubular pressure ($P_t$)
  - Glomerular capillary surface area or permeability ($K_f$)
- Regulation of GFR is achieved by neural or hormonal unput to the afferent/efferent arteriole resulting in changes to the glomerular capillary pressure
- Autoregulation ensures fluid + solute excretion remain reasonably constant (without which urine production + ion loss will vary)
  - To decrease GFR, constrict the afferent arteriole or dilate the efferent arteriole
  - To increase CFR, construct the efferent arteriole or dilate the afferent arteriole
- Autoregulation is required e.g. during exercise, as bp increases but you don’t want to be producing large amounts of urine
- **Mechanisms of autoregulation**
  - Myogenic mechanism – reflex response to blood pressure increase
    - Arterial pressure pressure rises → afferent arteriole stretches →arteriole contracts → (vessel resistance increases)→ blood flow reduces and GFR remains constant
  - Tubuloglomerular feedback – macula densa response to NaCl increase
- NaCl conc in tubular fluid sensed by macula densa in juxtaglomerular apparatus → release ATP as signaling molecule which signals afferent arteriole to vasoconstrict → reduced filtration (with associated negative feedback loop)

**Clinical relevance of GFR**
- In a normal individual carrying out a daily routine, GFR will be maintained at 120ml/min
- Severe haemorrhage → decreased GFR (result of decreased blood pressure)
- Obstruction in nephron tubule → decreased GFR (due to increased hydrostatic pressure in tubule = opposing pressure)
- Reduced plasma protein concentration → increase GFR (due to increased oncotic pressure = opposing pressure)
- Small increase in blood pressure, e.g. during exercise → GFR remains constant (result of autoregulation)

**Renal Clearance**
- As substances in the blood pass through the glomerulus, they are filtered to different degrees
- The extent to which substances are removed from the blood is called clearance
- Clearance is defined as the number of litres of plasma completely cleared of the substance per unit time (C)
  - C = U x V / P
  - C = clearance (ml/min)
  - U = concentration of substance in urine
  - V = rate of urine production
  - P = concentration of substance in plasma
- NB: clearance is affected by the processes following ultrafiltration, unlike GFR
- However, if a molecule is freely filtered (i.e. neither reabsorbed nor secreted following its ultrafiltration), clearance = GFR (i.e. amount excreted = amount filtered)
  - Example of this is *inulin*: a plant polysaccharide that is measurable in both urine + plasma and gives a clearance value of 120ml/min = GFR
  - However in humans, GFR is estimated from creatinine clearance
    - Creatinine is a waste product from creatine in muscle metabolism
    - The amount of creatinine is fairly constant, thus if renal function is stable, the amount of creatinine in urine is stable (120ml/min)
    - A low creatinine clearance may indicate renal failure
    - A high plasma creatinine may also indicate renal failure

**Renal plasma flow (RPF)**
- The volume of plasma (component of blood) reaching the kidney per unit time
- PAH (para aminohippurate) is completely removed from the plasma passing through the kidney, therefore its clearance = renal plasma flow = 625ml/min
- With other substances, the amount of the substance appearing in the urine reflects the combined effects of filtration, reabsorption + secretion, so that:
  - Amount excreted = amount filtered – amount reabsorbed + amount secreted
  - Thus most solutes have a clearance of < 120ml/min (GFR) = controlled excretion

**Renal diagnostics**
- A fall in GFR is the cardinal feature of renal disease
- If GFR falls, excretory products will build up in the plasma
- A raised plasma concentration of creatinine is diagnostic of renal disease
- Excretion of many other substances will also be impaired in renal failure, including some drugs. This needs to be taken into account when calculating drug doses (use in pharmokinetics)
1. In the context of renal function, define the terms reabsorption + secretion. Explain the meaning of transcellular and paracellular transport.

2. Draw a diagram of the wall of the early proximal tubule showing the following: tubular fluid, luminal membrane, basolateral membrane, peritubular capillary, tight junction, Na+/K+ “pump” and one example of each of the following: an ion-selective channel, co-transport of two solutes, counter-transport of two solutes.

3. Explain how active sodium transport acts as a driving force for the reabsorption of water + many other ions and molecules.

4. Describe the main routes for Na+ entry into tubular cells in the thick ascending limb of the loop of Henle, in the distal convoluted tubule and in the principal cells in the cortical collecting tubule.

5. Contrast the osmolarity of the tubular fluid:
   a. In Bowman’s space
   b. At the end of the proximal tubule
   c. Emerging from the loop of Henle

Introduction

- The kidney is a central regulator of homeostasis. On an average day we consume 20-25% more water and salts than we need to. Thus in order to maintain homeostasis, we need to lose this excess as well as other waste products e.g. urea.
- However too much water + small molecules are able to pass through the fenestrae of the glomerular capillaries, thus in order to remove excess/waste whilst retaining “good stuff” – glomerular filtration is followed by controlled reabsorption + secretion. This results in a final urine production which is associated with maintenance of solute balance, plasma concentration + pH.
- In reality, 99% of the ultrafiltrate is reabsorbed into the peritubular capillaries.

Osmolarity

- One of the key concepts involved in tubular function, osmolarity is defined as “a measure of the osmotic pressure exerted by a solution across a perfect semi-permeable membrane”
- Osmolarity is dependent on the number of solute particles, not the nature of the particles (with each particle/ion counted separately) – plasma osmolarity must be carefully controlled during reabsorption/secretion
  - NB: plasma osmolarity is the concentrations of all the different solutes in plasma added together
- Normal plasma osmolarity = 285-295mosmol/l
- Normal urine osmolarity = 50-1200mosmol/l
- From this you can see that plasma osmolarity is carefully controlled, as changes in plasma osmolarity may affect all the cells in the body due to the varying osmotic pressure exerted across their semi-permeable membranes
  - However intake of the different solutes from the external environment varies considerably, therefore in order to maintain a relatively constant plasma osmolarity, the effect is that the urine osmolarity varies hugely.

<table>
<thead>
<tr>
<th>Solute</th>
<th>Plasma</th>
<th>Urine (per 24hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>~140</td>
<td>50-200</td>
</tr>
<tr>
<td>Chloride</td>
<td>~150</td>
<td></td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>~24</td>
<td></td>
</tr>
<tr>
<td>Potassium</td>
<td>~4</td>
<td>40-100</td>
</tr>
<tr>
<td>Glucose</td>
<td>~3-8</td>
<td>~0</td>
</tr>
<tr>
<td>Calcium</td>
<td>~2</td>
<td>2-8</td>
</tr>
<tr>
<td>Creatinine (waste)</td>
<td>6-16</td>
<td></td>
</tr>
<tr>
<td>Urate (waste)</td>
<td>~1</td>
<td>2-6</td>
</tr>
<tr>
<td>protein</td>
<td>~1</td>
<td>&lt;150mg/24h</td>
</tr>
</tbody>
</table>
Transport across the renal tubular wall

- Single epithelial layer with tight junction at apical (luminal facing) membrane
- Lateral intercellular spaces between cells at basolateral membranes
- Peritubular capillary in close association with the basal membrane
- Movement across the renal tubular wall may be in either direction, and may be transcellular (across the cell) or paracellular (between the cells)
  - Reabsorption occurs in an apical-basal direction (from tubular fluid in lumen → peritubular capillary)
  - Secretion occurs in a basal-apical direction (from peritubular capillary → tubular fluid in lumen)

Methods of transport in the tubules:
- Active – active transport
- Passive – osmosis, co-transport (symporters), counter transport (antiporters), passive movement down a concentration or electrical gradient

Passive movement may involve lipophilic or hydrophilic molecules. This can be applied to all biological systems
- Movement of lipophilic molecules is protein independent, and the rate of movement is thus directly proportional to solute concentration
- Movement of hydrophilic molecules is protein dependent, thus the rate of movement has a maximum (due to the transport maxima of the protein carriers)
  - Passive movement of hydrophilic molecules is regulated by varying the number of protein carriers/channels embedded in the membrane
- The rate of active movement also has a maximum, as again involves carrier proteins. There are two types of active movement which occur across a cell.
  - Directly coupled to ATP hydrolysis, i.e. substance is moved into the cell using the energy from ATP hydrolysis (usually occurs at apical membrane)
  - Indirectly coupled to ATP hydrolysis, i.e. the substance is moved out of the cell using the energy from ATP hydrolysis (at basal membrane), and this creates a concentration which causes passive movement of the substance into the cell at the apical membrane
    - This is more important in tubular function
- Water “pumps” do not exist, therefore transcellular movement of water to an area of high osmolarity is via aquaporins, or water can move across the tight junctions (paracellular movement)
- Carrier proteins have a “transport maxima” which influences the max rate of the transport of the solute. However, this transport maxima is sufficient so that in a biological system, the transport system is rarely overloaded
  - Clinical correlation – glycosuria: the transport system for the reabsorption of glucose is overloaded, thus not all the glucose is reabsorbed from the tubular fluid + some is excreted in the urine

NB: Secretion – mechanism by which substances move from the peritubular capillaries into the tubular fluid (may be passively or actively transported). The most important ions which are secreted are H+ and K+. Choline, creatinine, penicillin + other drugs are also secreted, and thus excreted in the urine.

Regional specification of the nephron tubules

- Reabsorption is not uniform
- Most reabsorption (60-70%) occurs in the proximal convoluted tubule. This includes:
  - 100% glucose, amino acids + nutrients
  - 65% sodium
  - 90% bicarbonate
  - Water + anions then follow along a concentration/electrochemical gradient (maintaining plasma osmolarity)
More ion reabsorption occurs in the loop of Henle (i.e. 25% Na). However only the descending limb of the loop is permeable to water; therefore as tubular fluid moves through the loop, the osmolarity of the urine decreases.

In the early distal convolutes tubule, further ion reabsorption + water transport occurs (8% Na)

The late distal convoluted tubule + renal collecting duct as the final regulators. Variable reabsorption + secretion occurs depending on the body’s needs. This is regulated by aldosterone + vasopressin (ADH)

Cells of the different regions of the nephron tubules thus vary (suited to function)

- Lots of mitochondria are present in the PCT, ascending loop of Henle, + early DCT – indicates lots of active transport occurring
- Large brush border present on all regions except the collecting duct; large surface area means more interaction with tubular fluid = more passive reabsorption

The proximal convoluted tubule

- Site of most reabsorption, including Na+, Cl-, amino acids, glucose, proteins, water, urea, uric acid, vitamins, lactic acid, K+, Mg2+
  - H+ is also secreted
  - Creatinine, sulfates, phosphates + nitrates are not reabsorbed, and continue in the the tubular fluid

- NB: reabsorption of proteins – involves binding of the proteins on protein-receptors on the apical membrane, formation of pit + internalisation of the protein-receptor complex into a vesicle. The receptor then dissociates + is recycles; proteins are then broken down into amino acids before transport into the blood

- the majority of reabsorption that occurs in the PCT is driven indirectly by the Na/K pump present on the basolateral membrane. This pumps Na+ out of the cell into the peritubular capillary, therefore keeping intracellular [Na] low and [K] high (the cell is also then negative → electrochemical gradient)

- the concentration + electrical gradients thus favour Na movement into the cell, and this movement couples the uphill movement of glucose + amino acid into the cell (involving symporter proteins) and H+ out of the cell (involving antiporter carriers)

- urea + water then follows passively

- PCT reabsorption thus occurs via indirect ATP hydrolysis (driven by the Na/K pump) – this is used to reabsorb glucose, amino acids, sodium, potassium, calcium, vitamin C + uric acid

- The fact the reabsorption is dependent on the Na/K pump – means the reabsorption of all solutes/water are sensitive to metabolic poisons

- Secretion – there is some secretion in the PCT, which is important for the excretion of some drugs, as well as the movement of some drugs to more distal parts of the nephron to act there.
Loop of Henle

- The loop can be divided into the descending and ascending limb
- The **descending limb** is thinner, and highly permeable to water (which is passively reabsorbed between the squamous epithelium)
  - There are few mitochondria in the descending limb as ions are not actively transported
- The **ascending limb** is thicker and more involved in ion transport, therefore contains many more mitochondria.
  - Consists of a cuboidal epithelium with few microvilli, but is impermeable to water
  - Again, the Na/K pump on the basal membrane creates a concentration + electrical gradient, which drives the symporter on the apical membrane. This symporter transports 1 Na, 1 K and 2 Cl ions into the cell (making the inside of the cell negative compared to the outside)
  - However, there is also a K+ channel on the apical membrane which allows K+ to leak into the lumen along its concentration gradient. However this exaggerates the electrical gradient thus driving paracellular movement of the cations Na, K, Ca + Mg
  - Loop diuretics block the Na/K/Cl co-transporter
- On leaving the loop, 85% water + 90% of Na and K have been reabsorbed. However the fact that the ascending limb is impermeable to water means the tubular fluid is also hypo-osmolar compared to the plasma

Distal convoluted tubule (proximal end)

- Consists of a cuboidal epithelium with few mitochondria, complex basolateral membrane interdigitations with Na/K pumps + numerous mitochondria
- Na+ dependent uptake of Cl occurs (by co-transporter)
- Ca2+ entry into cell – from tubular fluid driven by electrochemical gradient + from plasma (by Na+/Ca antiporter)
- Thiazides = diuretics which target the Ca2+ channels → knock on effect on plasma calcium
- There is also a specialisation of the DCT where it meets the glomerulus = macula densa (forms part of juxtaglomerular apparatus) – detects changes in the [Na] of the tubular fluid filtrate

The Distal DCT + Cortical Collecting duct

- “fine” tuning of the filtrate in order to maintain homeostasis (plasma osmolarity, pH etc) occurs here
- Regulation is under hormonal control (aldosterone + ADH/Vasopressin)
- Consist of two types of cells: principle cells + intercalated cells
- The principle cells of the DCT are under the control of aldosterone, whereas the principle cells of the cortical collecting duct are under the control of ADH/Vasopressin
Principle cells are important in sodium, potassium + water balance (mediated via Na+/K+ pump on basolateral membrane).

In the principle cells of the DTC, the apical sodium channel is sensitive to aldosterone, and is linked to a K+ channel also on the apical membrane.

Intercalated cells exist between each principle cell, and are important in acid-base balance, mediated by an H-ATP pump on the apical membrane.

The principal cells of the cortical collecting duct have a tight epithelium, therefore tightly regulate water movement (driven by osmolality gradient).

Clinical application of knowledge – defects of tubular function

- There are 3 single gene defects which affect tubular function:
  o Renal tubule acidosis
  o Bartter syndrome
  o Fanconi syndrome

Renal tubular acidosis
- Caused by an inability to acidify the urine below pH5.5 → hypechloremic metabolic acidosis of the blood
- Other symptoms include impaired growth + hypokalemia
- Mainly a defect in the distal renal tubule → failure of H+ ion secretion even when conditions are favourable for secretion
- In a normal tubular cell, H+ secretion occurs simultaneously to HCO₃⁻ transport out of the cell into the blood. This is driven by a reaction catalysed by carbonic anhydrase.
- Failure of H+ secretion has 2 possible causes:
  o malfunction of bicarbonate transport out of tubular cell into blood → accumulation of product therefore limiting carbonic anhydrase activity.
  o A mutation in the carbonic anhydrase enzyme could also occur.

Bartter syndrome
- Definition = excessive electrolyte secretion
- Caused by mutation in the Na/Cl/K co-transporter on ascending limb of Henle, or mutation in the K+ channel
- Causes severe salt loss, moderate metabolic alkalosis, hypokalemia, renin + aldosterone hypersecretion
- Antenatal barter syndrome = more severe form of disease → premature birth + polyhydramnios

Fanconi Syndrome
- Defined by an increased excretion of low molecular weight proteins caused by a failure of protein reabsorption
- Also increased excretion of uric acid + glucose phosphate
- Caused by a defect in a Cl- channel involved in protein-receptor vesicle recycling → reduction of protein receptors therefore proteins cannot be reabsorbed
- E.g. of detrimental consequence = excessive amounts of cytokines flowing through tubular system → immune response
1. State the meaning of the term osmolarity
2. State the minimum and maximum osmolarity of the urine in humans and indicate the nephron sites responsible for the production of:
   a. Dilute urine
   b. Concentrated urine
3. Explain why the final concentration of urine depends on:
   a. The osmolarity of the medullary + papillary interstitum
   b. The permeability of the collecting ducts to water
4. Explain the mechanisms by which the medullary + papillary interstitium becomes hypertonic as a result of the accumulation of NaCl and urea
5. Describe how changes in plasma osmolarity influence the release of vasopressin (ADH) from the posterior pituitary, using the term hypothalamic osmoreceptors
6. Describe the action of vasopressin on the collecting ducts, and hence explain how urine volume is regulated in accordance with the state of hydration of the body
7. Describe how changes in plasma osmolarity and volume influence thirst

Osmolarity + Urine

Definition of osmolarity: a measure of the solute concentration in a solution

Units = osmoles/litre (1 mole of dissolved solute per litre, often measured as mosmol/l)

The greater the number of dissolved particles, the greater the osmolarity (considers the sum of the concentrations of all the different solutes added together)

Water flows across a semi-permeable membrane from a region of low osmolarity to a region of high osmolarity

If a cell is hyperosmotic compared to the surrounding solution, water moves in and the cell swells

If a cell is hypoosmotic compared to the surrounding solution, water moves out and the cell shrinks

We operate in a constant osmolarity environment, thus the regulation of water and salt balance are inter-related in order to maintain osmotic homeostasis, i.e. if we increase the salt concentration, osmolarity increases but then water follows the salt so the osmolarity returns to the original level.

On an average day we consume 20-25% more water and salt than we need. This results in 3 reasons for urine production:

- Removal of excess removal (to prevent oedema + hypertension)
- Removal of excess water (to prevent ECF from becoming hypoosmotic causing cell swelling)
- Removal of excess salt (to prevent ECF from becoming hyperosmotic causing cell shrinkage)

Water

- Normal plasma osmolarity is regulated between 285-295 mosmol/l
- Water is the most abundant component of plasma and ECF, with sodium being the most prevalent solute (~1450mmol/l). Other components include chloride, bicarbonate, potassium, glucose, calcium + proteins.
- Urine production is used to regulate the plasma osmolarity + ECF volume through the balance of water + salt
- Approx. 40L of water in the body, with approx. 65% forming intracellular fluid, and 35% extracellular
  - ECF consists of interstitial fluid, plasma, lymph + transcellular fluid (e.g. CSF)

**How do we get rid of water?**
- Sweat - ~450ml/day (production is uncontrollable, but varies with fever, climate + physical activity)
- Faeces - ~100ml/day (production is uncontrollable, but varies depending on solidity of faeces)
- Respiration - ~350ml/day (production is uncontrollable, varies with physical activity)
- Urine output - ~1500ml/day (largest component; variable but CONTROLLABLE)
  - Looking at the colour of urine (dipstick test) can be used to assess concentration and hence how well hydrated an individual is

**Control of water excretion**

- Within the tubular system, water removal occurs in all regions except the ascending limb of the loop of Henle
- Approx. 125ml/min (180L/day) of water passes through the fenestrae of the glomerular capillaries
- The bulk of the water is reabsorbed in the PCT (70%) – this is because most of the solute pumping occurs here, and water follows to maintain osmotic balance
- The rest of the water reabsorption is variable, with approx. 30% occurring in the descending limb of the loop of Henle + 20% in the DCT – water output from the renal collecting duct is thus very variable (0.7-1.4ml/min or 1-2L/day)
- Water reabsorption in the in the convoluted tubules is driven by ion pumping, therefore to account for the variation in water output through the kidney nephron, consider the loop of Henle + renal collecting duct
- In order to maintain plasma osmolarity, urine must be concentrated above the normal plasma osmolarity – this is done by producing a region of hyperosmotic interstitial fluid (from the cortex to the inner medulla, the osmolarity of the interstitial fluid increases from 290-1200mosmol/l)

**Generating the gradient**
- Counter current system is created in order to establish a gradient, by regulating where water movement can occur (i.e. varying the water permeability)
  - Urea also makes up a significant portion of the gradient
- **The loop of Henle**
  - The thin descending limb is permeable to water, and the cells are involved in a lot of water reabsorption (large brush border with few mitochondria)
  - The thick ascending limb is permeable to salt but not water, and the cells are involved in a lot of ion pumping (tight junctions with many mitochondria)
  - As tubular fluid flows through the ascending limb, it generates a salt gradient of approx. 200 mosmol/l → and interstitial fluid of ~400 mosmol/l
  - To equilibrate that, water is reabsorbed from the descending limb, decreasing the interstitial fluid osmolarity restoring the equilibrium
  - However at the bottom of the loop + the beginning of the ascending limb, more salt is pumped out generating a further difference of ~200 mosmol/l – thus the tubular fluid leaving the loop of Henle is hypoosmolar (the water reabsorption in the descending limb is not sufficient to fully equilibrate the IF conc)
  - The collecting duct is then permeable to water under presence of ADH, therefore as the collecting duct transverses the renal medulla urine is concentrated as water moves out of the tubular fluid to maintain the IF osmolarity

- **Role of urea**
  - the concentration gradient generated in the loop of Henle cannot be created by salt alone
  - Urea has 2 regions of the kidney nephron which are permeable to it, and the movement of urea continues in a cycle:
    - The bottom of the loop/beginning of the ascending limb of Henle is permeable to urea, therefore it enters the tubular fluid thus resulting in a more hyposmolar tubular fluid
    - The renal collecting duct is then also permeable to urea, thus it is removed and reabsorbed

- **Renal medullary blood flow**
  - All the tubular cells require oxygen + nutrients in order to maintain function. However an ordinary blood supply would result in a loss of the hyperosmotic IF gradient as excess salt would move into the capillaries
  - This gives a need for a specialised blood supply – the vasa recta
  - The vasa recta is a looped blood system which follows the tubules, but is permeable to water and solutes
  - This means that in the descending limb, water diffuses out/solutes diffuse into the limb from the vasa recta. But in the ascending limb the reverse happens; therefore the concentration gradient is maintained on leaving the loop of Henle

**The Distal Convoluted tubule + the collecting duct**

**Vasopressin**
- Also known as antidiuretic hormone (ADH)
- Peptide hormone (9 aa long) – synthesis in hypothalamus, packaged into granules then secreted from neurohypophysis (PPG)
- Binds to specific receptors on basolateral membrane of the principal cells
- This causes insertion of aquaporins into the cells luminal membrane, Aquaporins are an active water uptake system which are stored in vesicles unless under the influence of ADH (therefore water uptake in the tubule is regulated). This increases the permeability of the duct to water.
- ADH also stimulates urea transport from the inner medullary collecting duct into the thin ascending limb of loop of Henle

**What triggers ADH release?**
- Release is regulated by hypothalamic osmoreceptors which respond to an increase in plasma osmolarity >300 Mos
- Baroreceptors also act as a secondary signal, stimulated by a fall in BP/blood volume
- Ethanol inhibits ADH → increase in urine volume + dehydration
Water intake

- Water load $\rightarrow$ a decrease in plasma osmolarity $\rightarrow$ sensed by hypothalamic osmoreceptors which decrease ADH release $\rightarrow$ decrease in water permeability of collecting duct $\rightarrow$ increase in urine flow rate + volume (increased fluid loss will tend to raise plasma osmolarity – negative feedback)
  - Basically sodium reabsorption occurs without water reabsorption $\rightarrow$ low urine osmolarity (50mosmol/l) + diuresis
  - Urea is also not recycled as much, therefore the concentration gradient generated in the loop of Henle is smaller which reduces water reabsorption
- Dehydration $\rightarrow$ decreased plasma osmolarity $\rightarrow$ sensed by hypothalamic osmoreceptors $\rightarrow$ thirst + ADH release $\rightarrow$ increased collecting duct water permeability $\rightarrow$ decreased urine flow rate
  - Increased urea recycling $\rightarrow$ larger gradient in loop of Henle $\rightarrow$ more water reabsorption

NB: Feedback Control via ADH keeps plasma osmolarity in a normal range – also determines urine output + water balance

Diabetes insipidus

- Disorder of water balance, caused by:
  - No/insufficient ADH production
  - Mutant ADH receptor $\rightarrow$ no detection
  - Mutant aquaporin $\rightarrow$ no response to ADH binding
- Results in polyuria (>30l/day) + polydipsia (unremitting thirst)

Control of Sodium + Potassium excretion

As discussed in the previous lecture, water balance is used to regulate plasma osmolarity. It can then be said that the level of salt determines the ECF volume (plasma makes up part of the ECF volume). If we know the number of mosmoles of salt in the ECF, as well its concentration; we can determine the volume of ECF.

As sodium is the most prevalent solute in the ECF, the knock on effect is that it is most important in regulating ECF volume.

Humans are closed systems, therefore changes in the volume of the ECF in turn affects the blood volume + pressure

- Increased dietary sodium $\rightarrow$ increased osmolarity $\rightarrow$ increased ECF volume (water moves into an area of higher osmolarity) $\rightarrow$ increased blood volume + pressure
  - water retention also leads to increase in body weight
- Decreased dietary sodium $\rightarrow$ decreased osmolarity $\rightarrow$ decreased ECF volume (Water moves into an area of high osmolarity) $\rightarrow$ decreased blood volume + pressure
  - water loss also leads to decrease in body weight
- In reality, the body can’t let the ECF osmolarity change outside of the regulated range of 285-296mosmol/L, therefore sodium levels must be regulated. This is done by balancing the sodium excretion according to our dietary intake

Control of sodium excretion

- The bulk of sodium is reabsorbed in the PCT (65%) through co-transport mechanisms which also drive the reabsorption of bicarbonate, glucose and amino acids.
- 25% more is absorbed in the loop of Henle, 8% in the DCT
- Reabsorption in the collecting duct is variable, but up to 2%
• NB: These percentages are true for any volume of filtrate. For example of GFR is increased, the Na reabsorption rate increases (more filtrate into Bowman’s capsule, therefore greater number of Na ion in the tubule at a given time, thus increasing the concentrating gradient across the tubular membrane and increasing reabsorption)
  o This is only true up to the transport maxima of the co-transporters on the luminal membrane (apical) + the Na/K pump on the basolateral membrane

❖ Increasing sodium retention
• Aim is to reduce the amount going into the glomerulus. This is achieved by increasing sympathetic activity (causing greatest vasoconstriction in the afferent arteriole) which thus reduces the GFR.
  o Increased sympathetic activity also stimulates Na channels in the PCT, thus increasing Na reabsorption in the PCT
  o Increased sympathetic activity also stimulates the juxtaglomerular apparatus, which stimulates the mechanisms which drive Angiotensin II synthesis.
    ▪ Angiotensin II also stimulates Na channels in the PCT, thus increasing Na reabsorption in the PCT
    ▪ Angiotensin II also drives Aldosterone synthesis, which increases Na and water reabsorption in the DTC + collecting duct
• Because the uptake of Na in the PCT has been increased, the filtrate reaching the juxtaglomerular apparatus will have a lower salt concentration. This is detected, and further drives angiotensin II production.

❖ Decreasing sodium reabsorption
• Involves atrial natriuretic peptide (ANP), which acts as a vasodilator (predominantly on the afferent arteriole), stimulating increased GFR
  o ANP also reduces the activity of Na uptake channels in the PCT, JGA + CT

Importance of the Renin-Angiotensin-Aldosterone system
• By the time the filtrate reaches the JGA, its salt concentrated is monitored by the macula densa cells of the juxtaglomerular apparatus
• A reduced filtrate Na concentration drives Renin synthesis, which converts angiotensinogen (angiotensinogen synthesised in the liver) to angiotensin I
  o Renin synthesis is also stimulated by decreased blood pressure, fluid volume + increase beta1-sympathetic activity
  o Renin synthesis is inhibited by an increase in blood pressure, fluid volume, decreased beta1-sympathetic activity + ANP
• Angiotensin converting enzyme (present in the lungs) converts angiotensin I → angiotensin II

Effects of angiotensin II
• Proximal convoluted tubule – increased sodium channel activity → increased sodium uptake → increased water reabsorption → increased ECF/plasma volume → increased blood pressure
• Vascular system – vasoconstriction → increased blood pressure
• Adrenal gland – aldosterone synthesis

Aldosterone
• Steroid hormone synthesised + released from the adrenal cortex in response to Angiotensin II, decreased blood pressure (via baroreceptors) and decreased osmolarity of the ultrafiltrate
• Aldosterone stimulates the principal cells of the distal convoluted tubule/cortical collecting duct
Increased sodium reabsorption (+ thus water reabsorption)
- Increased potassium secretion
- Increased hydrogen secretion

- Aldosterone excess leads to hypokalaemic alkalosis (reduced potassium concentration in the blood, in addition to reduced hydrogen concentration which increases the pH)
- Aldosterone binds to intracellular receptors, which causes a change in conformation which trives the receptor-aldosterone complex translocation into the nucleus.
  - In the nucleus, the complex binds to specific regions of DNA, acting as a transcription factor:
    - Increase expression of the apical/luminal Na channel
    - Also promotion of activity via regulatory proteins (the proteins are converted from low to high affinity transporters)
    - Increased formation of the Na/K ATPase pumps
  - Positive feedback also occurs, so that there is an increased effect of the aldosterone-receptor binding to the DNA

**Diseases of aldosterone secretion**

**Hypoaldosteronism**
- Reabsorption of sodium in the distal nephron is reduced, leading to an increased urinary loss of sodium and therefore water.
- ECF volume thus falls, with a compensatory increase in Renin, Ang II + ADH
  - This results in low BP, dizziness + salt craving. Salt is required for heart function, therefore palpitations also occur.

**Hyperaldosteronism**
- Reabsorption of sodium in the distal nephron is increased, leading to a reduced urinary loss of sodium and therefore water
- EVF volume thus increases, with a compensatory decrease in Renin, Ang II + ADH
- A compensatory increase in atrial/brain natriuretic peptide occurs
- This causes hypertension, muscle weakness, polyuria + thirst

**Liddle’s Syndrome**
- Autosomal dominant disorder characterised by early, and frequently severe, hypertension. Also associated with low plasma renin activity, metabolic alkalosis due to hypokalemia, and hypoaldosteronism
- Caused by a mutation in the aldosterone activated sodium channel in the principal cells of the DCT/CT, the result of which is a permanently activated channel leading to increased sodium retention

**Relationship between ECF + blood pressure**
- There are two sets of baroreceptors, which respond to blood pressure.
  - One set exist in a low pressure environment – the atria, right ventricle + pulmonary vasculature
    - These respond to a decrease + increase in blood pressure
  - The other exists a high pressure side – carotid sinus, aortic arch, juxtaglomerular apparatus (+ pulmonary vasculature)
    - These only respond to a decrease in blood pressure
- Responses to low blood pressure – receptors on both the LOW + HIGH pressure side signal through afferent fibres to the brainstem, which increases sympathetic activity + ADH release
  - The JGA cells on the high pressure side also respond by increases renin release
- Response to high blood pressure – receptors on the LOW pressure side respond to atrial stretch by release of ANP + BNP
ANP (atrial natriuretic peptide)
- Small peptide made in the atria (also brain natriuretic peptide, BNP, made) which is released in response to increased atrial stretch following increased blood pressure
- Actions of ANP include:
  - Vasodilation of renal (and other systemic) blood vessels (increases GFR)
  - Inhibition of sodium reabsorption in the PCT + CT
  - Inhibition of renin + aldosterone release
  - Ultimately reduces blood pressure

ECF volume expansion (with corresponding increased blood pressure)
Effect on:
- **Sympathetic nervous system** – reduced sympathetic activity increases GFR + reduces renin secretion from the juxtaglomerular apparatus
- **Brain** – reduced ADH production causes an increase in Na + water excretion from the collecting duct
- **Heart** – increased release of ANP + BNP has an effect on the kidney nephron; increasing Na + water excretion from the collecting duct.
  - These also have an effect on the **adrenal gland**, to reduce aldosterone secretion which in turn reduces sodium reabsorption in the DCT/cortical collecting duct
  - Also an effect on macula densa cells of JGA – reduced renin secretion
- **Juxtaglomerular Apparatus** – volume expansion has a direct effect on renin secretion (as well as the influence of reduced sympathetic activity + ANP/BNP from atria)
  - Reduced renin secretion \(\rightarrow\) reduction in angiotensinogen conversion to Angiotensin I, with a corresponding decrease in Angiotensin II production

ECF volume contraction (with corresponding decrease in blood pressure) – reverse occurs

Sodium, BP and Diuretics

Regulating sodium is central to the function of diuretics

ACE Inhibitors
- Work by inhibiting the angiotensin I converting enzyme, with a corresponding reduction in Angiotensin II levels
- This in turn reduces Aldosterone secretion
- Reduction in angiotensin II \(\rightarrow\) reduced sodium channel activity in the PCT \(\rightarrow\) reduced sodium uptake with corresponding decrease in ECF volume + blood pressure
- Reduction in aldosterone \(\rightarrow\) increased sodium reabsorption in the DCT/cortical collecting duct with a corresponding decrease in ECF volume + blood pressure
- However note that the effects of reduction in Ang II + aldosterone are not confined to the kidney:
  - E.g. Reduced Angiotensin II causes reduced vasoconstriction

Diuretics
- There are different types of diuretic drugs, all which act to increase the rate + volume of urine production. The different diuretics have different sites of action along the nephron.
  - Osmotic diuretics and carbonic anhydrase inhibitors - act on the proximal convoluted tubule
  - Loop diuretics – act on the thick ascending limb of loop of Henle
  - Thiazides – act on the proximal part of the distal convoluted tubule
  - K+ spacing diuretics – act on the distal part of the DCT + cortical collecting duct

❖ Osmotic diuretics
Osmotic diuretics are compounds which are present in the tubular filtrate, increasing its osmolarity therefore water retention in the urine occurs to maintain osmotic balance.

Mannitol is filtered into the glomerulus, but cannot be reabsorbed – its presence leads to an increase in the osmolarity of the filtrate. To maintain osmotic balance, water is retained in the urine.

Glucose is usually completely reabsorbed from the tubular filtrate into the blood. However in certain conditions such as diabetes mellitus, the concentration of glucose in the blood exceeds the transport maxima of the Na-glucose co-transporters in the PCT. When this happens, glucose remains in the filtrate leading to the osmotic retention of water in the urine + associated glycosuria.

**Carbonic anhydrase inhibitors**

- Carbonic anhydrase is an enzyme present in the cells of the PCT, which leads to Na reabsorption + increased urinary acidity (through reduced conversion if bicarbonate into water + carbon dioxide).
- Carbonic anhydrase inhibitors act to inhibit the actions of carbonic anhydrase.
- In the PCT, carbonic anhydrase acts both in the tubular fluid and in the cells of the PCT.
  - HCO$_3$- (bicarbonate ions) bond with H+ ions in the tubular fluid to form H$_2$CO$_3$. Carbonic anhydrase acts to convert the H$_2$CO$_3$ to H$_2$O + CO$_2$ (these can then easily diffuse into the PCT cell).
    - NB: CO$_2$ also diffuses into the cell from the plasma.
  - In the cell, the H$_2$O + CO$_2$ are then converted back to H$_2$CO$_3$ (through the action of carbonic anhydrase). This then splits into its former H$^+$ and HCO$_3^-$ ions.
    - The H$^+$ ions are then transported back into the tubular fluid, coupled with the transport of Na from the fluid (i.e. sodium reabsorption).
    - The HCO$_3^-$ then diffuse freely into the ECF, thus increasing the basicness of the ECF.

**Loop diuretics**

- E.g. furosemide – blocks the triple luminal transport on the cells of the ascending loop of Henle, thus increasing the osmolarity of luminal fluid leading to increased urine water retention.

**Thiazides**

- Work on inhibiting the Na/Cl co-transporter in the distal convoluted tubule.
- This increases the osmolarity of the tubular fluid, thus reducing the amount of water reabsorbed.

**K$^+$ sparing diuretics**

- These are diuretics which do not promote the secretion of potassium into the urine; thus, potassium is spared and not lost as much as in other diuretics. The term "potassium-sparing" refers to an effect rather than a mechanism or location; nonetheless, the term almost always refers to two specific classes that have their effect at similar locations:
  - Aldosterone antagonists – e.g. spironolactone. Prevents aldosterone action of increasing sodium reabsorption in the late DCT + cortical collecting duct.
  - Epithelial sodium channel blockers – e.g. amiloride.

**Potassium Regulation**

- Potassium is the main IC ion (150mmol/l).
- Low EC ion (3-5mmol/l).
- Extracellular K$^+$ has effects on excitable membranes, e.g. nerves + muscles.
  - High EC K$^+$ - involved in depolarisation (along with a high IC [Na$^+$]); generation of action potentials. Therefore may be involved in tachycardia + heart arrhythmias.
  - Low EC K$^+$ - also involved in heart arrhythmias, particular asystole (absence of heart contractions).
Potassium mainly comes from diet. By eating meat, you effectively are eating cells, thus eating IC space which contains lots of potassium. This leads to increased plasma potassium, which must be taken up into tissues
- This tissue uptake is driven by insulin (+ aldosterone and adrenaline)

Tissue uptake can be seen as the immediate response to dietary K+, which involves the Na/K pump transporting potassium out of the plasma into the cell.
- Potassium then leaks out of the cell via potassium channels, into the EC space surrounding cells

**Potassium handling by the kidneys**
- ~70% of potassium reabsorption occurs in the PCT, with ~30% of the filtered load reaching to loop of Henle
- Further reabsorption occurs in the loop of Henle, with ~10% remaining to reach the DCT
- Potassium secretion then occurs in the principle cells of cortical collecting duct, and this varies from 1-80% of the initial filtered load (prior to reabsorption). Secretion is stimulated by:
  - Increased plasma [K$^+$]
  - Increased aldosterone
  - Increased tubular flow rate
  - Increased plasma pH
- On a cellular level, potassium secretion is driven by the basolateral Na/K pump, and the apical/luminal K$^+$ channel
  - The cell membrane potential also drives the secretion.
  - Aldosterone stimulates the action of both the basolateral Na/K pump, and the apical/luminal K$^+$ channel
  - An increase in tubular flow rate also increases K secretion. The rate increase is detected by cilia on principal cells, linked to the PDK1 enzyme which activation increases the intracellular calcium.
    - This increases stimulates the activity of the luminal K+ channel, causing more potassium to be excreted

**Potassium imbalances**

- **Hypokalemia**
  - Low plasma potassium levels – one of the most common electrolyte imbalances (common in 20% hospitalised patients)
  - Caused by:
    - Diuretics
    - Surreptitious vomiting (intentional in secret, e.g. in eating disorders)
    - Diarrhoea
    - Genetics - Gitelman’s syndrome; mutation in the Na/Cl transporter in the distal nephron

- **Hyperkalemia**
  - High plasma potassium levels – present in 1-10% hospitalised patients
  - Seen in:
    - K+ sparing diuretics
    - ACE inhibitors
    - Elderly
Mechanism of acid-base balance

1. What is the normal physiological pH range, and why is its normal value so important for life?
2. Why is HCO₃⁻/CO₂ system so important for plasma pH?
3. What is the definition of buffer and what do they do in our body?
4. How do the kidneys contribute to systemic acid-base balance?
5. What are the mechanisms for H⁺ excretion in kidneys?
6. How do the various segments of the nephron contribute to the process of reabsorbing the filtered HCO₃⁻?
7. How do the kidneys form new HCO₃⁻?
8. What are the major mechanisms by which the body defends itself against changes in acid-base balance?
9. What is the difference between simple metabolic and respiratory acid-base disorders, and how are they differentiated by blood gas analysis?

Outline of lecture: basic definitions, controlled pH value + its importance, acid-base balance regulation, cellular balance system, hydrogen ion excretion, bicarbonate reabsorption, new bicarbonate formation, basic acid-base disorders, clinical implications

- **Acid**: a substance that can release H⁺ ions in solution
- **Base**: a substance that can accept H⁺ ions in solution
- **Buffer**: a substance which can release or accept H⁺ ions in solution, resulting in minimal changes to pH
- **pH**: a logarithmic measurement of H⁺ ion concentration; indicates acidity of the solution. pH = -log[H⁺]
Controlled pH value and its importance

- \([H^+]\) maintained in very narrow limits at low conc:
  - Normal EC = 40nmol/l
  - This is equal to pH=7.40
  - Normal plasma pH range = 7.34-7.45
- Outside pH range 7.2-7.6 regarded as serious pathological condition
  - Range of pH compatible with life = 7.80-6.80 ([H+] = 16-160nmol/l)
- Urine pH range = 4.0-8.5

The control of pH is dependent on the dissociation of hydrogen bicarbonate involving the enzyme carbonic anhydrase. In fact the pH of the plasma is dependent on both H+ concentration and bicarbonate ion concentration.

To determine this, we use Henderson-Hasselbach equation

\[
\text{pH} = \text{pKa} + \log \left( \frac{[\text{HCO}_3^-]}{\alpha \text{PCO}_2} \right)
\]

Why is control of pH so important?

- Metabolic reactions are highly sensitive to pH or ion concentration
- This is because H+ ions change the shapes of proteins, including the enzymes which catalyse all the metabolic reactions in the body

Acid-base balance regulation + cellular buffer system

Basic steps
1. Extracellular and intracellular buffers
2. Control of partial pressure of CO₂ in blood by alterations in the rate of alveolar ventilation
3. Control of plasma HCO₃⁻ concentration by changes in renal H⁺ excretion

Buffering process
- The principle buffer in the blood is H₂CO₃ <-> H⁺ + HCO₃⁻
- The principle buffer in the intracellular fluid is H₂PO₄⁻ <-> H⁺ + HPO₄²⁻
- In metabolic acidosis, 80-85% of the acid load is buffered in cells
- In metabolic alkalosis, only 30-35% of the OH⁻ load is buffered in cells
- In respiratory acidosis/alkalosis, “all buffering is intracellular”
- The most important EC buffering system is:
  - H⁺ + HCO₃⁻ <-> H₂CO₃ <-> H₂O + CO₂
  - HCO₃⁻ is independently regulated by renal H⁺ excretion
  - PCO₂ is also independently regulated by changes in the rate of alveolar ventilation

Buffering at a local level
- H₂SO₄ + HCl are produced during metabolism, but do not circulate as free acids
- They are immediately buffered in the ECF by HCO₃⁻
  - H₂SO₄ + 2NaHCO₃ → Na₂SO₄ + 2H₂CO₃ → 2H₂O + CO₂
  - HCl + NaHCO₃ → NaCl + H₂O + CO₂
- These reactions minimise increase in EC H⁺, but excess must be excreted by the kidney to prevent progressive depletion of HCO₃⁻

Hydrogen ion excretion

Sources of H⁺ ions in the body
- Physiologically – carbohydrates + fats, sulphur-containing amino acids, arginine histidine + lysine
Pathologically – hypoxia, carbohydrates + fats, diabetes, ketoacids
Volatile acids – produced from metabolism of carbohydrates + fats, result in carbon dioxide production which is lost through respiration
Non-volatile acids – derived from metabolism of proteins, result in H+ ions that are excreted by the kidneys

Renal H+ excretion
- The kidneys must excrete 50-100mmol of non-carbonic acids generated each day
- Involves different mechanisms at various parts of the nephron (PCT, thick ascending LoH, CD)
- Overview:
  - All bicarbonate ions filtered into urine reabsorbed
  - Secreted H+ ions are then excreted, either with:
    - Filtered buffers e.g. phosphates + creatinine
    - Manufactured buffer e.g. ammonia; manufactures from glutamine in the PCT

Renal H+ pumps
- In the PCT, ions are secreted into the lumen by Na/H exchanger
  - Bicarbonate ions are then returned to the systemic circulation by the Na/HCO3 co-transporter
- In the collecting duct, the luminal pump is mediated by active H+-ATPase and CL-HCO3 exchanger in basolateral membrane
- The net effect is the excretion of 1H+ion (buffered by phosphate) + addition of 1 HCO3- to the plasma

Regulation of excretion
To increase or decrease H+ secretion, there are both primary + secondary mechanisms (secondary stimuli/mechanisms are not directed at maintaining acid-base balance)

- To increase H+ secretion:
  - Primary – decrease plasma bicarbonate concentration + increase pp of arterial CO2
  - Secondary – increase filtered load of bicarbonate, decrease ECF volume, increase Ang II, increase aldosterone, hypokalaemia
- To decrease H+ secretion
  - Primary – increase plasma bicarbonate concentration + decrease pp of arterial CO2
  - Secondary – decrease filtered load of bicarbonate, increase ECF volume, decrease aldosterone + hyperkalaemia

Bicarbonate reabsorption
- ~80% is reabsorbed in the PCT, with the remaining being reabsorbed in the thick ascending loop of Henle + outer medullary collecting duct – this is known as segmental reabsorption
- There is a net reabsorption of 1 filtered Na+ and 1HCO3-

New Bicarbonate formation
- Reason for new formation = bicarbonate reabsorption < bicarbonate lost during buffering of non-volatile acids
- In the liver, amino acids are broken down into glutamine + urea; this glutamine is converted to ammonium ions + alpha-ketoglutarate in the kidney
- The alpha-ketoglutarate is then converted to bicarbonate ions in the kidney

Ammonium excretion
- Ability to excrete H+ ions as ammonium adds important degree of flexibility to renal acid-base regulation
- NH3 produced in tubular cells predominantly from glutamine
Some of excess NH₃ diffuses into tubular lumen
Excreted H⁻ combines with NH₃ to form NH₄⁺

Basic acid-base disorders

Metabolic acidosis
- Low plasma pH and HCO₃⁻
- Caused by
  - Addition of non-volatile acids (e.g., ketoacidosis in diabetes)
  - Loss of non-volatile alkalis (e.g., diarrhoea)
  - Failure to reabsorb sufficient HCO₃⁻ (renal failure)
- Respiratory compensation occurs by raised ventilation due to peripheral chemoreceptor stimulation
  - pCO₂ falls by ~1.2 mm Hg for every 1 mmol/l fall of HCO₃⁻
- Renal excretion of net acid increases if possible

Metabolic alkalosis
- Raised plasma pH and HCO₃⁻
- Caused most commonly by loss of non-volatile acid (e.g., vomiting)
- Also caused by raised aldosterone
- Compensation occurs by
  - Reduced ventilation: pCO₂ rises by 0.7 mm Hg for every 1mmol/l rise of plasma HCO₃⁻
  - Renal excretion of excess HCO₃⁻, but this can be limited if low blood volume with Na⁺ and Cl⁻ depletion

Respiratory acidosis
- Low plasma pH and high pCO₂
- Caused by reduced alveolar ventilation or impaired gas diffusion
- Renal compensation occurs by increasing HCO₃⁻ and NH₄⁺ secretion (takes several days)
  - In intervening acute phase, cellular buffering minimises changes to plasma pH
  - For change of pCO₂ of 10 mmHg, plasma HCO₃ increases by ~1mmol/l in acute phase and by ~3.5mmol/l in chronic phase
- pH rises back towards, but not above normal

Respiratory alkalosis
- Elevated plasma pH and reduced pCO₂
- Caused by increased alveolar ventilation
- Renal compensation occurs by decreasing HCO₃⁻ reabsorption and NH₄⁺ secretion, but this takes several days
  - In acute intracellular buffering phase, plasma HCO₃⁻ decreases by 2mmol/l for drop of pCO₂ of 10 mm Hg
  - In chronic phase, plasma HCO₃⁻ decreases by 5mmol/l
  - pH falls back towards normal, but not below normal

Analysis of acid-base disorders
- Analysis of an acid-base disorder directed at identifying the underlying cause
- Treatment can be initiated
- Med. history and associated physical findings often provide valuable clues about nature and origin of an acid-base disorder
- Required arterial blood analysis

GOOD SUMMARIES ON PP
What happens when the kidneys stop?

Loss of excretory function → accumulation of waste products
Loss of homeostatic function →
Disturbance of electrolyte balance
Loss of acid-base control
Inability to control volume homeostasis
Loss of endocrine function

NB: the clinical features are determined by the rate of deterioration

Causes:

**Lethargy + anorexia**
- Accumulation of nitrogenous waste products, hormones, peptides and other ‘middle-sized’ molecules (Mol Wt 2-5000)
- Acidosis
- Hyponatraemia
- Volume depletion (low blood pressure)
- Anaemia

**Salt + water imbalance**
- Inability to decrease sodium excretion (i.e. increase sodium reabsorption) when sodium depleted.
- Osmotic diuresis - caused by high concentration small MW waste substances, e.g., urea.
- This inappropriately high loss of salt and water results in volume depletion which causes the low blood pressure
- Salt and water loss usually found in patients with tubulointerstitial disorders in which the concentrating mechanisms have been damaged
- It is more usual for patients with renal dysfunction to have difficulty in excreting salt and water. This leads to a tendency to retain sodium
  - Hypertension
  - Oedema
  - Pulmonary oedema

**Implications:**

**Acidosis**
- Caused by decreased excretion of $\text{H}^+$ ions and by retention of acid bases
- Buffered by $\text{H}^+$ ions passing into cells in exchange for $\text{K}^+$ ions – therefore aggravates tendency to hyperkalaemia
- Another compensation mechanism is increasing CO$_2$ loss through the lungs - Kussmahl respiration (air hunger)
- Exacerbates anorexia and increases muscle catabolism

**Hyperkalaemia**
- Caused by failure of distal tubule to secrete potassium
- Exacerbated by acidosis - causes shift of potassium from intracellular to extracellular space
- Can cause cardiac arrhythmias and arrest
- Clinical features of hyperkalaemia are dependent on the chronicity of the hyperkalaemia
Metabolism
- Decreased erythropoietin production in renal failure results in anaemia
- Low 1-25 Vit D levels result in poor intestinal calcium absorption, hypocalcaemia (short term) and hyperparathyroidism (longer term)
- Increased cardiovascular risk

Acute or chronic loss of function

<table>
<thead>
<tr>
<th>Acute</th>
<th>Chronic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaemia</td>
<td>Anaemia</td>
</tr>
<tr>
<td>Acidosis</td>
<td>Acidosis</td>
</tr>
<tr>
<td>Tendency to Hyperkalaemia</td>
<td>Tendency to Hyperkalaemia</td>
</tr>
<tr>
<td>Hypocalcaemia</td>
<td>Renal osteodystrophy</td>
</tr>
<tr>
<td><strong>Renal size unchanged</strong></td>
<td><strong>Renal size often reduced</strong></td>
</tr>
<tr>
<td>Acute metabolic complications</td>
<td>Chronic uraemic symptoms</td>
</tr>
<tr>
<td>Tendency to hyponatraemia</td>
<td>Tendency to hyponatraemia</td>
</tr>
<tr>
<td>Volume usually overloaded $\rightarrow$ Oedema, hypertension</td>
<td>Volume usually overloaded $\rightarrow$ Oedema, hypertension</td>
</tr>
<tr>
<td>Previously normal creatinine</td>
<td>Previously abnormal creatinine</td>
</tr>
</tbody>
</table>

Treatment/Management

Initial management
- Intravenous normal saline to correct fluid depletion
- Intravenous sodium bicarbonate to correct acidosis
- Intravenous insulin and dextrose to lower plasma potassium (by driving K$^+$ ions back into cells)
- dialysis

Assessing GFR
- **UREA**
  - Poor indicator
  - Confounded by diet, catabolic state, GI bleeding, drugs, liver function etc
- **CREATININE**
  - Affected by muscle mass, age, race, sex etc. Need to look at the patient when interpreting the result
- **CREATININE CLEARANCE**
  - Difficult for elderly patients to collect an accurate sample
- **ESTIMATED GFR**
  - Equation which automatically calculates GFR from serum creatinine
  - Easiest equation uses age and ethnicity (MDRD equation)
  - Alternatives can include weight, albumin etc
  - Generally unreliable for well preserved GFR (>60ml/min)
- **INULIN CLEARANCE**
  - Laborious - used for research purposes only
- **RADIONUCLIDE STUDIES**
  - EDTA clearance etc
  - Reliable but expensive

Long-term management
- Glomerular Filtration Rate (GFR) measure
- Remains on regular haemodialysis
- Low potassium diet and fluid restriction
- Erythropoietin injections to correct anaemia
- 1.25 Vitamin D supplements to prevent hyperparathyroid bone disease
1. Briefly explain, in the context of renal function, what is meant by a “transport maximum”, and why it occurs

When renal cells transport solutes out of or into tubular fluid, they move specific substances in one direction only involving specific membrane proteins, which act as transmembrane transporters. Each type of transporter has an upper limit on how fast it can work; the transport maximum (Tm) – measured in mg/min. In physiology, the transport maximum refers to the point at which increases in concentration do not result in an increase in movement of a substance across a membrane.

E.g. glucose

The body wants to retain glucose as a substrate; therefore the renal handling of glucose only needs to be considered in terms of filtration, reabsorption + excretion, i.e. there is no secretion of glucose back into the tubular fluid.

Glucose filtration – through the fenestrated glomerular capillaries results in no change in glucose concentration in the filtrate compared to the blood, therefore the rate of glucose filtration increases proportionally to the plasma glucose concentration.

Glucose reabsorption – involves the Na+ Glucose co-transporter on the apical membrane, which transports glucose into the renal cells from the tubular fluid. These transporters have a transport maximum, therefore rate glucose reabsorption only increases proportionally to plasma glucose concentration up to a point, above which all the transporters are occupied and the rate can no longer increase.

Glucose excretion – should be 0, but above a certain plasma glucose concentration; not all the glucose can be reabsorbed, and hence some is excreted (glycosuria). The rate of excretion increases above the Tm point.

2. Suppose Tm for glucose reabsorption = 2mmol/min, and the glomerular filtration rate = 100ml/min (0.1l/min). Show on the axes below the relationship between:
   a. The rate of glucose filtration
   b. The rate of glucose reabsorption
   c. The rate of glucose excretion
3. **Explain the phenomenon of “splay”**

In reality, the plasma concentration at which glucose begins to be excreted in the urine (threshold) is NOT identical to the plasma concentration at which the maximum rate of glucose reabsorption is first reached. In fact, the former is normally substantially lower than the latter. This phenomenon is known as splay.

![Graph showing the phenomenon of splay](image)

4. **What is the normal plasma glucose concentration?**

3.6-5.8 mmol/l

5. **Name the common disorder in which the primary abnormality is nothing to do with renal function but can lead to significant amounts of glucose appearing in the urine**

Diabetes – dysfunction of insulin production/regulation from beta cells within islets of Langerhans in pancreas, which normally act in conjunction with glucagon to regulate plasma glucose concentrations.

6. **In renal glycosuria, significant amounts of glucose are present in the urine as a result of renal defect. How do you think the graphs you have drawn in answer to question 2 will differ from normal in someone with this disorder?**

The defect may occur in the glucose co-transporters in the proximal convoluted tubule, therefore glucose reabsorption does not occur nearly as efficiently, and the rate of glucose reabsorption does not increase to combat an increased rate of glucose filtration \(\rightarrow\) glycosuria.

---

**The Renal Cortico-Medullary Osmotic Gradient**

**CAL Practical Session**

The ability of the kidney to produce hypertonic urine depends on the high osmolarity of the interstitial fluid in the renal medulla. This in turn depends on three properties of the loops of Henle:

1. Countercurrent flow: down the descending limb and up the ascending limb
2. Descending limb: permeable to water; relatively impermeable to solutes
3. Ascending Limb: Water impermeable; NaCl actively transported out of the tubular fluid into the interstitium
These properties alone are enough to explain high osmolarity in the medulla.

The figure below represents a loop of Henle with the renal cortex at the top and the bend of the loop deep in the medulla.

To start with assume that there is no gradient and that the fluid everywhere in the loop and the interstitium is roughly isotonic: osmolarity = 290 mosmol/l

<table>
<thead>
<tr>
<th>Descending</th>
<th>Interstitium</th>
<th>Ascending</th>
</tr>
</thead>
<tbody>
<tr>
<td>290</td>
<td>290</td>
<td>290</td>
</tr>
<tr>
<td>290</td>
<td>290</td>
<td>290</td>
</tr>
<tr>
<td>290</td>
<td>290</td>
<td>290</td>
</tr>
<tr>
<td>290</td>
<td>290</td>
<td>290</td>
</tr>
</tbody>
</table>

Active transport of NaCl out of the ascending limb raises interstitial osmolarity.

Assume that the difference between osmolarity between the ascending limb and the interstitium at ANY HORIZONTAL LEVEL of the loop is achieved as a result of active NaCl transport is 200 mosmol/l

<table>
<thead>
<tr>
<th>Descending</th>
<th>Interstitium</th>
<th>Ascending</th>
</tr>
</thead>
<tbody>
<tr>
<td>290</td>
<td>390</td>
<td>190</td>
</tr>
<tr>
<td>290</td>
<td>390</td>
<td>190</td>
</tr>
<tr>
<td>290</td>
<td>390</td>
<td>190</td>
</tr>
<tr>
<td>290</td>
<td>390</td>
<td>190</td>
</tr>
</tbody>
</table>

Water moves out of the descending limb down its osmotic gradient, thus raising the osmolarity of the tubular fluid in the descending limb and lowering that of the interstitium.

<table>
<thead>
<tr>
<th>Descending</th>
<th>Interstitium</th>
<th>Ascending</th>
</tr>
</thead>
<tbody>
<tr>
<td>340</td>
<td>340</td>
<td>190</td>
</tr>
<tr>
<td>340</td>
<td>340</td>
<td>190</td>
</tr>
<tr>
<td>340</td>
<td>340</td>
<td>190</td>
</tr>
<tr>
<td>340</td>
<td>340</td>
<td>190</td>
</tr>
</tbody>
</table>

Fluid flows down the descending limb and up the ascending limb; new isotonic fluid enters the loop.

<table>
<thead>
<tr>
<th>Descending</th>
<th>Interstitium</th>
<th>Ascending</th>
</tr>
</thead>
<tbody>
<tr>
<td>290</td>
<td>340</td>
<td>190</td>
</tr>
<tr>
<td>340</td>
<td>340</td>
<td>190</td>
</tr>
<tr>
<td>340</td>
<td>340</td>
<td>190</td>
</tr>
<tr>
<td>340</td>
<td>340</td>
<td>340</td>
</tr>
</tbody>
</table>

So if we go once round the cycle of:

1. Active NaCl transport out of the ascending limb
2. Osmotic equilibrium between the descending limb and the interstitium, and
3. Countercurrent flow we get the above diagram.

*Obviously in the real world these processes all take place simultaneously, but it makes life easier to think of them as happening one after the other.*

If we go around again we get this,

<table>
<thead>
<tr>
<th>Descending</th>
<th>Interstitium</th>
<th>Ascending</th>
</tr>
</thead>
<tbody>
<tr>
<td>290</td>
<td>328</td>
<td>165</td>
</tr>
<tr>
<td>328</td>
<td>352</td>
<td>165</td>
</tr>
</tbody>
</table>
And again, we get this,

<table>
<thead>
<tr>
<th>Descending</th>
<th>Interstitium</th>
<th>Ascending</th>
</tr>
</thead>
<tbody>
<tr>
<td>290</td>
<td>318</td>
<td>159</td>
</tr>
<tr>
<td>318</td>
<td>343</td>
<td>197</td>
</tr>
<tr>
<td>343</td>
<td>374</td>
<td>290</td>
</tr>
<tr>
<td>374</td>
<td>421</td>
<td>421</td>
</tr>
</tbody>
</table>

And yet again, we get this,

<table>
<thead>
<tr>
<th>Descending</th>
<th>Interstitium</th>
<th>Ascending</th>
</tr>
</thead>
<tbody>
<tr>
<td>290</td>
<td>314</td>
<td>170</td>
</tr>
<tr>
<td>314</td>
<td>344</td>
<td>232</td>
</tr>
<tr>
<td>344</td>
<td>388</td>
<td>321</td>
</tr>
<tr>
<td>388</td>
<td>448</td>
<td>448</td>
</tr>
</tbody>
</table>

Thus, if we go around the cycle 4 times, this is what we end up with. Note that while we started with the same osmolarity everywhere we have finished with

(a) Interstitial osmolarity near the bend of the loop well above the starting value of 290 mosmol/l – i.e. HYPERTONIC

(b) Osmolarity in the fluid emerging from the loop of Henle well below 290 mosmol/l – i.e. HYPOTONIC

This is obviously a very simplified model of the loop of Henle.

We can complicate it further by considering 50 horizontal levels in the loop (instead of 4) and by going round the cycle 100 times (instead of 4).

This graph shows the calculated values of the interstitial osmolarity at 50 different levels after 100 cycles.
Thus, active transport out of the ascending limb creates a relatively small (200 mosmol/l) gradient at any one level of the loop, but as a result of countercurrent flow this difference gets MULTIPLIED to result in a large gradient between the cortex and the inner medulla.

Unfortunately this does not fully explain the high osmolarity of the renal medulla. This is obviously a very simple model and there are a couple of complications.

NaCl and H\(_2\)O are reabsorbed from the loop of Henle into the interstitium.

In addition, both solutes and water – the latter dependant on the concentration of vasopressin (ADH) - are reabsorbed from the collecting ducts.

These reabsorbates can’t just accumulate forever in the interstitium (or the kidney would swell up and burst)

The vasa recta provide the blood supply to the medulla.

As blood flows out of the cortex down the descending vasa recta through the increasingly hypertonic medulla, solute will diffuse in.

If this hypertonic, solute-laden blood left the kidney at this point the cortico-medullary gradient would virtually disappear.

But of course it doesn’t. Instead the blood goes round the bend and everything starts happening in reverse. As the blood heads back up the ascending vasa recta the surrounding interstitium becomes less and less hypertonic. As a result, much of the NaCl that diffused in on the way down will now diffuse back out again.

This means that the medulla can be supplied with O\(_2\) and nutrients and yet the high osmolarity of the medulla can be maintained.

It is important to appreciate that this countercurrent flow and solute movement into and out of the vasa recta does not CREATE the cortico-medullary gradient, but prevents the blood supply washing away the high solute content in the medulla.

However, the amount of solute that diffuses into the vasa recta on the way down is not quite matched by the amount that diffuses out on the way up. The blood flowing through the medulla DOES remove some solute – and the higher the rate of blood flow, the more will be removed. In fact, what we end up with is the medullary osmolarity at which the amount of solute removed (per minute) nicely matched the amount reabsorbed from the loops of Henle and collecting ducts, this results in a stable cortico-medullary gradient and effectively deals with that problem – but unfortunately there is another more intractable one.

**INNER MEDULLA**

The high medullary osmolarity depends on active NaCl transport out of the ascending limb of the loop of Henle. This certainly happened in the THICK ascending limb in the cortex and outer medulla. However, there is no THICK ascending limb of the loop of Henle in the INNER medulla and there seems to be no active transport of NaCl out of the THIN ascending limb.
UREA

In the early segments of the nephron some urea is reabsorbed (in the proximal tubule) and some secreted (in the thin limbs of the loop of Henle). However, the interesting bit is what happened further down. It seems that the whole of that part of the nephron has a low permeability to urea; i.e. little can cross the tubule wall. If, in any part of the nephron, water is reabsorbed with little or no urea reabsorption, then tubular fluid concentration of urea ill rise.

This happens in the presence of vasopressin throughout most of the length of the collecting ducts. (In the whole of the latter – vasopressin greatly increases water permeability).

If water is absorbed without urea, the urea concentration of the tubular fluid will go up and up as it passes down the collecting ducts and will be very high by the time it reaches the innermost part of the inner medulla, where things change – because there urea CAN cross the tubule wall (down its concentration gradient) and enter the interstitial fluid.

This hypothesis can explain the high concentration of urea in the medulla and help to explain the inner medullary concentration gradient.

This accumulation of urea in the medullary interstitium is indirectly dependant on active transport in the thick ascending limb of Henle's loop. The crucial fluid reabsorption on which it depends only happens because:

   a. The interstitial fluid around the cortical collecting ducts (shown here in green) is isotonic while the fluid coming out of the loops is hypotonic because of active NaCl transport out of the thick ascending limb.
   b. Fluid reabsorption in the medullary collecting ducts only occurs because of the high medullary osmolarity – again as a result of active NaCl transport out of the thick ascending limb.

Questions

1) Active transport of NaCl out of the descending limb of the loop of Henle is crucial to the development of the cortico-medullary osmotic gradient. **FALSE**
2) Active transport of NaCl out of the thin ascending limb on the loop of Henle is crucial to development the cortico-medullary osmotic gradient. **FALSE – There is no significant transcellular active transport out of either (descending or ascending) of the thin limbs.**
3) Active transport of NaCl out of the thick ascending limb of the loop Henle is crucial to the development the cortico-medullary osmotic gradient. **TRUE**
4) The descending limb of the loop Henle is virtually impermeable to water. **FALSE**
5) The thin ascending loop of Henle is virtually impermeable to water. **TRUE**
6) The thick ascending limb of the loop of Henle is virtually impermeable to water. **TRUE – Both the thin and thick ascending limb of the loop of Henle ARE almost impermeable to water.**
7) The tubular fluid entering the loop of Henle is markedly hypertonic. **FALSE**
8) The tubular fluid entering the loop of Henle is markedly hypotonic. **FALSE – The epithelium of the proximal tubule is quite ‘leaky’. In this context, this means that any difference in osmolarity between the cortical interstitial fluid and the tubular fluid rapidly disappears as a result of osmotic water movement across the wall of the tubule. So the tubular fluid remains close to that of plasma, i.e. it is roughly ISOTONIC when it leaves the proximal tubule and enters the loop of Henle.**
9) The tubular fluid leaving the loop of Henle is markedly hypertonic. **FALSE**
10) The tubular fluid leaving the loop of Henle is markedly hypotonic. **TRUE – As a result of active transport of NaCl out of the (water-impermeable) thick ascending limb, the osmolarity of the**
tubular fluid is reduced below that of the surrounding interstitium. Since in the cortex the surrounding fluid is isotonic with plasma, the tubular fluid emerging from the cortical loops of Henle will be HYPOTONIC.

11) The descending vasa recta are water permeable but impermeable to NaCl and urea. FALSE

12) The ascending limbs of the vasa recta actively transport NaCl into the surrounding interstitium thus helping to create the cortico-medullary osmotic gradient. FALSE – Like most capillaries (other than brain capillaries), the walls of the vasa recta are permeable to small solutes as well as water. Na\(^+\) and Cl\(^-\) diffuse passively in on the way down (down their concentration and electrochemical gradients) and then back out again on the way up – but they are not actively transported across the blood vessel walls.

13) Like NaCl and urea, water moves into the descending vasa recta and then moves out of the ascending vasa recta. FALSE - Water will move OUT of the descending vasa recta (into the increasingly hyperosmotic interstitium, down its osmotic gradient) and then back IN again on the way up. A steady state is achieved in which the removal of water from the medulla by the vasa recta nicely balances the amounts reabsorbed from the medullary loops of Henle and collecting ducts.

14) An increase in the rate of blood flow through the vasa recta will tend to reduce the osmolarity of the medullary interstitium. TRUE - Although the loop shape of the vasa recta helps to preserve the high solute concentration in the medulla, the flow of blood still does tend to dissipate the osmotic gradient to some extent. If the blood flow increases, a new steady state will eventually be achieved, BUT with a lower medullary osmolarity.

15) When you are dehydrated, you produce highly concentrated urine to restore normal fluid balance. In this situation the tubular fluid emerging from the loops of Henle is no longer hypotonic. FALSE - Since there is still active NaCl transport out of the (water-impermeable) thick ascending limb under these conditions, the fluid coming out of the loops will still be hypotonic. The crucial events in this context happen further downstream.

16) When you drink a lot of water and vasopressin levels fall, one consequence is a reduction in medullary interstitial osmolarity. TRUE - The extreme case of this is the Brattleboro rat, which is unable to synthesise vasopressin, with the result that these animals produce roughly their own body weight of dilute urine each day (think about that!). The medullary osmolarity of these animals is far below normal. This is a special case as the animals are chronically deprived of vasopressin, which complicates matters, but the following will apply even during an acute reduction in circulating vasopressin levels - such as will occur after you drink a large volume of water. Water reabsorption in the collecting ducts will be minimal with the result that the tubular fluid urea concentration will rise less than is normally the case. As a result, less urea will be reabsorbed from the inner medullary collecting ducts. This will tend to lower medullary interstitial hyperosmolality.