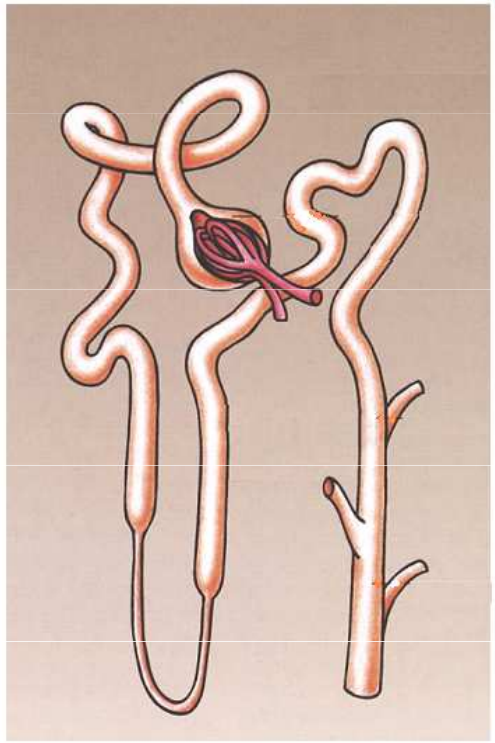


Renal functions

Biochemistry II
Lecture 10

2008 (J.S.)

The main functions of the kidneys

- **Maintaining of the composition, osmolality, and volume of ECF**
- **Excretion of nitrogenous catabolites** (**urea, uric acid, creatinine and various hydrophilic drugs or toxins into the urine.**
- **Control of acid-base balance.**
- **An endocrine function** (**erythropoietin, renin, urodilatin, calcitriol**).

The excretory function of the kidneys includes
filtration of the plasma in the glomeruli – **glomerular filtration**,
transport of water and solutes from the tubular lumen into the blood – **tubular resorption**,
and transport of substances from tubular cells to the lumen – **tubular secretion**,

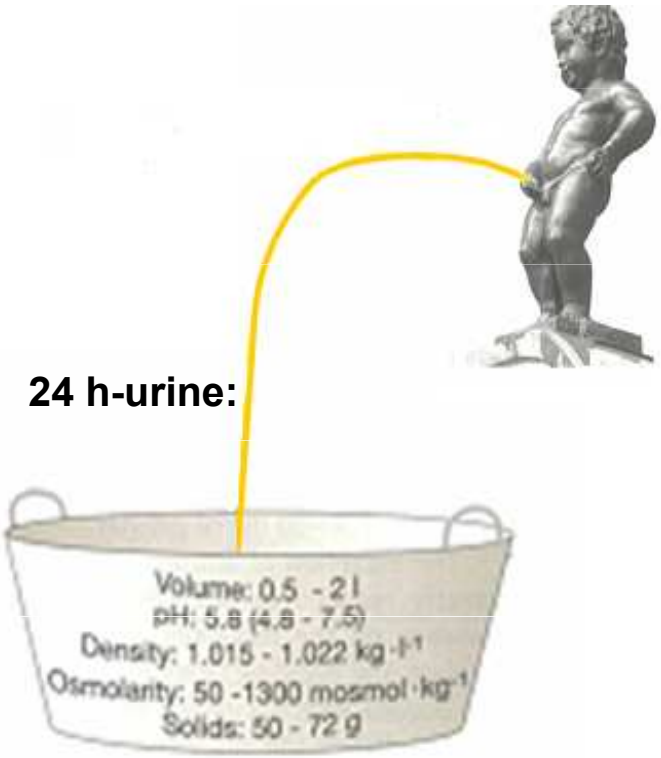
The blood flow through the kidney is about 1.2 – 1.8 l / min.

The urine

Urinary excretion of selected compounds

depends on the dietary intake.

Approximate ranges of amounts excreted daily in adults:



Inorganic ions	mmol / d	g / d
Na^+	120 – 240	2.8 – 5.6
K^+	45 – 90	1.8 – 3.6
Ca^{2+}	1.2 – 10	0.05 – 0.4
Mg^{2+}	2 – 6	0.05 – 0.14
Cl^-	120 – 240	4.3 – 8.6
Phosphates	16 – 48	0.5 – 1.5 (P)
SO_4^{2-}	8 – 35	0.3 – 1.1 (S)

Nitrogenous compounds			% of total nitrogen
NH_4^+	30 – 50	0.5 – 0.9	~ 5
Urea	330 – 600	20 – 30	~ 84
Creatinine	9 – 16	1.0 – 1.8	~ 4
Uric acid	1.5 – 6	0.25 – 1.0	~ 4
Amino acids	3.5 – 14	0.4 – 1.7	~ 1
Other	–	–	< 1

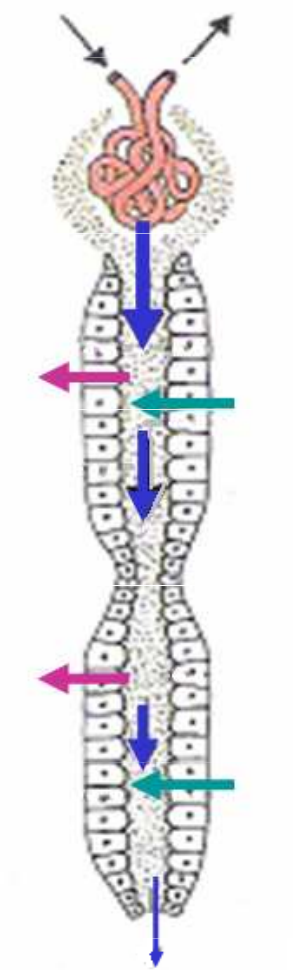
The **osmolality** is mostly much higher than that of blood plasma, it varies from about 80 to 1200 mmol / kg_{H_2O}. The osmolality of the glomerular filtrate is about 300 mmol / kg_{H_2O} , therefore the **maximal increase in urine osmolality is approximately fourfold.**

There are substantial differences in the increases of particular **solute concentrations**.

Approximate values of urine / plasma concentration ratio

Na^+	1.0
K^+	10
Ca^{2+}	1.3
Cl^-	1.2
Phosphate	15
NH_4^+	700
Urea	100
Creatinine	100
Uric acid	10

Amounts of solutes excreted into the urine during a given period



The amount of a solute excreted into the urine depends on

- the amount that has been **filtered** in the glomeruli

$$n_{\text{filtered}} / t = c_{\text{plasma}} \times V_{\text{GF}} / t = c_{\text{plasma}} \times \text{GFR}$$

(GFR is glomerular filtration rate, l / d or ml / s)

- the amount reabsorbed in the tubules n_{abs} / t, and
- the amount secreted from the tubular cells n_{secr} / t.

The total amount excreted during a given period equals

$$c_{\text{urine}} \times V_{\text{urine}} / t = c_{\text{plasma}} V_{\text{GF}} / t + (n_{\text{secr}} - n_{\text{abs}}) / t$$

From the equation can be calculated the fraction of the amount excreted into the urine from the amount that has appeared in the glomerular filtrate within a given period – the **fractional excretion E/F**.

The clearance of substances from plasma

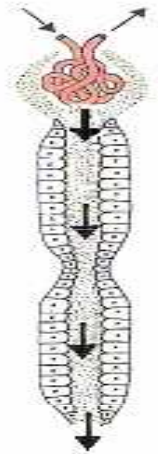
The quantity **renal clearance** is commonly used to express the efficiency of the elimination of a particular solute from blood plasma into the urine,

Renal clearance, simply **clearance of a solute X** ($\mathbf{C_x}$) is the ratio of the amount of a solute X that is excreted in a unit of time into the urine ($c_{urine} \times V_{urine} / t$) to its concentration in blood plasma c_{plasma} :

$$C_x = c_{urine} \times V_{urine}\, t^{-1} / c_{plasma} \qquad \text{(in ml / s or l / d)}$$

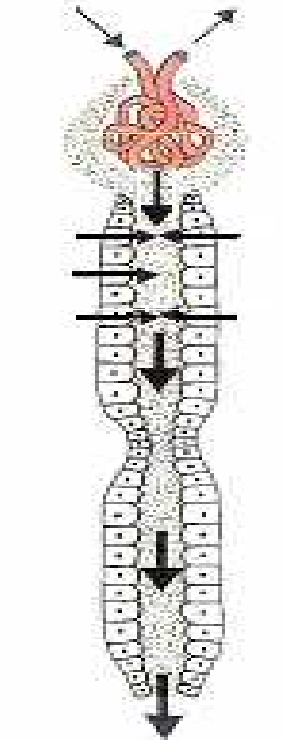
Thus **the clearance** of a solute X may be understood as **the volume of plasma that is completely cleared of that substance in a unit of time**.

Clearance is a very informative quantity.
For example, the clearance of the compound that is completely filtered and neither reabsorbed nor secreted in the renal tubules (as **inulin** or, with certain limitations, **endogenous creatinine**) is equal to the **glomerular filtration rate GFR**, the volume of filtrate formed in a unit of time.



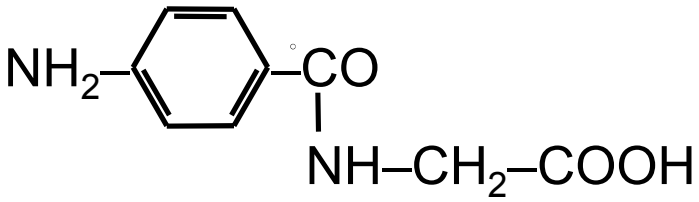
Another example:

p-Aminohippuric acid is an aromatic acid that is **both filtered** in the glomeruli **and secreted** by the cells of proximal renal tubules, so that the blood plasma is **completely cleared** of it during the sole passage through the renal vessels.



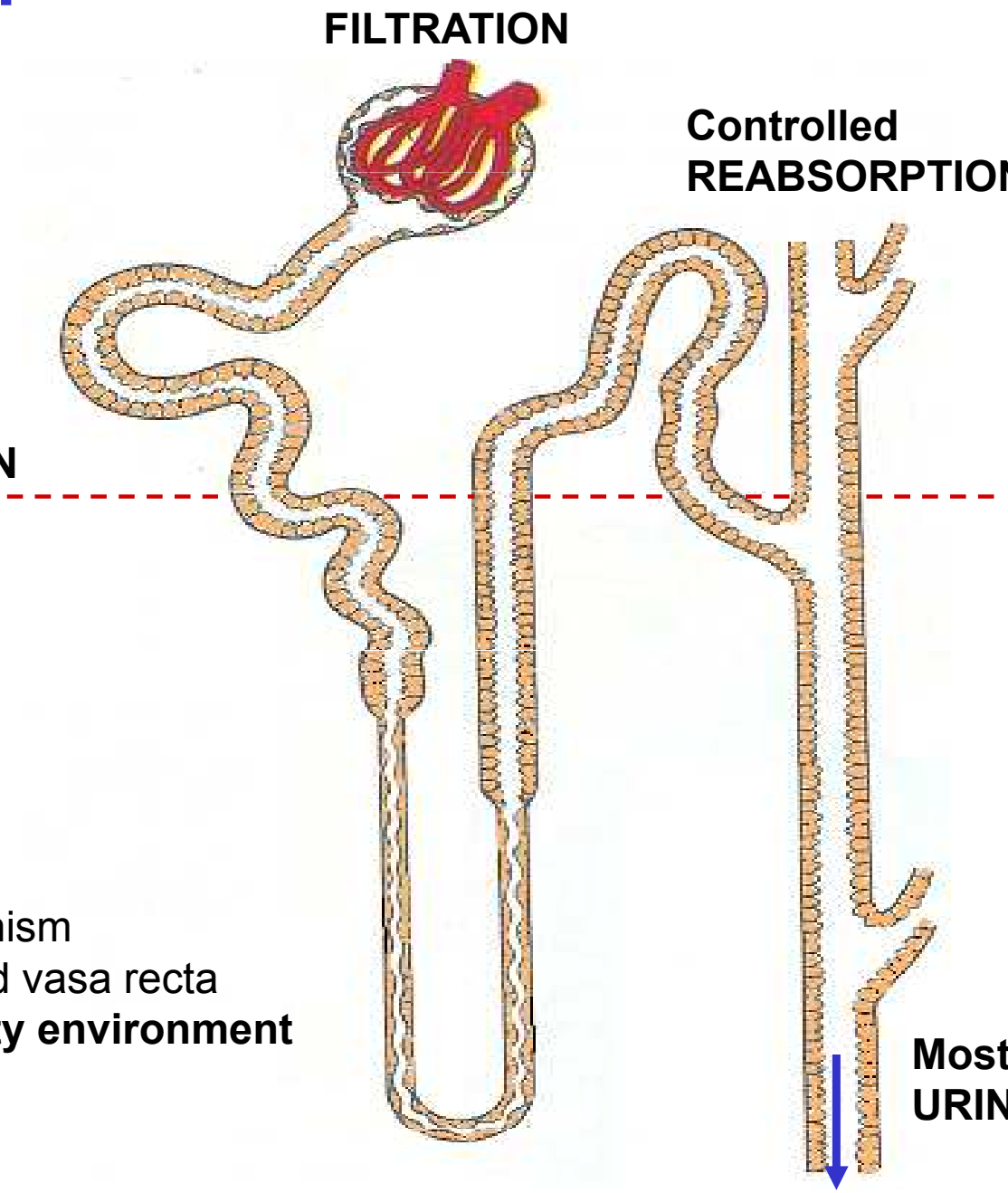
If *p*-aminohippurate (PAH) is applied intravenously till the steady concentration is reached, concentrations of the compound can be measured in plasma and urine and the **clearance C_{PAH}** calculated. Its value corresponds to the **renal plasma flow** in a time unit.

The renal plasma flow is 8 – 13 ml / s
(500 – 800 ml / min).



***p*-aminohippuric acid**
(**PAH**, ***p*-aminobenzoylglycine**)

The nephron

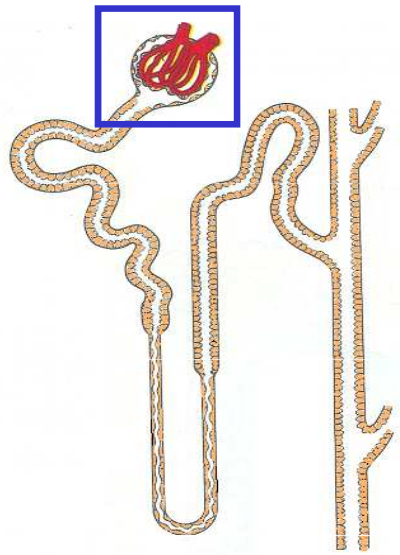


Countercurrent mechanism
of the loop of Henle and vasa recta
forms a **high osmolality environment**
in the medulla

Glomerular filtration

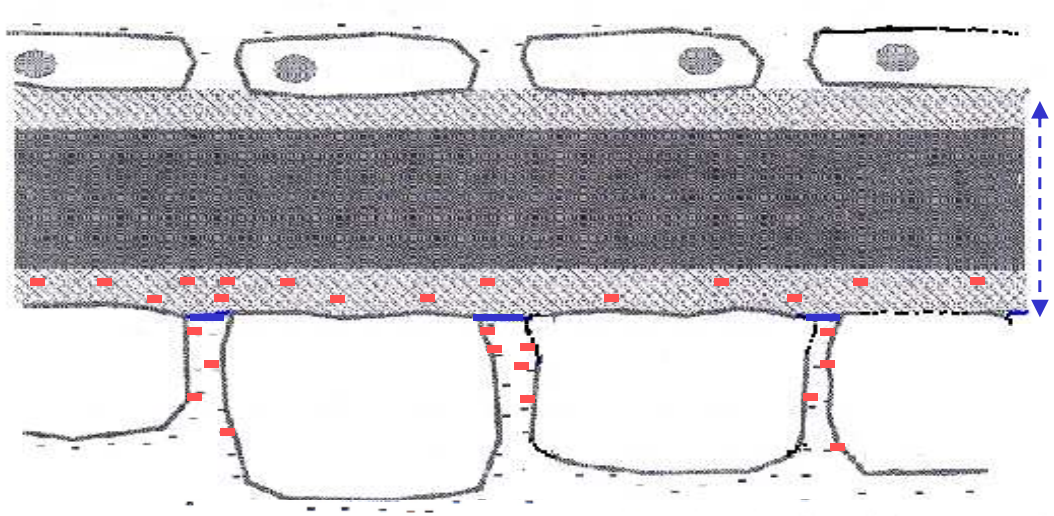
In renal glomeruli, the blood plasma is filtered.
The composition of **ions and small molecules** in glomerular filtrate is quite similar to that of plasma.

A common statement that glomerular filtrate is a protein-free fluid is not right. Low concentrations of proteins are present (predominantly of those with M_r < 30 000), about 10 – 30 mg per litre of filtrate, but most of proteins are reabsorbed in the tubules.



The glomerular filtration barrier

capillary lumen (plasma)



Bowman´s space (tubular fluid)

fenestrated endothelial layer
(about 30 % of the basement membrane not covered by the endothelial cells)

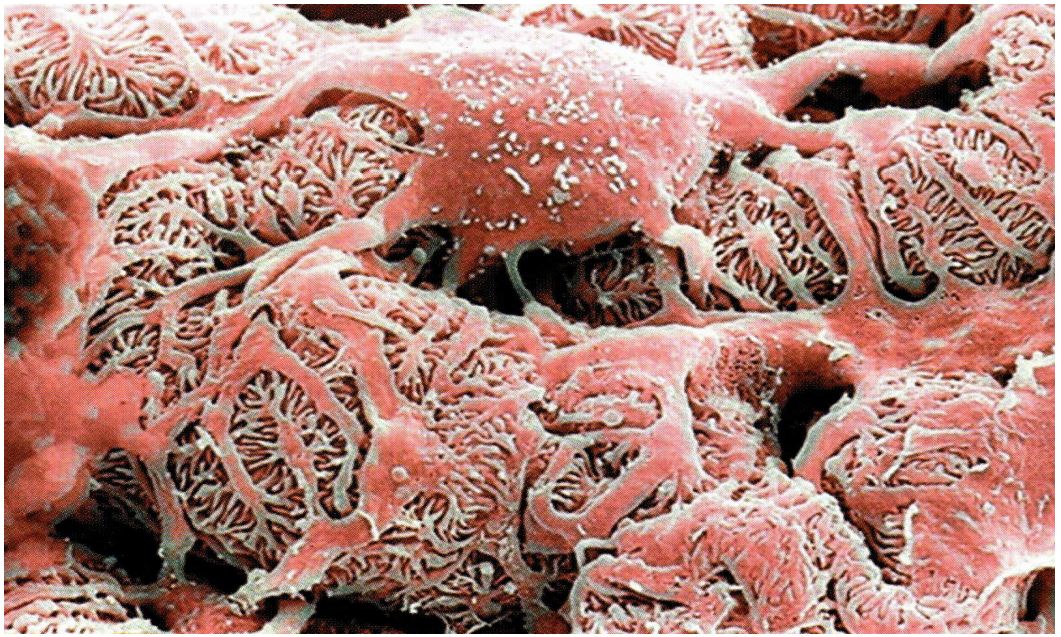
the thick basement membrane
~ 300 nm

the slit membrane (pore size ~ 5 nm)

the processes of podocytes
(visceral epithelium) and
filtration slits

The basement membrane consists of type IV collagen fibres and certain amount of laminin and proteoglycans. Heparan sulfate in the membrane and sialic acid containing glycoproteins in the filtration slits have **negative electrical charges.**

Podocytes with their interdigitating foot processes separated by filtration slits cover the basement membranes of capillary walls (scanning electron micrograph).



Plasma proteins with a M_r above approx. 120 000 are excluded by **filtration**. Proteins with a M_r above 65 000 and anionic character (albumin and transferrin) are prevented from entering the urine by an **electrostatic filter**, concentration of albumin in the filtrate is about 4 mg/l (0.01 % plasma concn.).

Low-molecular plasma proteins (M_r 10 000 – 60 000) cross the filtration barrier, their concentration in glomerular filtrate can reach 50 – 90 % concentration in plasma.

Healthy adults excrete **less than 150 mg of total urinary protein per 24 h**:

albumin	7 – 11 mg / d (**less than 20 mg / d**),
transferrin	< 1 mg / d,
α_1-acid glycoprotein	< 10 mg / d,
α_1-microglobulin	1 – 5 mg / d,
retinol-binding protein	< 0.5 mg / d,
β_2-microglobulin	< 0.3 mg / d, etc.

Proteinuria – **more than 300 mg of total urinary protein per 24 h** .

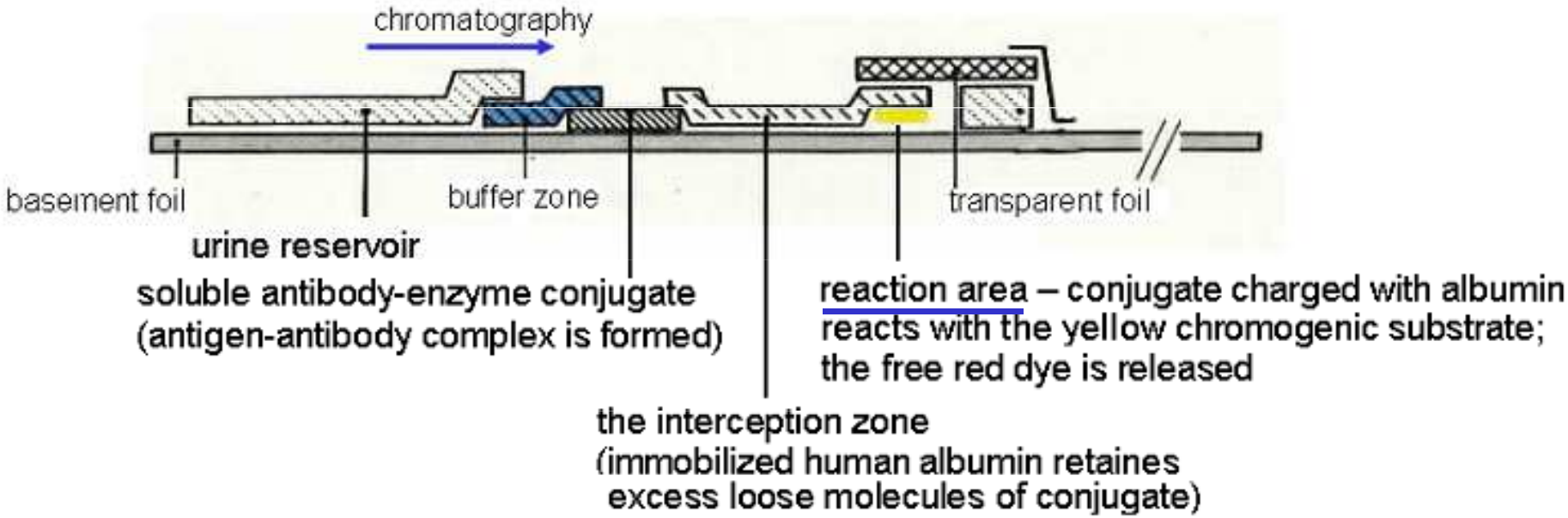
Glomerular proteinuria – the normal glomerular barrier to plasma proteins is disrupted, proteins with molecular mass higher than 65 000 are present in the urine (albumin 67 000, transferrin 79 000, immunoglobulins > 150 000). Low-molecular proteins are reabsorbed in the tubules.

Tubular proteinuria – the cause is in incomplete proximal tubular reabsorption (in the presence of normal glomerular permeability), normally filtered low-molecular-mass plasma proteins appear in the urine in increased amounts.

Tubular proteinuria can occur alone or in association with glomerular proteinuria.

Test for microalbuminuria (Micral-test®)

Microalbuminuria is excretion of albumin in the range **from 30 mg / d** to 200 – 300 mg /d (to obvious proteinuria). It often predicts development of nephropathy. It is important to detect microalbuminuria early, because kidney damages are minimal and are still reversible. Measurements of urinary albumin is especially important for diabetic and hypertensive patients.



Glomerular filtration rate (GFR)

is the volume of glomerular filtrate formed in a unit of time.

Normal range **1.33 – 2.33 ml / s** i.e. 80 – 140 ml / min
(115 – 200 l / d)

The ideal marker for GFR

– would appear endogenously in the plasma at a constant concentration,
– would be freely filtered at the glomerulus,
– would be neither reabsorbed nor secreted by the renal tubule, and
– would undergo no extrarenal elimination from the body.

Clearance of inulin or with certain limitations also **clearance of endogenous creatinine** are equal to **GFR**.

Clearance of inulin

is a very **exact measure of GFR**, suitable more for scientific research than for routine use.
Inulin is a plant (or synthetic) polyfructosan. It must be infused intravenously, about 50 mg/kg body weight. The samples of blood as well as urine cannot be taken before a steady concentration of inulin in plasma is reached.

Creatinine clearance (C_{creat})

serves as an routine estimate of GFR for more than 60 years, because it is a very simple test..

Creatinine is excreted predominantly through glomerular filtration. There is no tubular reabsorption, but (namely at higher plasma concentrations) the amount of creatinine secreted in the tubules increases.

Clearance of creatinine depends on age and gender, timed samples of urine must be collected, and the analytical method is not quite specific.

Creatinine clearances have slightly higher values when compared with inulin clearances (about 2.33 and 2.00, resp.).

Calculation:

Entries - c_{plasma}(creatinine) in mmol/l (normal value about 115 µmol/l)
c_{urine}(creatinine) in mmol/l
V_{urine} / t in **ml** / **s**

$$C_{creat} = \text{uncorrected } \mathbf{GFR} = c_{urine} \times V_{urine}\, t^{-1} / c_{plasma} \quad \text{(ml/s)}$$

Glomerular filtration rate corrected to the standard body surface area 1.73 m^2:

$$\mathbf{GFR}_{corr} = \mathbf{GFR} \times A / 1.73 \quad \text{(ml/s)}$$

Body surface $A = 0.167 \times \sqrt{h \times w}$ (in m^2)

Estimate of GFR from cystatin C concentration in serum

Cystatin C is a low-molecular protein ($M_r \approx 13\,400$) that acts as an inhibitor of cysteine proteinases and is released uniformly from all nuclear cells into the circulating blood. Its **concentration is stable** (e.g., it doesn't depend on inflammatory processes).

Like other low-molecular weight proteins, cystatin C is eliminated from the plasma exclusively by glomerular filtration and decomposed in tubular cells.

Cystatin C concentration in serum is indirectly related to GFR.

The interindividual variability in cystatin C concentration is lower than that of creatinine, what enables the early detection of decrease in GFR.

Concentration of cystatin C in serum or plasma is determined by means of an immunoturbidimetric method or ELISA. There is no need to collect urine.

The estimate of GRF corrected to the standard body surface area is calculated in adults as

$$GFR_{cystatin}\ (\text{ml / s}) = 1.41 \times \rho\ (\text{cystatin C, mg/l})^{-1.68},$$

in adolescents under the age of 14 multiplied by the "praepubertal" factor 1.384 .

Estimates of GFR_{corr} from the clearance of creatinine are progressively substituted by $GFR_{cystatin}$.

Glomerular filtration - laboratory investigations

Serum creatinine concentration

~ 100 μmol/l (55 – 120 μmol/l)

Rough estimates of GFR from the creatinine concentration were derived in the past (e.g. the Cockcroft-Gault formula).

Glomerular filtration rate.

1.5 – 2.5 ml/s – estimated either from serum cystatin C concentration or as creatinine clearance corrected to body surface area 1.73 m^2.

Serum urea concentration

2.5 – 6.6 mmol/l

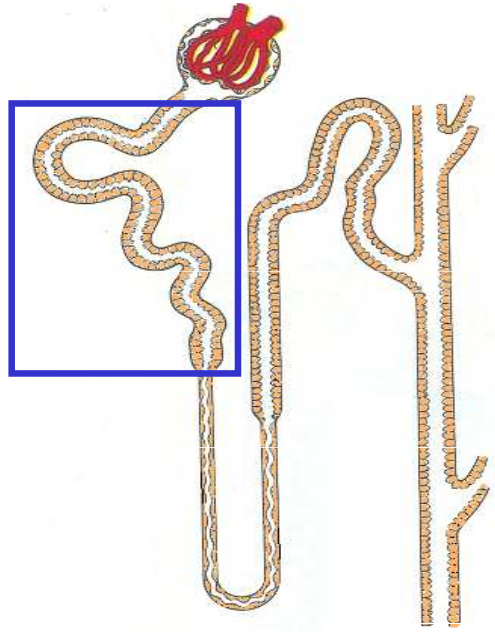
High serum urea in any cause of impaired renal perfusion, reduced GFR, or obstruction to urine outflow.

Detection of proteinuria / microalbuminuria

Quantification of urinary protein excretion
(albumin < 20 mg/d, total protein < 150 mg/d)

Serum albumin concentration, determination of proteinuria type

The functions of renal tubules



The proximal tubule

Efficient reabsorption of most amino acids,
nearly all glucose, unless its supply is greater
than the capacity of the transfer into the cells
- a threshold approximately at 10 mmol/l,
most Na^+ (50 – 60 %), K^+, Cl^-, phosphates, HCO_3^-, etc.,
driven by Na^+,K^+-ATPase located within the basolateral membranes.

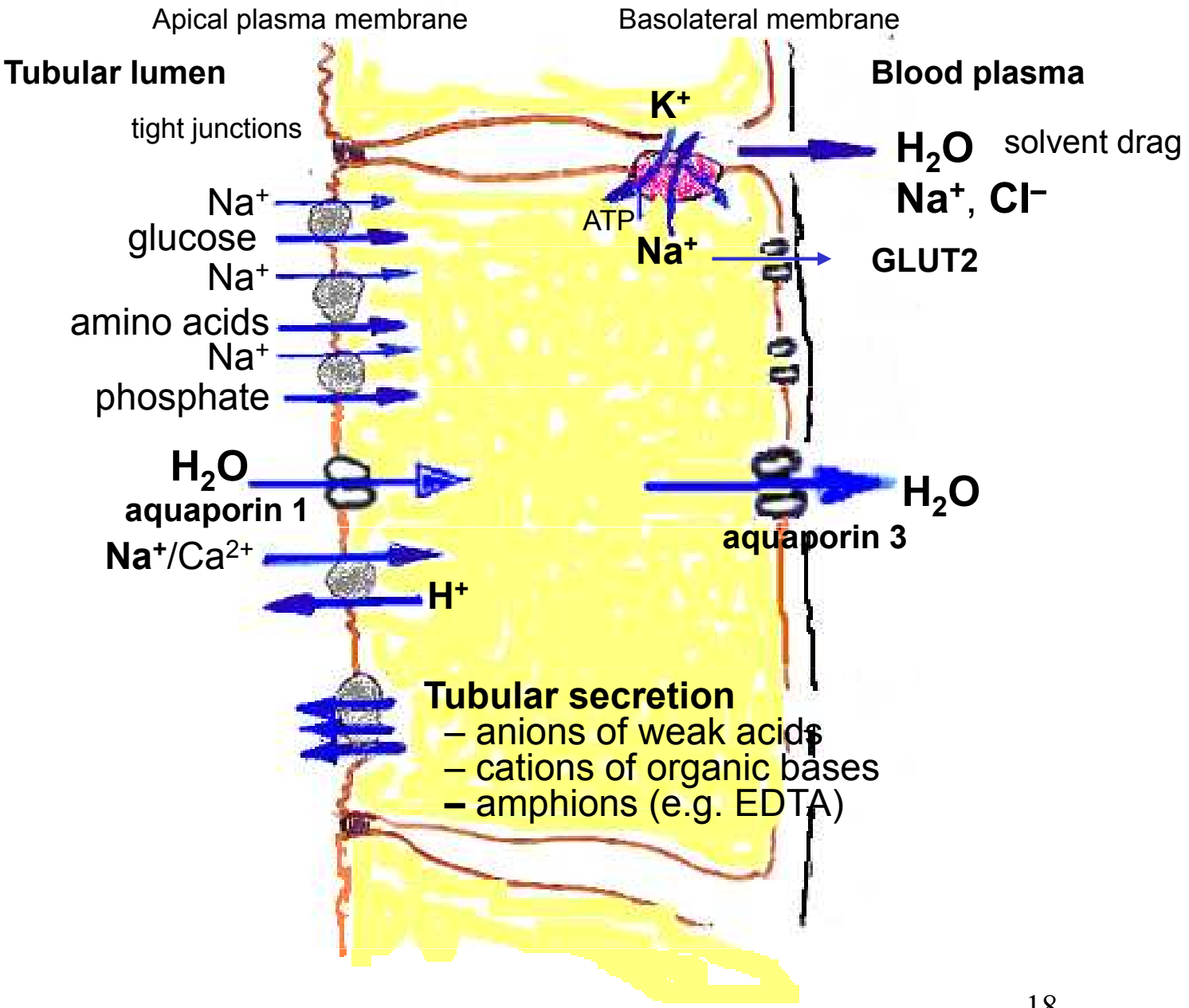
Passive reabsorption of water (70 – 80 %, isotonic and "obligatory",
independent on ADH).

Tubular secretion of organic ions, basic drugs.

Proximal tubule

Reabsorption of
- 70 – 80 % water
- 50 – 60 % Na^+

Reabsorption of
(not shown here)
- > 90 % K^+,
- 85 % Cl^-,
- 90 % HCO_3^- ,
- 50 – 70 % phosphates,
- 65 % Ca^{2+},
- urate,
- small proteins, etc.



The loop of Henle and vasa recta

form the osmotic gradient between the renal cortex and medulla.

High osmolality of the medullary peritubular interstitial fluid is essential for the efficient reabsorption of water in the collecting ducts, for concentration of urine. The osmolality increases from the cortex to the medulla, and it is maintained by the **countercurrent mechanism** that consists of

- **countercurrent multiplication of the loop of Henle**, and
- passive **countercurrent exchange** of water and urea between vasa recta, the descending loop of Henle, and collecting ducts.

In the thick ascending limb, which is impermeable for water, Na^+,K^+-ATPase drives the active ion transport out from tubules (increase in osmolality of interstitial fluid). .
In the thin descending limb, water moves freely from the tubules into the hyperosmolal interstitial fluid.

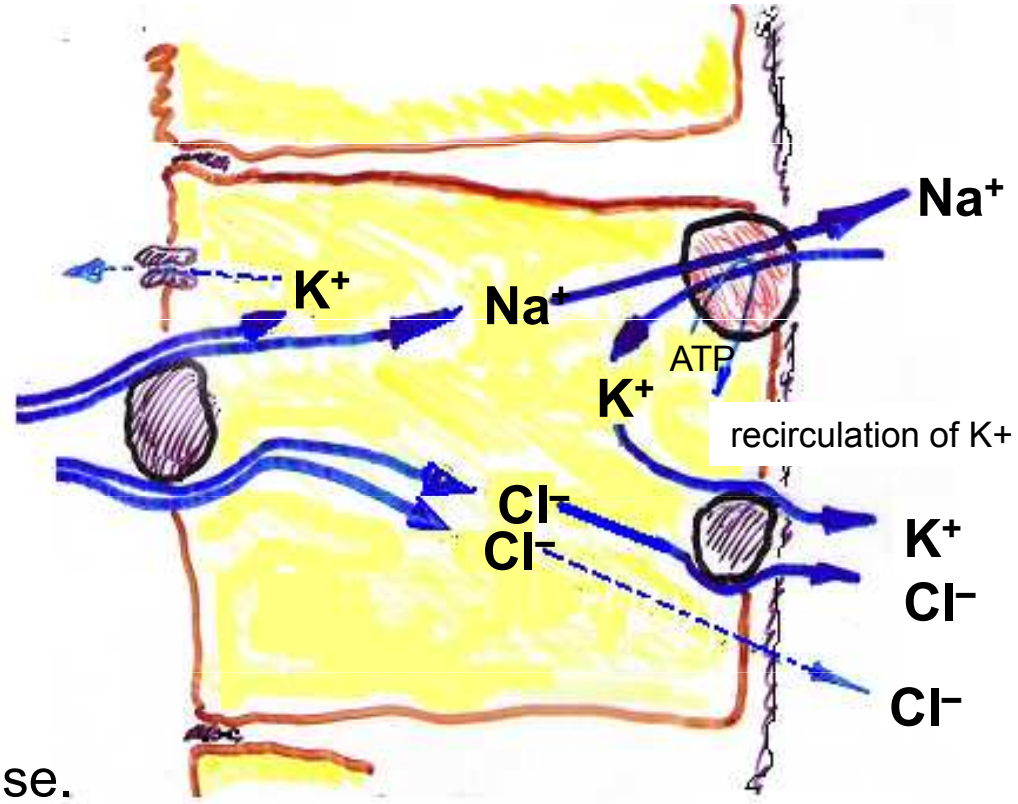
The medullary parts of collecting ducts are permeable for water (controlled by ADH) and urea, so that water may be efficiently reabsorbed (similarly to the thin descending limb of the loop) into the hyperosmolal interstitial fluid and drained to the cortex by vasa recta. There is also a high concentration of urea in the collecting ducts, urea freely diffuses into the interstitial fluid, into the thick ascending parts of the loop of Henle and vasa recta (urea recycling).

The thin descending limb of the loop

There are numerous aquaporins in the plasma membrane (AQP 1 in the apical part) that make the membranes **freely permeable to water**, which moves out of the lumen into the hyperosmolal surrounding tissue; **ion transporters are absent**.

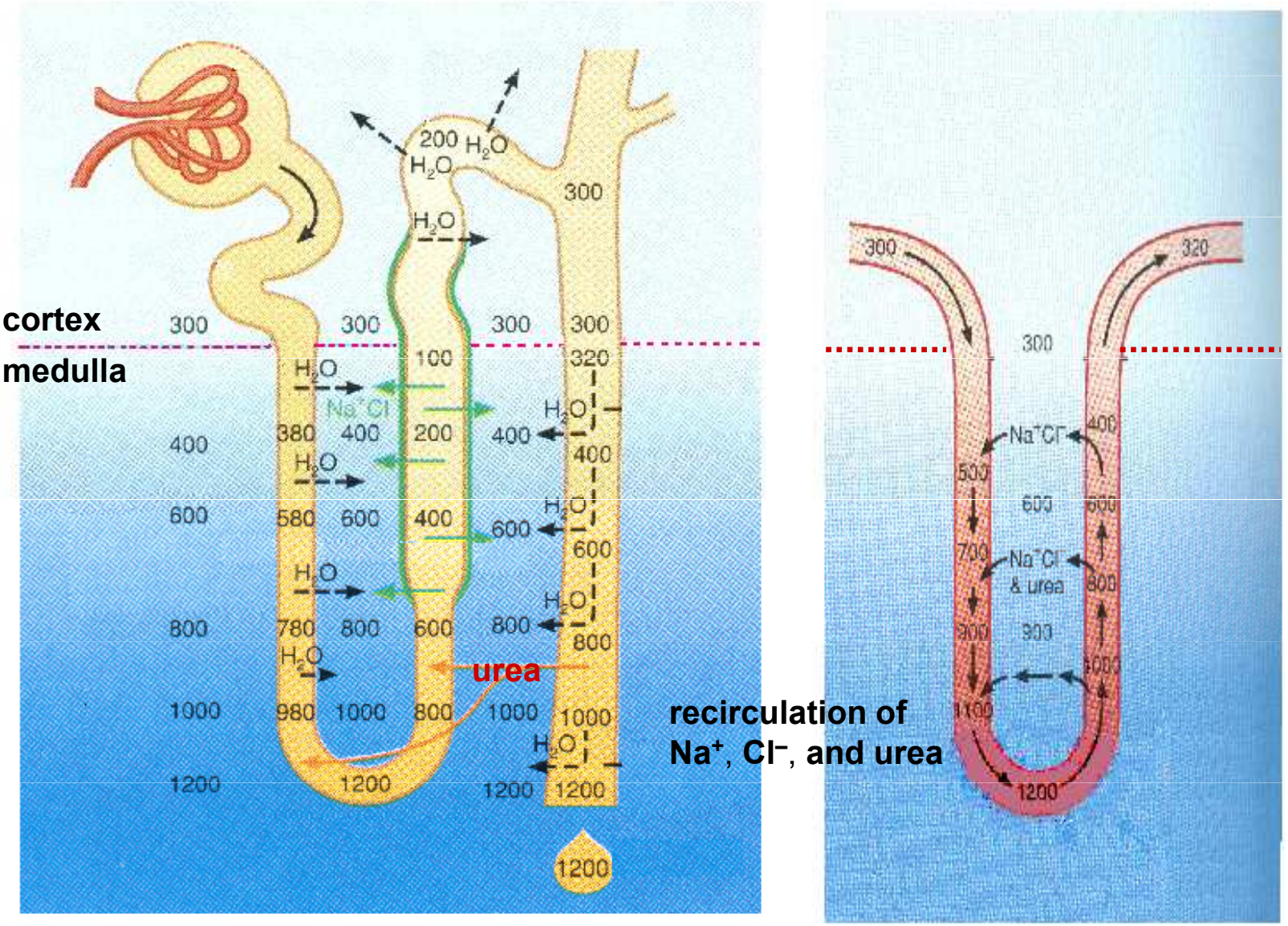
The thick ascending limb of the loop

Apical membrane of the thick ascendent limb of the loop is **impermeable for water**.

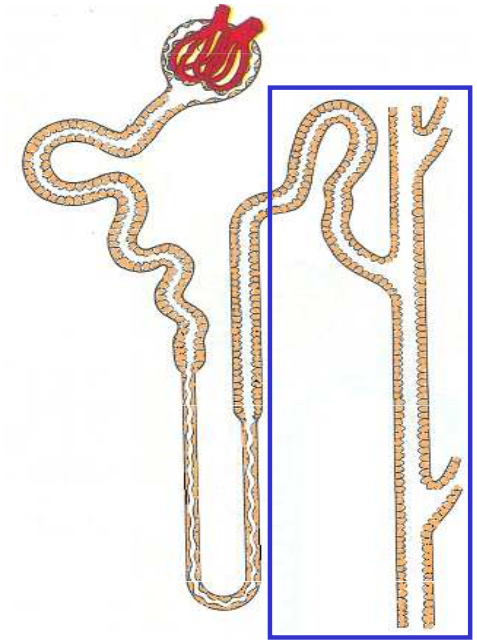


Na^+, K^+, and Cl^- are pumped out **against the concentration gradient** into the hyperosmolal interstitial fluid (the process is driven by Na^+,K^+-ATPase.

Osmolality of the tubular and peritubular interstitial fluid

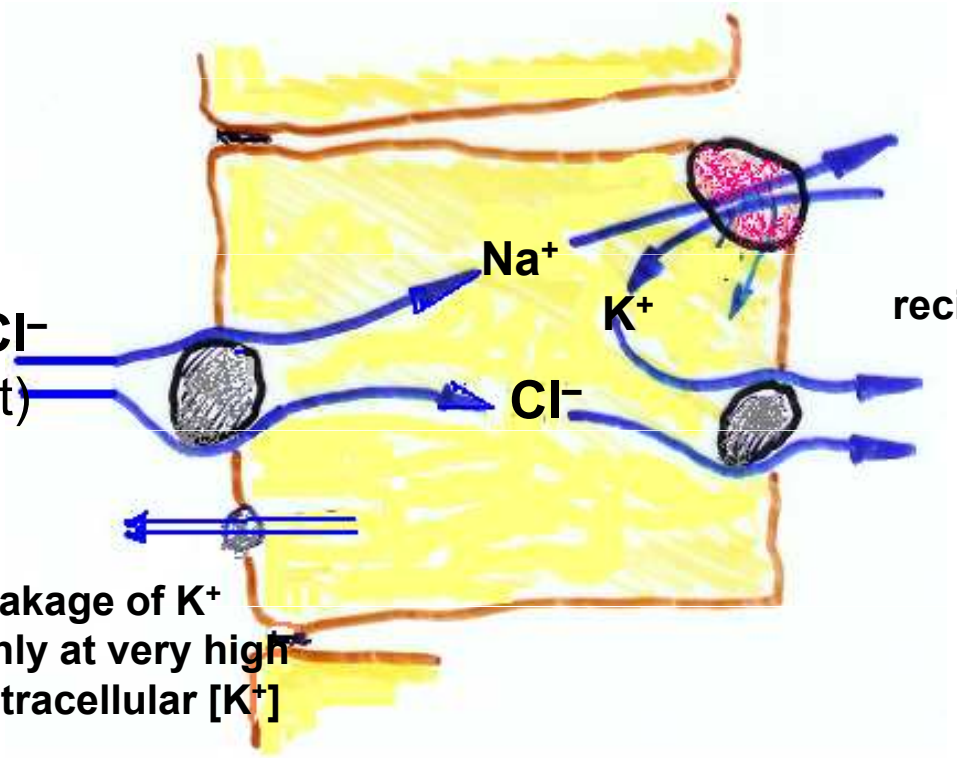


Distal tubules and collecting ducts



- Passive reabsorption of water into the hyperosmolal interstitium (controlled by antidiuretic hormone) – the **final concentration of urine**.
- Resorption of Na^+ (driven by Na^+,K^+-ATPase) and
- tubular secretion of K^+ (most of K^+ excreted into the urine) – both under the control of aldosterone or natriuretic peptides
- Excretion of Ca^{2+} and phosphates under the control of parathyrin and calcitonin.
- A part of tubular secretion of H^+ in the form of $H_2PO_4^-$ and NH_4^+ depends on the acid-base status.

Distal tubule



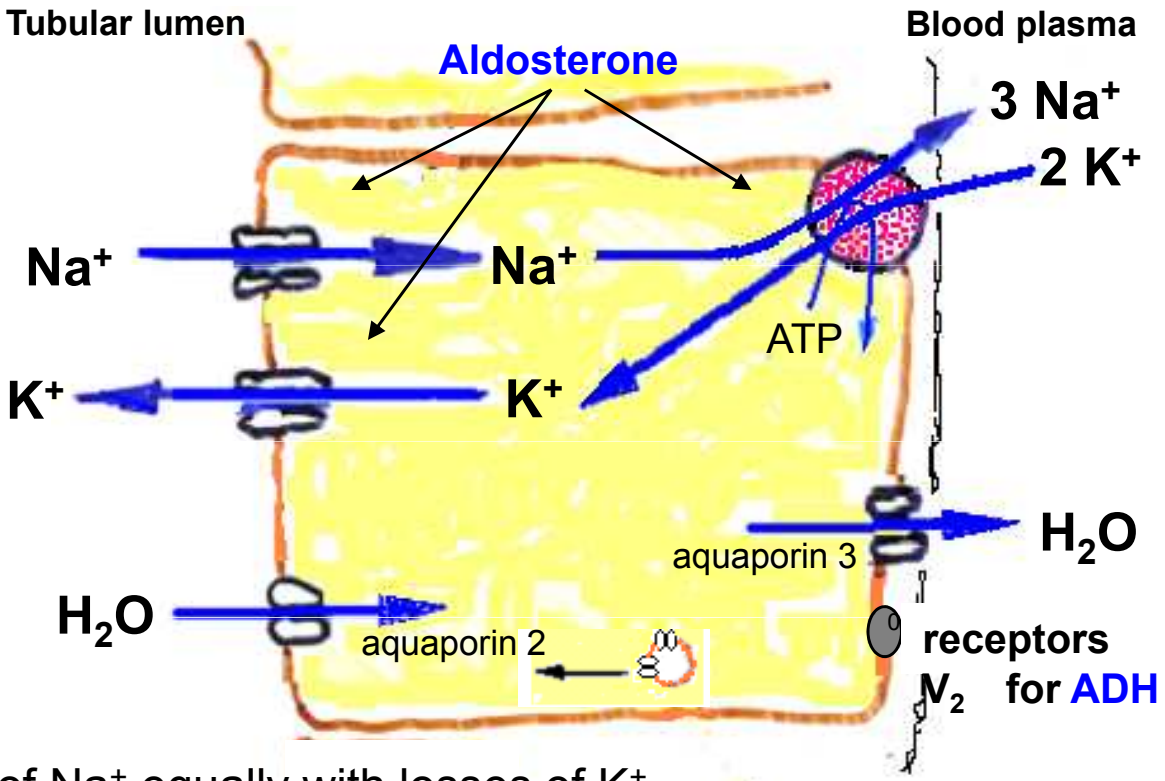
The cells of distal tubules represent the sites of **Ca^{2+} reabsorption** (about 10 % of total reabsorbed) and **phosphate leakage** controlled by **parathyrin** and to a less extent by **calcitonin**.

Collecting ducts

The principal tubular cells

In spite of reabsorption of only about **3 % of total filtered Na^+ ions** in the collecting ducts, changes in that relatively small percentage are decisive in natriuresis, elimination of Na^+ from the body.

Both Na^+ and K^+ channels are inhibited by "potassium saving" diuretics amilorid and spironolacton.



Aldosterone enlarges reabsorption of Na^+ equally with losses of K^+ by exposition of Na^+ and K^+ channels and also by partial stimulation of Na^+,K^+-ATPase.

Antidiuretic hormone (ADH, Arg-vasopressin) increases reabsorption of water. It binds onto membrane V_2 receptors and activation of proteinkinases A is a cause of exposition of aquaporin AQP-2 in the apical membrane.

Natriuretic peptides inhibit Na^+ and K^+ channels (in addition to the vasodilating effect) so that they support Na^+ excretion and retention of K^+.

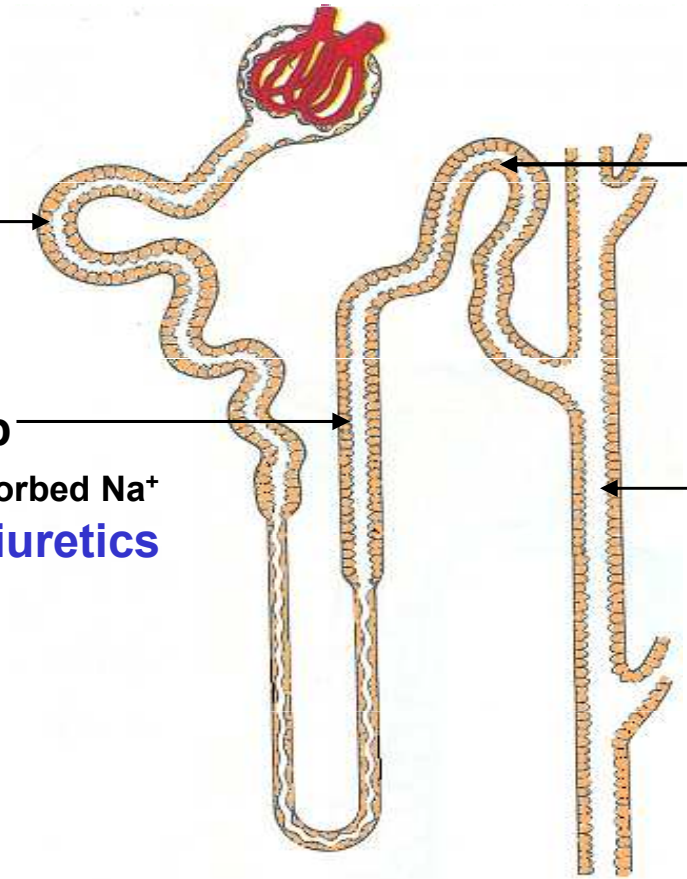
The intercalated cells

have their major role in excretion of H^+ and reabsorption of HCO_3^-.

Natriuresis - the renal excretion of sodium ions

Diuretics are drugs that support elimination of water from the body by stimulating natriuresis through different mechanisms. Most diuretics influence the cells from the tubular fluid (the apical part of plasma membranes).

proximal tubules
50 - 60 % reabsorbed



distal tubules
5 – 8 % of total reabsorbed Na^+
inhibition by **thiazide diuretics**
(partly by "loop" diuretics, too)

thick ascending limb
30 – 40 % of total reabsorbed Na^+
inhibition by **"loop" diuretics**
(e.g. furosemide)

collecting ducts
2 – 3 % of total reabsorbed Na^+
(regulated by aldosterone)
inhibition by
potassium- saving diuretics
(e.g. amiloride, spironolactone)

Tubular functions - laboratory investigation

Capacity of the kidneys top concentrate the urine

(formerly tested by thirsting – healthy subjects up to osmolality 800 – 1100 mmol/kg_{H2O} or density 1026 – 1032 g/l)

Osmolar clearance C_{osm}

should not exceed 0.05 ml/s to prevent solute losses

Fractional excretion of solutes E/F_{osm} normal values ≤ 0.035 (≤ 3.5 %)

Clearance of electrolyte-free water (EWC) C_{EW}

negative values – amount of water which has to be added to the urine to become isotonic with the plasma

positive values – amount of water which has to be relieved from the urine to become isotonic with the plasma

the usual range – 0.002 ± 0.008 ml/s

(positive values only temporarily in healthy persons after high water intake)

Urinary Na^+ output

must be proportional to the intake, about 100 – 200 mmol/d.

Fractional excretion of Na^+ E/F_{Na+} normal range 0.004 – 0.012 (**0.4 – 1.2 %**)

Fractional excretion of K^+ E/F_{K+} normal range 0.04 – 0.19 (**4 – 19 %**)

The endocrine function of the kidney

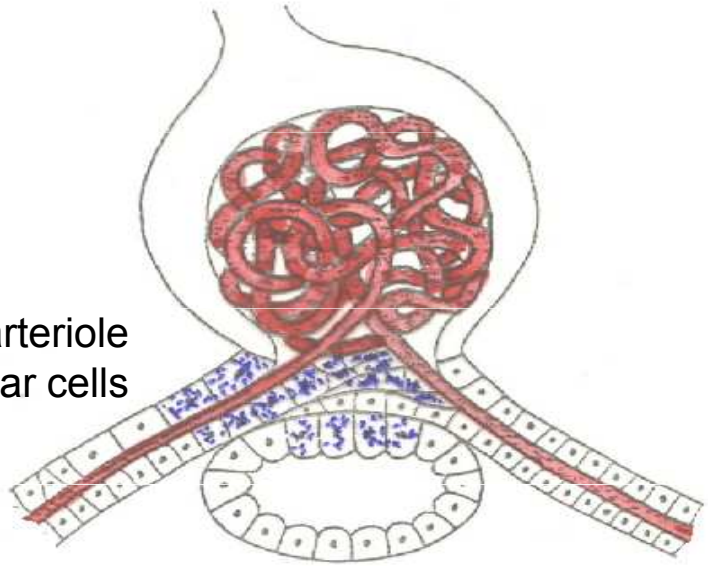
Erythropoietin (EPO) is the primary regulatory factor of erythropoiesis (formation of red blood cells in the bone marrow). EPO is a glycoprotein (165 AA, M_r ~ 34 000), released in the kidney (about 10 % also in the liver) by splitting of the precursor protein by the action of a specific proteinase. Hypoxia and decrease in blood haemoglobin stimulate the release of EPO from the kidneys.

Calcitriol (1α,25-dihydroxycalciol) originates from circulating calcidiol (25-hydroxycalciol) by hydroxylation in the renal tubular cells.
The specific hydroxylating system is stimulated by parathyrine.

Urodilatin is the natriuretic peptide synthesized in the kidney (4 amino acid residues more than ANP, M_r ~ 3 500) being more paracrine, it is secreted into the urine. Urodilatin regulates water and sodium reabsorption in the collecting duct by inhibition of Na^+ channels and vasodilation.

Renin is a proteinase secreted from the juxtaglomerular cells that starts the renin-angiotensin system – RAS: ./.

The juxtaglomerular apparatus and the renin-angiotensin system



The juxtaglomerular apparatus responds to decreased renal perfusion pressure by secreting a proteinase, **renin**, that uses angiotensinogen (one of the plasma glycoproteins in the α_2-globulin fraction) as its substrate and liberates **angiotensin I** (a decapeptide) from angiotensinogen. Angiotensin I is transformed into a octapeptide **angiotensin II** (by angiotensin-converting enzyme, **ACE**) that stimulates the synthesis and release of a mineralocorticoid **aldosterone**.

The macula densa also senses the tubular chloride concentration.

Control of acid-base balance by the kidneys

In healthy persons,
50 – 100 mmol H^+ / d (from non-volatile acids) have to be excreted.

Although the urine is more acidic than blood plasma, excretion of free H^+ ions in this way (actual acidity) may be neglected: Only 0.01 mmol H^+ is excreted in one litre urine pH 5.0 .

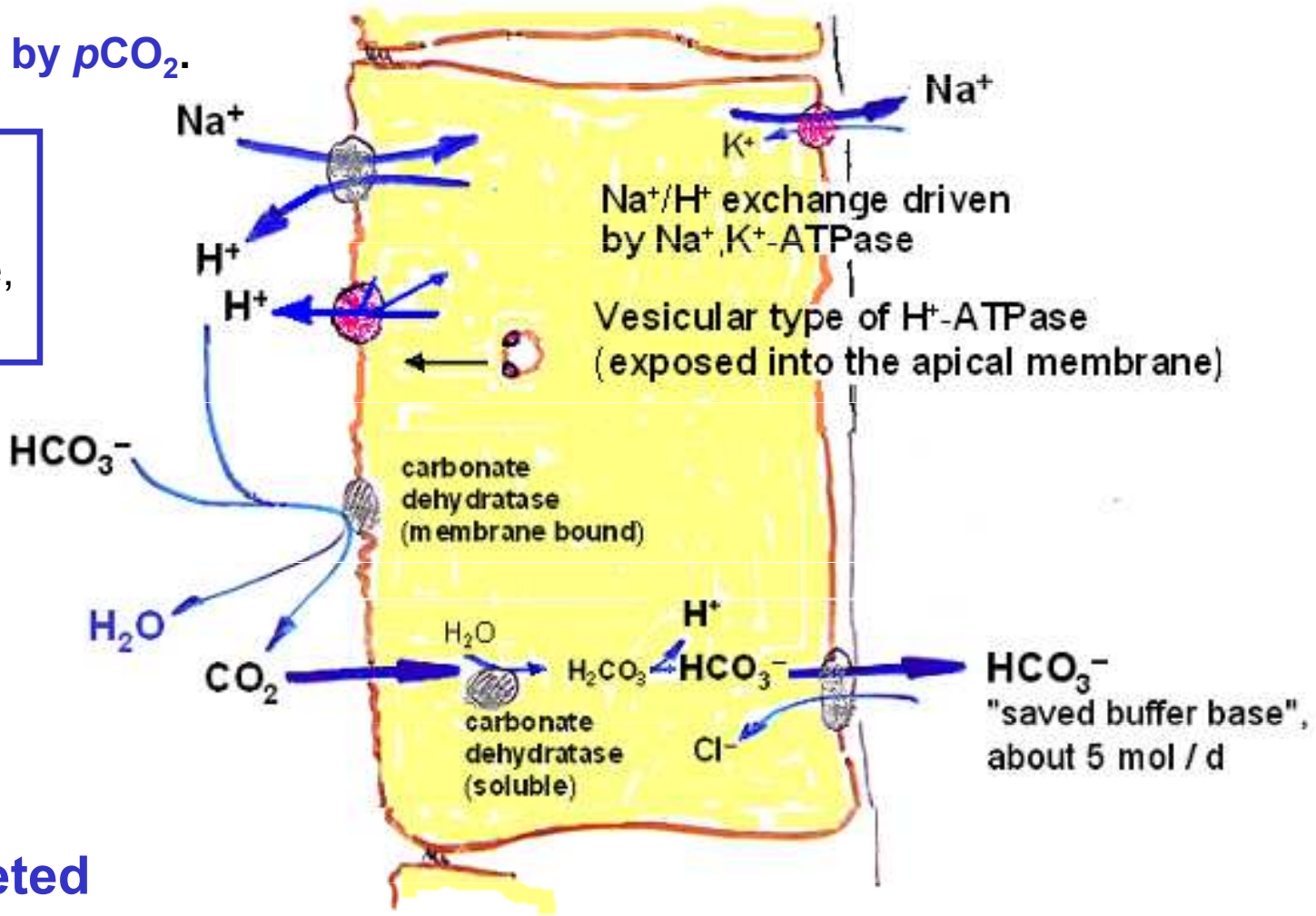
- Tubular cells excrete H^+ ions in the form of **$H_2PO_4^-$ ions** (H^+ bound onto HPO_4^{2-}), the urine so becomes slightly acidic - this titratable acidity represents approximately **10 – 30 mmol H^+ / d** eliminated from the body.
- Tubular cells excrete **NH_4^+ ions** formed from NH_3 by binding H^+ ; NH_3 is released from glutamine (and glutamate). About **30 – 50 mmol H^+ / d** are excreted in this way.
- **Reabsorption of HCO_3^- ions** from tubular fluid saves the main plasma buffer base.
  In alkalosis, **HCO_3^- ions are secreted** into the urine.

Reabsorption of HCO_3^-

90 % **HCO_3^- reabsorbed in the proximal tubule** (**pars recta**),
10 % **in the collecting ducts** (**by intercalated cells**)

Reabsorption is **regulated by *p*CO_2.**

H^+ ions transported into the tubular fluid are not excreted into the urine, they give water.



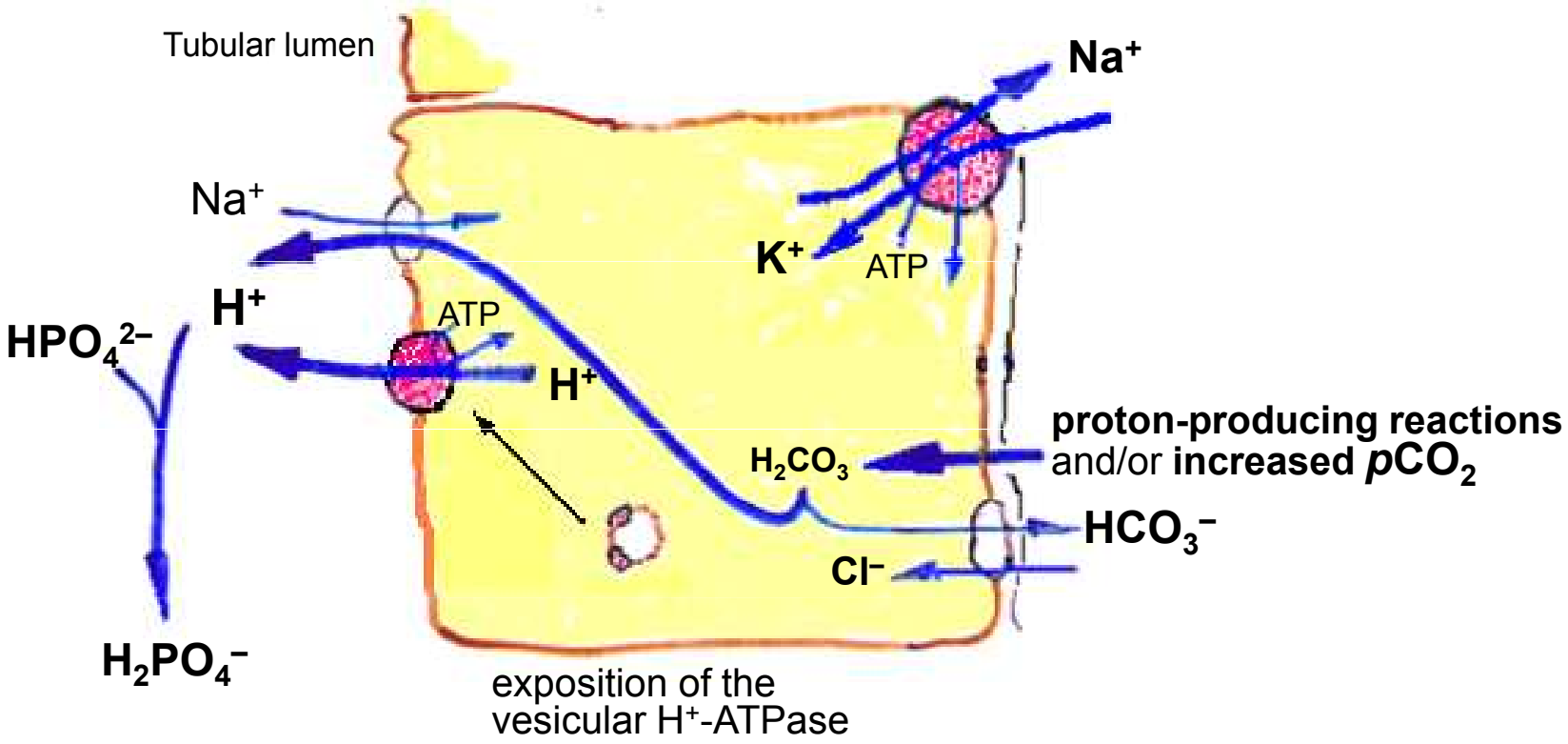
HCO_3^- ions are secreted in alkalosis:

When [HCO_3^-] in blood exceeds 28 mmol/l, Na^+/H^+ antiport is diminished and Cl^-/HCO_3^- antiporters are inserted into the apical membrane of **separate base-secreting tubular cells** by a triggered exocytosis. A steady state with a maximal HCO_3^- excretion is reached in the course of several days.

Excretion of H^+ in the form of $H_2PO_4^-$

Cells of straight part of proximal tubules and **intercalating cells of collecting ducts**

H^+ ions bound onto hydrogen phosphate changes the ratio $[HPO_4^{2-}] / [H_2PO_4^-]$, so that they result in only a slight decrease in pH value. The amount of H+ excreted in this way can be measured as "titratable" acidity of the urine.

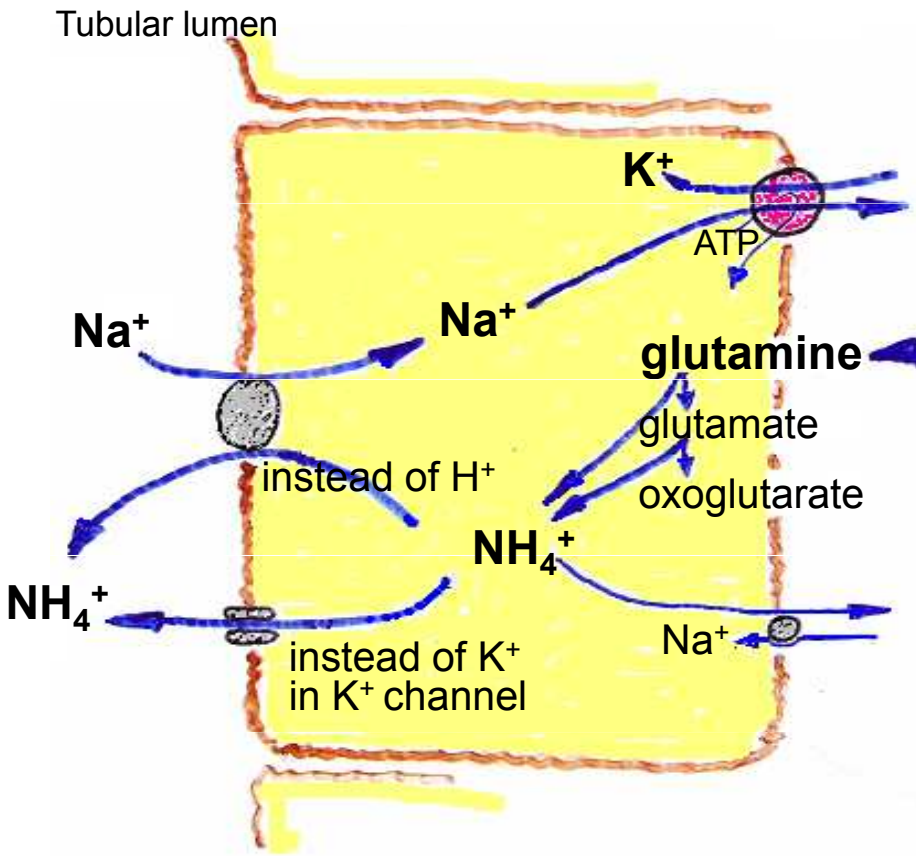


Under physiological conditions, **10 – 30 mmol H^+/d** are excreted in this way. The increase of that amount is limited by the accessibility of HPO_4^{2-} in the tubular fluid to a maximum of about 40 – 50 mmol/d, i.e. to the urinary pH 4.5).

The excretion of H^+ in the form of NH_4^+

Proximal tubules, **distal tubules**, and **intercalated cells of collecting ducts**

– without any decrease in pH value of the urine.



production of glutamine in the liver preferred **in acidosis**

transfer of NH_4^+ into the blood **in alkalosis**

(NH_4^+ is the substrate for ureosynthesis that is a proton-producing process)

Under physiological conditions, **30 – 50 mmol H^+/d** are excreted in this way.
In an extreme acidosis, the excretion of NH_4^+ may reach (stepwise during 5 days) 200 – 500 mmol/d; NH_4^+ can accumulate in the renal medullary interstitium.