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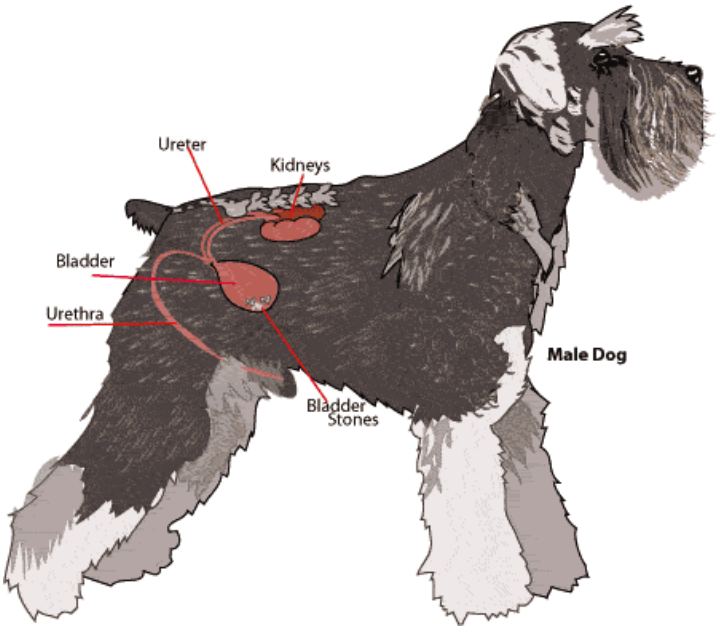
THE UNIVERSITY OF MELBOURNE
FACULTY OF VETERINARY & AGRICULTURAL SCIENCES

Structure and Function of the Kidney 2

Veterinary Bioscience: Metabolism and Excretion



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Learning objectives

1. Describe how glomerular filtration occurs, why it is important and the factors which regulate it
2. Explain the process of autoregulation and the factors and hormones that regulate it.
3. Explain how GFR can be measured and used as an assessment of renal function
4. Describe how urine is formed and modified through glomerular filtration, tubular reabsorption and secretion
5. What is the composition of normal urine
6. Describe the transport mechanisms that are responsible for Na^+ reabsorption by the nephron
7. Explain the coupling of water reabsorption to Na^+ in the proximal tubule
8. Describe how organic anions and cations are secreted. Why is this important

Autoregulation

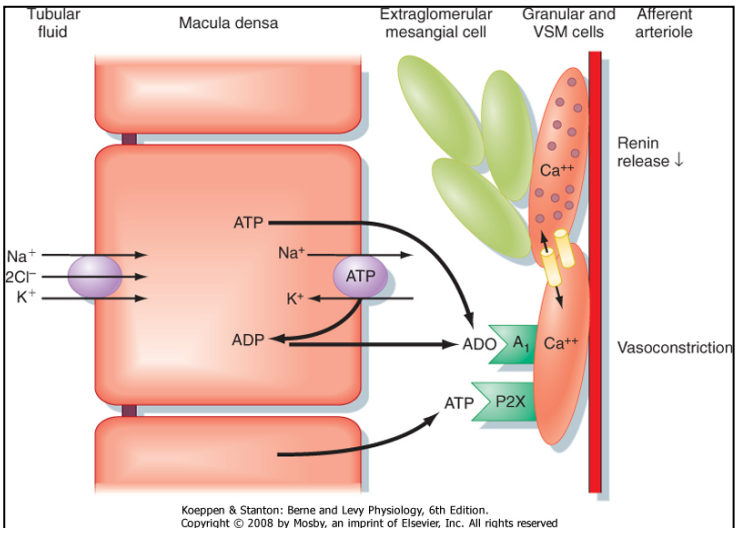
Renal blood flow & GFR are regulated by an autoregulation process

Only small changes in renal blood flow and GFR occur when the systemic blood pressure changes between 80 and 180mmHg. Resistance in the interlobular arteries and afferent arterioles (through either dilation or contraction) are automatically adjusted when the blood pressure changes.

Autoregulation mechanisms

1. Myogenic mechanism. Increased stretch (due to increased blood pressure) opens pores for calcium ions to get into the smooth muscle cells resulting in contraction of vascular smooth muscle and afferent arteriole constriction.

2. Tubulo-glomerular feedback. An increase in arterial pressure causes an increase in the GFR causing total fluid flow through the nephron to increase. It is thought that macula densa cells can detect this increased flow by detecting altered NaCl concentrations which results in the generation of vasoconstrictor chemicals (eg adenosine & ATP) which acts on smooth muscle cells via A1 receptors in adjacent afferent arteriole smooth muscle cells to cause the release of Ca2+ and vasoconstriction and reduces GFR.



Tubulo-glomerular feedback mechanism.

Regulators of renal blood flow and GFR

GFR and RBF in spite of autoregulation can be independently regulated to maintain blood volume. For example in haemorrhage resulting in a loss of effective circulating volume the amount filtered by the kidney is reduced to maintain blood volume. In this case autoregulation may be overridden by sympathetic nerves and angiotensin II to alter GRF. In times when renal blood flow is comprised vasodilators such as PGE2, PGI2 and nitric oxide are produced to protect the kidney from severe vasoconstriction.

Key concept:

When GFR is increased then increase filtration and removal of Na+ and H_2O from the body

Reduced GFR then conserve Na+ and H_2O

Sympathetic control

Sympathetic stimulation of afferent arterioles can cause changes in renal hemodynamics when systemic arterial pressure is altered. For example following haemorrhage a decrease in systemic arterial pressure is detected by baroreceptors in the carotid sinus and aortic arch resulting in stimulation of renal sympathetic nerves. Noradrenalin is released and binds to $\alpha 1$ adrenergic receptors on afferent arterioles causing vasoconstriction and a reduction in GFR occurs as well as an increase in total peripheral resistance.

Angiotensin II

Angiotensin II = powerful vasoconstrictor. Stimulus for angiotensin II production is secretion of the proteolytic enzyme renin by juxtaglomerular cells of kidney.
Renin is synthesised and secreted in response to:

1. Decreased pressure in the afferent glomerular arterioles
2. Decreased NaCl in tubular fluid sensed by cells of the macula densa.
3. Sympathetic stimulation of juxtaglomerular cells.

All of these stimuli are related to a decrease in blood volume, which is the physiological parameter defended by the renin-angiotensin-aldosterone system (see later for description).
Angiotensin II acts via AT1 receptor to vasoconstrict afferent and efferent arterioles. As the molecule constricts both afferent and efferent arterioles, it gives less of a decrease in GFR than in RBF. Decreased RBF increases oncotivc forces so partially inhibits GFR. Angiotensin II also contracts glomerular mesangial cells decreasing the vascular luminal surface area, which lowers Kf and, consequently GFR.

Prostaglandins

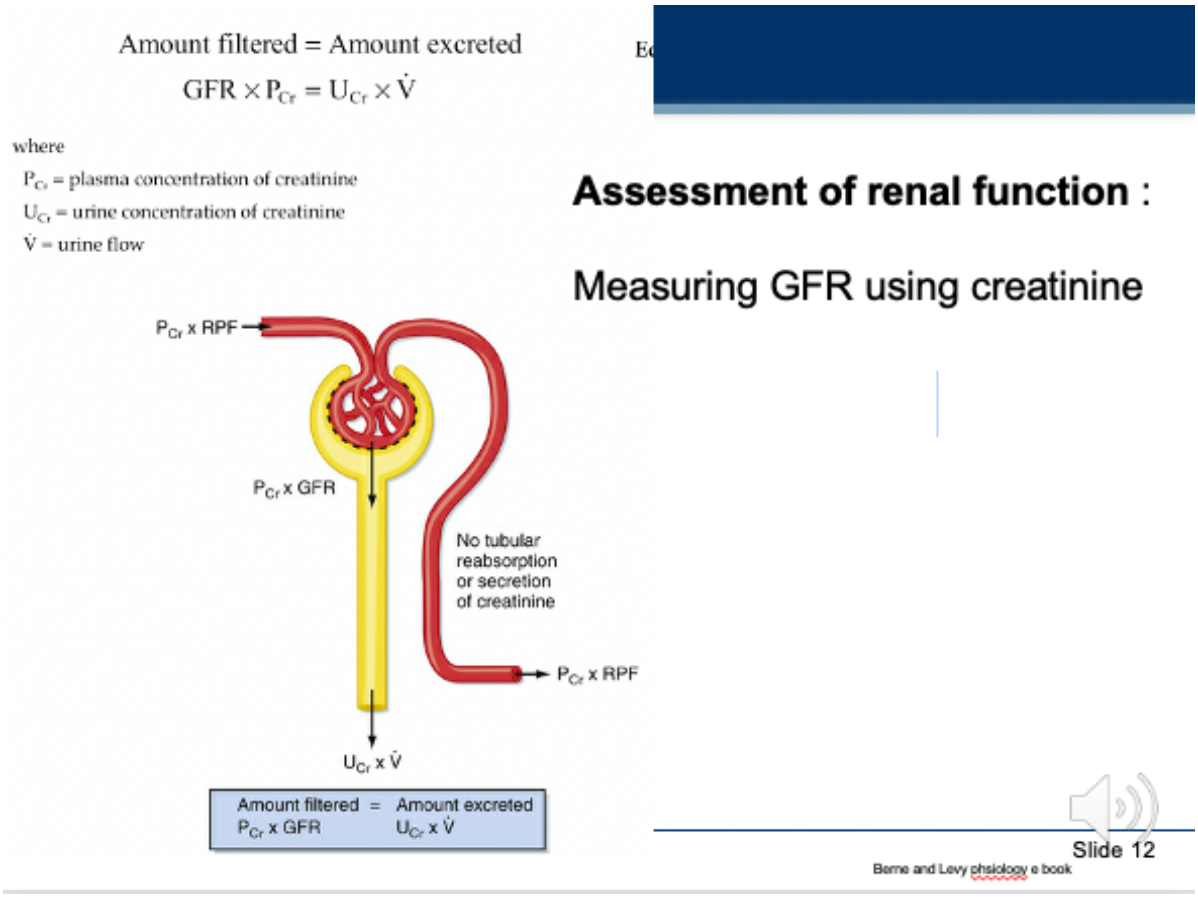
PGE2 and PGI2 are potent vasodilators, which act on afferent arterioles to reduce the constrictor effects of sympathetic stimulation and angiotensin II. This allows for a balance between (a) the requirement for increased total peripheral resistance to maintain systemic arterial pressure and (b) the likelihood of kidney damage were renal vasoconstriction is too severe.

Nitric oxide (NO)

Vasodilator derived from endothelial cells. Associated with a drop in blood pressure or flow.
NO produced by endothelial cells via synthesis of NO synthetase.
Stimulus for release includes acetylcholine, histamine, shear stress and bradykinin.
NO then diffuses into and activates guanylate cyclase & cGMP in smooth muscle cells which activates protein kinase G and induces vasodilation by dropping Ca2+ levels within the cell.

Atrial natriuretic peptide

Produced by cardiac myocytes following an increase in ECV.



URINE FORMATION AND MODIFICATION

Renal function consists of three basic processes:

1. Glomerular filtration: filtration of blood. The glomerular filtrate contains solute concentrations similar to those in plasma but excludes medium to high molecular weight proteins and formed cellular components.
2. Tubular reabsorption: movement of fluid and solutes from tubular lumen to peritubular capillary. Fluid and solutes filtered at the glomerulus are largely recovered by tubular reabsorption.
3. Tubular secretion: movement of solutes from the peritubular capillary to the tubular lumen.

Glomerular Filtration

1. Urine formation begins with glomerular filtration, which occurs as a result of the bulk flow of fluid from the glomerular capillaries through a filtration barrier into the Bowman's space. Filtration is directly related to glomerular perfusion pressure that is determined by systemic blood pressure, as well as afferent and efferent arteriolar tone.
2. Glomerular filtration rate (GFR) = Volume of fluid filtered from the glomeruli into Bowman's space per unit time is large. In humans ~ 125ml/min (180L/day).
1.

Table 1 Composition of Urine (Rose 2001).

Substance	Concentration
Na^+	50- 130 mEq / L^+
K^+	20- 70 mEq / L^+
NH_4^+	30- 50 mEq / L
Ca^{++}	5- 12 mEq/ L^+
Mg^{++}	2- 18 mEq/ L^+
CI^-	50- 130 mEq / L^+
Pi	20- 40 mEq / L^+
Urea	200- 400 mM
Creatinine	6-20 mM
pH	5.0- 7.0
Osmolality	500- 800 mOsm/kg H_2O
Glucose	0
Amino acids	0
Protein	0
Blood	0
Ketones	0
Leukocytes	0
Billirubin	0
Volume excreted/day	0.5 -1.5 L

Tubular reabsorption

Most of the useful plasma components, eg H_2O, inorganic ions eg Na^+, Cl- and organic nutrients eg glucose & proteins that are filtered by the glomerulus are reabsorbed. Amounts excreted in the urine represent very small fraction (<1%) of the filtered loads.

Tubular reabsorption across the epithelial cell occurs via diffusion & osmosis **not by bulk flow** as there is limited hydrostatic and oncotic pressure gradients across the tubule. Reabsorption is via paracellular or transcellular routes.

Transport processes across tubular epithelial cells

1. Simple diffusion down electrochemical grdients
2. Facilitated diffusion
    a. channels
    b. symporters or antiporters
3. Active transport (ATP)
4. Osmosis.
5. Endocytosis (for proteins).

Renal handling of NaCl

NaCl is freely filtered in the glomerulus and the concentration in the filtrate of NaCl is similar to blood (Na+ 135-145 mEq/L). Most reabsorption of NaCl occurs in the proximal tubule and loop of Henle with fine regulated reabsorption occurring in the distal tubule and collecting duct.

Na reabsorption is a primary active process (dependent upon Na-K-dependent ATPase pumps located in the basolateral membrane). Creates a favourable gradient between tubule lumen and cell cytoplasm. Reabsorption of Cl may be passive and/or active, depending upon the nephron segment, but is coupled to reabsorption of Na.

Proximal tubule

Of the filtered NaCl 65% is reabsorbed in the proximal tubule. Reabsorption of Na on the luminal membrane occurs via either co-transport with glucose, amino acids, lactate and phosphate ions or alternatively via counter-transport with H^+ Passive diffusion of water

occurs via osmosis (ie water follows NaCl). At the end of the proximal tubule, the osmolarity of the tubular fluid is approximately iso-osmolar with plasma.

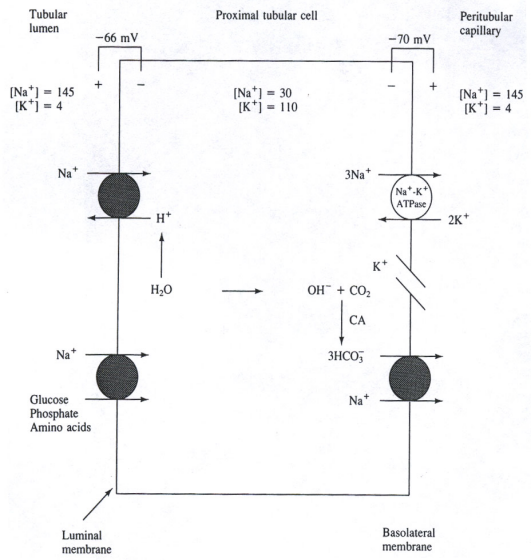


Fig 2 Major transporters in proximal convoluted tubule from Rose 2001

Loop of Henle

The descending limb of the loop of Henle does not reabsorb Na+ or Cl- but the thick and thin ascending limb reabsorb 25% filtered sodium. In the thick ascending limb a Na-K-2Cl symport carrier is used.

Distal convoluted tubule

Na is reabsorbed via a Na-Cl symport carrier

Collecting duct (principal cells)

Have a more limited reabsorptive capability via Na channels regulated by hormones such as aldosterone (increases reabsorption) and atrial natriuretic peptide (inhibits).

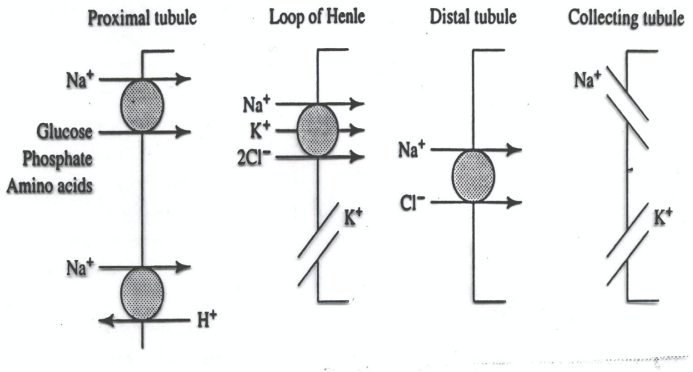


Fig 3 . Na transport in nephron Rose 2001

H_2O permeability varies from tubular segment to segment.

Water reabsorption is by diffusion (osmosis) and is dependent upon sodium reabsorption.

- Proximal tubule: very **permeable** to water. Small gradients suffice to move large quantities of water,
- Distal tubule: very **impermeable** to water almost no water reabsorbed, regardless of osmotic gradient
- Collecting ducts: permeability to water subject to physiological control (e.g., ADH).

Water transport via osmosis is coupled to solute reabsorption. Starling's forces govern the movement of solutes from interstitial fluid (after they have crossed epithelial cell barrier) into peritubular capillaries. An increase in interstitial pressure and a reduction in PGC within the peritubular capillaries favour movement of solutes and water into blood

Renal handling of organic substances.

1. Proximal tubule is major site for reabsorption organic nutrients
    a. eg glucose, amino acids, phosphate, sulfate, lactate etc.
2. Active transport across luminal membrane usually via co transport with Na+.

For glucose it is transported across the luminal membrane by a Na/Glucose cotransporter type 1 and across the basolateral membrane by diffusion and a Glut 5 uniport carrier.

Carriers exhibit a Transport Maximum.

The transport maximum (Tm) of a substance is the maximum tubular transport capacity for a substance. The membrane carrier proteins responsible for transport can become saturated.

a. A classic example involves glucose transport: eg if the concentration of glucose is too high, membrane protein binding sites in the proximal tubule become saturated, resulting in the excretion of glucose in the urine, as seen in diabetes
b.

Metabolism by the Tubules

Metabolism by the tubules is a fourth renal process which can contribute to urine formation.

Examples include:

(i) the synthesis of NH4+ from glutamine and

(ii) the synthesis of HCO3-

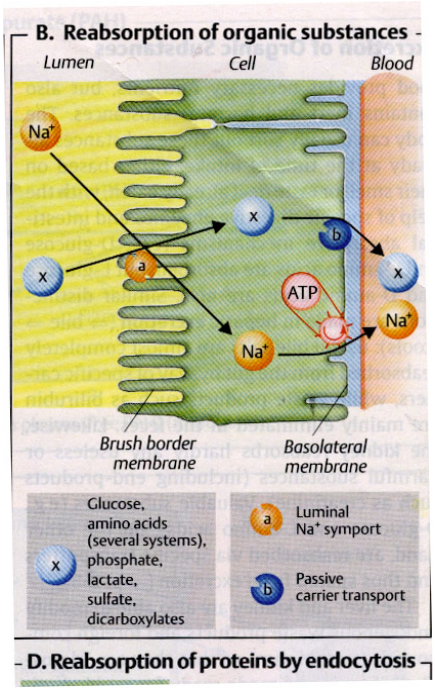


Fig 4 reabsorption of organic substances in proximal tubule. Despopoulos 2001

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Proteins and peptides

Although the concentration of protein filtered by the glomerulus is small ~10mg/ml the large GFR (125ml/min) means that the amount is still significant.

- These molecules undergo reabsorption in the proximal tubule.
- Large proteins undergo endocytosis and partial proteolysis.

- Small, linear peptides are handled quite differently: they are catabolised by peptidases located on the luminal plasma. Small dipeptides are transported by symport carriers with H+ and oligopeptides are co-transported with Na+.

Tubular Secretion

Tubular secretion is the movement of substances from peritubular capillaries into the tubular lumen. It begins with simple diffusion out of the peritubular capillaries into the interstitial fluid. The next step involves crossing either tight junctions (intercellular) or the basolateral and luminal membranes of the cell (intracellular). The most important substances secreted by the tubules are H+ and K+ but large number of organic ions, eg creatinine, or foreign chemicals, eg penicillin.

Organic anions (OA-)

1. OA- secretion occurs at the proximal tubule and includes drugs such as penicillin and barbiturates and endogenous substances such as oxalate and urate.
2. The transport processes are active and of very low specificity.
3. Many involve conjugation in the liver (to glucuronate, sulfate or glutathione).
4. Active transport across the basolateral membrane (to overcome inside negative charge) occurs using an organic anion antiporter in exchange for a-ketoglutarate followed by simple facilitated diffusion into lumen.

Organic cation secretion (OC)

Include endogenous substances adrenalin, histamine, choline, dopamine and drugs such as atropine, morphine. These organic cations diffuses passively from blood into epithelial cells via a polyspecific OC+ carrier. Active step is across luminal membrane of proximal tubular cells via direct ATP carriers or multispecific OC+/H+ antiporters

Key concepts

1. Autoregulation allows GFR and RPF to remain constant despite changes in arterial pressure but can be overridden in disease states
2. Clinically GFR can be estimated by measuring plasma and urine creatine levels
3. Increased GFR leads to loss Na and water whereas decreased GFR conserves Na and water
4. The four main nephron segments (PCT,LOH, DT and CD) are functionally different, have different transport molecules and undertake selective reabsorption of solutes and water and secretion of some solutes. This ultimately determines the composition and volume of urine.
5. Tubular secretion allows for the rapid removal of substances (drugs and toxins) from the blood (compared to glomerular filtration alone) and allows protein bound organic anions and cations substances to be removed

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